frontiers | Research Topics

The potential role of melatonin in the regulation of abiotic stress in plants

Edited by
Muhammad Ahsan Altaf, Milan Kumar Lal,
Rahul Kumar Tiwari, Safina Naz and Vijay Gahlaut

Published in
Frontiers in Plant Science

ISSN 1664-8714
ISBN 978-2-8325-3563-9
DOI 10.3389/978-2-8325-3563-9

About Frontiers

Frontiers is more than just an open access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers journal series

The Frontiers journal series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the *Frontiers journal series* operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews. Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the *Frontiers journals series*: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area.

Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers editorial office: frontiersin.org/about/contact

The potential role of melatonin in the regulation of abiotic stress in plants

Topic editors
Muhammad Ahsan Altaf — Hainan University, China
Milan Kumar Lal — Central Potato Research Institute (ICAR), India
Rahul Kumar Tiwari — Central Potato Research Institute (ICAR), India
Safina Naz — Bahauddin Zakariya University, Pakistan
Vijay Gahlaut — Chandigarh University, India

Citation
Altaf, M. A., Lal, M. K., Tiwari, R. K., Naz, S., Gahlaut, V., eds. (2023). *The potential role of melatonin in the regulation of abiotic stress in plants*. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-8325-3563-9

Table of contents

Frontiers in Plant Science

TYPE Editorial
PUBLISHED 12 September 2023
DOI 10.3389/fpls.2023.1271973

OPEN ACCESS

EDITED AND REVIEWED BY
Baris Uzilday,
Ege University, Türkiye

*CORRESPONDENCE
Milan Kumar Lal
milan2925@gmail.com
Rahul Kumar Tiwari
rahultiwari226@gmail.com

RECEIVED 03 August 2023
ACCEPTED 05 September 2023
PUBLISHED 12 September 2023

CITATION
Altaf MA, Lal MK, Tiwari RK, Naz S and Gahlaut V (2023) Editorial: The potential role of melatonin in the regulation of abiotic stress in plants.
Front. Plant Sci. 14:1271973.
doi: 10.3389/fpls.2023.1271973

Editorial: The potential role of melatonin in the regulation of abiotic stress in plants

Muhammad Ahsan Altaf[1], Milan Kumar Lal[2]*,
Rahul Kumar Tiwari[2]*, Safina Naz[3] and Vijay Gahlaut[4]

[1]Key Laboratory for Quality Regulation of Tropical Horticultural Crops of Hainan Province, Sanya Nanfan Research Institute, Hainan University, Sanya, China, [2]ICAR-Central Potato Research Institute, Shimla, Himachal Pradesh, India, [3]Department of Horticulture, Bahauddin Zakariya University, Multan, Pakistan, [4]Department of Biotechnology and University Center for Research and Development, Chandigarh University, Mohali, India

KEYWORDS

melatonin, abiotic stress management, photosynthesis, oxidative damage, root growth

Editorial on the Research Topic
The potential role of melatonin in the regulation of abiotic stress in plants

Melatonin (N-acetyl-5-methoxytryptamine) is a dynamic molecule with a diverse range of functions in plants. Initially, melatonin was discovered in the bovine pineal gland in 1958. Melatonin was discovered in higher plantsn 1995 (Dubbels et al., 1995). Afterward, the multiple functions of melatonin have shown its significant promise in the field of plant physiology (Shi et al., 2017). Recently, melatonin has gained recognition as a versatile natural protector for crop plants, effectively mitigating both abiotic and biotic stresses (Tiwari et al., 2020; Altaf et al., 2021a). Melatonin plays a significant role as an antioxidant in plants, thereby enhancing their ability to withstand various stressors such as heat, drought, heavy metal exposure, salinity, chilling temperatures, as well as viral and fungal infections (Altaf et al., 2021a; Tiwari et al., 2021a; Tiwari et al., 2021b).

Furthermore, melatonin exerts its influence on plant physiology by regulating essential processes like rhizogenesis (the formation of lateral roots and root primordia), carbon assimilation, stomatal conductance, photochemical efficiency of photosystems, RuBisCO accumulation, chlorophyll molecule breakdown, and the ascorbate-glutathione (AsA-GSH) cycle in stressed plants (Moustafa-Farag et al., 2020; Sharma et al., 2020). The predominant mechanism through which melatonin exerts its anti-stress effects is enhancing the plant's antioxidant defense mechanisms and aiding in scavenging ROS.

In the current scenario, climate change significantly influences agricultural productivity. Abiotic stress is the major agricultural constraint since it interferes with plant growth and yield (Hayat et al., 2023). Plants are exposed to various abiotic stresses, such as heavy metals, drought, temperature fluctuation, acid rain, salinity, and nutrient deficiency (Lal et al., 2023). Abiotic stresses significantly affect seed germination, flowering, root architecture, leaf photosynthesis, and seedling growth, ultimately reducing plant growth, yield, and quality (Rhaman et al., 2020). Therefore, it is crucial to have an area of research that includes plant physiological, morphological, and metabolic responses to determine the effect of abiotic stressors and identify possible defense mechanisms and mitigating approaches.

Application of plant growth regulators has become prevalent in agricultural crop production, enhancing yield quality and alleviating abiotic challenges' impact (Altaf et al., 2023b). Melatonin regulates various plant physiological functions such as seed germination, photosynthetic efficiency, root architecture system, mineral nutrient uptake, maintained redox homeostasis, secondary metabolites production and balanced antioxidant enzymes system in plants (Altaf et al., 2021b). Compelling evidence shows that melatonin positively regulates the abiotic stress tolerance in plants. The majority of these studies propose that melatonin plays a dual role in plant defense mechanisms. Firstly, it appears to act as the frontline defense against reactive oxygen species (ROS), effectively neutralizing free radicals and preventing their harmful effects. Secondly, melatonin is involved in the second line of defense by regulating the expression of various genes that respond to stress conditions. This dual function positions melatonin as a crucial factor in helping plants cope with environmental challenges (Altaf et al., 2023a).

This Research Topic focuses on the potential role of melatonin in regulating abiotic stress in plants. We aim to ask whether and how melatonin regulates plant growth and stress responses and how the melatonin network interacts with other signalling pathways. This Research Topic contains seven original research and two review articles.

Zhao and Hu described the potential functions of melatonin in plants, including seed germination, leaf photosynthesis, root architecture system, redox homeostasis, antioxidant defence system, and seedling health index, as well as the influence of melatonin on plant growth and stress response. Melatonin acts as a growth regulator and bio-stimulator, enhancing plant tolerance to abiotic stress by improving nutrient uptake, osmolyte production, and cellular membrane stability. The review by Zhao and Hu provides valuable insights into how melatonin can be used to develop stress-tolerant horticultural crops in changing environments. Ahmad et al. highlighted melatonin's interactions with nitric oxide and indole-3-acetic acid (IAA), which regulated physiological, morphological and metabolic functions. As a result of its endogenous application and regulatory roles involving NO and IAA, melatonin is capable of enhancing plant resilience and productivity under abiotic stresses.

The potential roles of melatonin were also revealed in different plant species under abiotic stress in this Research Topic. In common bean, Zhang et al. revealed that exogenous melatonin application significantly increased cell wall regulation pathway, plant growth, shoot length, and root morphological traits under salt stress. Melatonin treatment improved salt tolerance in over 65% of germplasm materials, and specific markers associated with cell wall synthesis enabled the prediction of melatonin-responsive varieties. This research suggests that melatonin can enhance salt tolerance in common bean by influencing the cell wall and providing markers for selecting stress-tolerant varieties. In barley, Jiang et al. found that rhizospheric melatonin supplementation favorably controlled the photosynthetic carbon assimilation and redox homeostasis in response to low temperatures. Similarly, exogenous melatonin reduced the impacts of drought stress in groundnuts by boosting endogenous melatonin concentration, which increased the antioxidant system and photosynthetic properties (Shreya et al.). Ghorbani et al. observed that melatonin application efficiently improved plant growth and biomass production, pigments content, proline production, antioxidant defence system, nitrogen metabolism, ion homeostasis, and reduced ROS production, electrolyte leakage and sodium accumulation in tomato seedlings under salinity stress. Awan et al. consistently noticed that melatonin markedly increased seed germination, biomass production, radical length, proline accumulation, and antioxidant enzymes system and reduced oxidative damage in soybean under salinity, heat and drought stress. In soybean, Jahan et al. found that spray treatment of melatonin considerably improved leaf gas exchange characteristics, enhancing water uptake efficiency, protected photosynthetic capacity, and balanced photosystems I and II and decreased ROS production under osmotic stress. Zulfiqar et al. revealed that preharvest melatonin treatment is viable for enhancing the cut tuberose's postharvest quality. Various melatonin concentrations were tested, and all treatments significantly extended vase life by up to 4 days. Melatonin also improves various physiological characteristics, including increased levels of soluble proteins and sugars, enhanced catalase activity, and reduced oxidative stress markers, suggesting that melatonin preharvest application may be a useful tool for improving tuberose flower quality after harvest.

While this research presents promising results, further research is needed to address underlying questions. The focus of this study on melatonin's role in regulating abiotic stress offers a new perspective and provides a basis for future research. It's crucial to recognize that, beyond this specific Research Topic, we eagerly anticipate uncovering additional novel insights in the future regarding the potential role of melatonin in plant stress regulation. Abiotic stressors have a unique physiological response to melatonin treatments, so future research should investigate how these treatments can be optimized for various crop species. This will enable tailored strategies to enhance stress tolerance in diverse agricultural contexts.

Author contributions

MA: Conceptualization, Writing – original draft, Writing – review & editing. ML: Conceptualization, Writing – review & editing, Writing – original draft. RT: Conceptualization, Writing – original draft, Writing – review & editing. SN: Writing – review & editing. VG: Writing – review & editing.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

Altaf, M. A., Shahid, R., Ren, M. X., Khan, L. U., Altaf, M. M., Jahan, M. S., et al. (2021a). Protective mechanisms of melatonin against vanadium phytotoxicity in tomato seedlings: insights into nutritional status, photosynthesis, root architecture system, and antioxidant machinery. *J. Plant Growth Regul.* 41, 3300–3316. doi: 10.1007/s00344-021-10513-0

Altaf, M. A., Shahid, R., Ren, M. X., Mora-Poblete, F., Arnao, M. B., Naz, S., et al. (2021b). Phytomelatonin: An overview of the importance and mediating functions of melatonin against environmental stresses. *Physiol. Plant* 172, 820–846. doi: 10.1111/ppl.13262

Altaf, M. A., Sharma, N., Singh, J., Samota, M. K., Sankhyan, P., Singh, B., et al. (2023a). Mechanistic insights on melatonin-mediated plant growth regulation and hormonal cross-talk process in solanaceous vegetables. *Sci. Hortic.* 308, 111570. doi: 10.1016/J.SCIENTA.2022.111570

Altaf, M. A., Sharma, N., Srivastava, D., Mandal, S., Adavi, S., Jena, R., et al. (2023b). Deciphering the melatonin-mediated response and signalling in the regulation of heavy metal stress in plants. *Planta* 257, 1–17. doi: 10.1007/S00425-023-04146-8

Dubbels, R., Reiter, R. J., Klenke, E., Goebel, A., Schnakenberg, E., Ehlers, C., et al. (1995). Melatonin in edible plants identified by radioimmunoassay and by high performance liquid chromatography-mass spectrometry. *J. Pineal Res.* 18, 28–31. doi: 10.1111/j.1600-079X.1995.tb00136.x

Hayat, F., Khanum, F., Li, J., Iqbal, S., Khan, U., Javed, H. U., et al. (2023). Nanoparticles and their potential role in plant adaptation to abiotic stress in horticultural crops: A review. *Sci. Hortic.* 321, 112285. doi: 10.1016/J.SCIENTA.2023.112285

Lal, M. K., Tiwari, R. K., Altaf, M. A., Kumar, A., and Kumar, R. (2023). Editorial: Abiotic and biotic stress in horticultural crops: insight into recent advances in the underlying tolerance mechanism. *Front. Plant Sci.* 14. doi: 10.3389/FPLS.2023.1212982/BIBTEX

Moustafa-Farag, M., Almoneafy, A., Mahmoud, A., Elkelish, A., Arnao, M. B., Li, L., et al. (2020). Melatonin and its protective role against biotic stress impacts on plants. *Biomolecules* 10, 1–12. doi: 10.3390/biom10010054

Rhaman, M. S., Imran, S., Rauf, F., Khatun, M., Baskin, C. C., Murata, Y., et al. (2020). Seed priming with phytohormones: an effective approach for the mitigation of abiotic stress. *Plants* 10, 37. doi: 10.3390/PLANTS10010037

Sharma, A., Wang, J., Xu, D., Tao, S., Chong, S., Yan, D., et al. (2020). Melatonin regulates the functional components of photosynthesis, antioxidant system, gene expression, and metabolic pathways to induce drought resistance in grafted Carya cathayensis plants. *Sci. Total Environ.* 713, 136675. doi: 10.1016/j.scitotenv.2020.136675

Shi, H., Love, J., and Hu, W. (2017). Editorial: melatonin in plants. *Front. Plant Sci.* 8. doi: 10.3389/FPLS.2017.01666/BIBTEX

Tiwari, R. K., Lal, M. K., Kumar, R., Chourasia, K. N., Naga, K. C., Kumar, D., et al. (2021a). Mechanistic insights on melatonin-mediated drought stress mitigation in plants. *Physiol. Plant* 172, 1212–1226. doi: 10.1111/ppl.13307

Tiwari, R. K., Lal, M. K., Kumar, R., Mangal, V., Altaf, M. A., Sharma, S., et al. (2021b). Insight into melatonin-mediated response and signaling in the regulation of plant defense under biotic stress. *Plant Mol. Biol.* 1, 1–15. doi: 10.1007/s11103-021-01202-3

Tiwari, R. K., Lal, M. K., Naga, K. C., Kumar, R., Chourasia, K. N. S. S., Kumar, D., et al. (2020). Emerging roles of melatonin in mitigating abiotic and biotic stresses of horticultural crops. *Sci. Hortic.* 272, 109592. doi: 10.1016/j.scienta.2020.109592

frontiers | Frontiers in Plant Science

TYPE Original Research
PUBLISHED 06 October 2022
DOI 10.3389/fpls.2022.998861

Rhizosphere melatonin application reprograms nitrogen-cycling related microorganisms to modulate low temperature response in barley

Miao Jiang[1,2], Fan Ye[1,3], Fulai Liu[4], Marian Brestic[5,6] and Xiangnan Li[1,3,7]*

[1]Key Laboratory of Mollisols Agroecology, Northeast Institute of Geography and Agroecology, Chinese Academy of Science, Changchun, China, [2]Key Laboratory of Agricultural Soil and Water Engineering in Arid and Semiarid Areas, Ministry of Education of China, Northwest A & F University, Yangling, China, [3]College of Advanced Agricultural Sciences, University of Chinese Academy of Sciences, Beijing, China, [4]Faculty of Science, Department of Plant and Environmental Sciences, University of Copenhagen, Tåstrup, Denmark, [5]Department of Plant Physiology, Slovak Agricultural University, Nitra, Slovakia, [6]Department of Botany and Plant Physiology, Czech University of Life Sciences Prague, Prague, Czechia, [7]Chinese Academy of Science (CAS) Engineering Laboratory for Eco-agriculture in Water Source of Liaoheyuan, Chinese Academy of Science, Changchun, China

Rhizospheric melatonin application has a positive effect on the tolerance of plants to low temperature; however, it remains unknown whether the rhizosphere microorganisms are involved in this process. The aim of this study was to investigate the effect of exogenous melatonin on the diversity and functioning of fungi and bacteria in rhizosphere of barley under low temperature. The results showed that rhizospheric melatonin application positively regulated the photosynthetic carbon assimilation and redox homeostasis in barley in response to low temperature. These effects might be associated with an altered diversity of microbial community in rhizosphere, especially the species and relative abundance of nitrogen cycling related microorganisms, as exemplified by the changes in rhizosphere metabolites in the pathways of amino acid synthesis and metabolism. Collectively, it was suggested that the altered rhizospheric microbial status upon melatonin application was associated with the response of barley to low temperature. This suggested that the melatonin induced microbial changes should be considered for its application in the crop cold-resistant cultivation.

KEYWORDS

melatonin, low temperature, *Hordeum vulgare*, microbial diversity, nitrogen cycling

Check for updates

OPEN ACCESS

EDITED BY
Milan Kumar Lal,
Central Potato Research Institute (ICAR), India

REVIEWED BY
Chengqiang Ding,
Nanjing Agricultural University, China
Guang Sheng Zhou,
Huazhong Agricultural University, China
Ajay Kumar,
Chaudhary Charan Singh Haryana Agricultural University, India
Xingang Zhou,
Northeast Agricultural University, China

*CORRESPONDENCE
Xiangnan Li
lixiangnan@iga.ac.cn

SPECIALTY SECTION
This article was submitted to Plant Abiotic Stress, a section of the journal Frontiers in Plant Science

RECEIVED 20 July 2022
ACCEPTED 15 September 2022
PUBLISHED 06 October 2022

CITATION
Jiang M, Ye F, Liu F, Brestic M and Li X (2022) Rhizosphere melatonin application reprograms nitrogen-cycling related microorganisms to modulate low temperature response in barley.
Front. Plant Sci. 13:998861.
doi: 10.3389/fpls.2022.998861

Introduction

Melatonin (*N*-acetyl-5-methoxytryptamine) is an important signal molecule regulating various physiological processes in plants under abiotic stress, including scavenging reactive oxygen species (ROS) as an antioxidant (Arnao and Hernández-Ruiz, 2009), activating antioxidant defense systems (Tan et al., 2012), modulating the expression of stress response genes (Li et al., 2021), and regulating photosynthetic carbon assimilation (Li et al., 2016). Though the original sites of melatonin biosynthesis are mitochondria and chloroplasts in higher plants (Tan and Reiter, 2019), both foliar and rhizospheric melatonin application has been shown to enhance the tolerance of plants to abiotic stress, such as low temperature and drought (Li et al., 2016; Nawaz et al., 2016). For instance, exogenous melatonin increases the tolerance to low temperature of bermudagrass by enhancing endogenous melatonin level and antioxidant enzyme activities (Fan et al., 2015). Spaying melatonin benefited the growth of barley plant under low temperature by promoting photosynthetic carbon assimilation, activating ROS scavenging capacity and optimizing carbohydrate metabolism (Zhao et al., 2015; Li et al., 2016). In addition to a direct effect on root and shoot physiology, rhizospheric melatonin application could also affect some other processes in soil, such as rhizospheric microbial status, which may play key roles in the process of melatonin-induced stress tolerance.

The rhizosphere is the home of an overwhelming number of microorganisms (Philippot et al., 2013), where the soil microorganisms interact most intensely with plant roots, establishing beneficial associations which could mitigate the adverse effects of abiotic stress on plant (Toju et al., 2018). For example, microbial populations that thrive when exposed to abiotic stress, known as “defense biomes”, can benefit plant stress tolerance (Liu et al., 2020). *A. chroococcum* has favorable effects on *Dodonaea viscosa* seedlings, resulting in improved plant growth and seed germination under salinity stress (Yousefi et al., 2017). *Pseudomonas* and *Mesorhizobium* strains assist to improve growth and symbiotic performance of liquorice (*Glycyrrhiza uralensis* Fish.) under abiotic stress (Egamberdieva et al., 2016). Plant-associated microbiomes play a role in determining plant fitness (Goh et al., 2013; Xu et al., 2018) and a number of microbial members have important functions as partners in the environmental adaptation and metabolisms in plants, such as enhanced nutrient uptake, nitrogen fixation and defense responses (Bano et al., 2021). Therefore, the species and abundance of rhizosphere microorganisms are closely related to plant growth and induction of stress tolerance (Mendes et al., 2013; Geng et al., 2018). In addition, as one of the most active components in the soil ecosystem, microorganisms drive the cycles of carbon, nitrogen, phosphorus and sulfur in soil, which also directly modulates plant growth and their responses to abiotic stress (Koide et al., 2011; Eisenhauer et al., 2012; Purahong and Krüeger, 2012).

Soil metabolites, including sugars, amino acids, organic acids, and phenolic compounds, can reflect the changes in important metabolic pathways for soil microbial community (Withers et al., 2020). Metabolic profiles in rhizospheric soils are comprised of a great variety of chemicals, which recruit specific microbial species to form complex relationships with plants (Philippot et al., 2013; Kuzyakov and Razavi, 2019), could be used to characterize soil functional alterations and facilitate the investigation of the link between soil microbial community and soil metabolites (Ding et al., 2021).

Low temperature induces a series of physiological changes in plants, including inactivation of many metabolic enzymes, disturbance of the metabolic regulations, and modifications of the carbohydrate metabolism and photosynthetic properties (Li et al., 2014a; Li et al., 2014b). To explore the relationship between melatonin and microbial diversity and its implications in modulating the response of low temperature stress in barley plant, the diversity of bacterial and fungal microbial, rhizosphere soil metabolome, the enzymatic profiling related to carbohydrate metabolism, redox homeostasis and melatonin metabolism in barley plants were investigated. It was hypothesized that (i) Rhizospherically applied melatonin would significantly alter the diversity of microbial community and re-program the rhizosphere soil metabolites under low temperature; (ii) Melatonin induced the changes of low temperature response in barley would be associated with the nitrogen-cycling related rhizosphere microorganisms.

Materials and methods

Plant material and cultivation conditions

The seeds of spring barley cv. Steptoe were sterilized with 80% ethanol and 1% sodium hypochlorite solution and washed with sterile water. Four seeds were sown in each pot (15 cm in diameter and 10 cm in height, with 2 drainage holes) with 1 kg soil. The characteristics of soil used were: pH 7.1, organic carbon 10.98 g kg^{-1}, total nitrogen 1.61 g kg^{-1}, available nitrogen 141 mg kg^{-1}, available phosphorus 62.8 mg kg^{-1}, available potassium 147 mg kg^{-1}. The plants were grown in the growth chamber at 26°C/20°C (day/night, 22,000 Lux/0 Lux, 12 h/12 h). The relative humidity in the growth chamber was 60% ± 5%. After 21 days of sowing, half of the plants were rhizospherically treated with 1 mmol/L melatonin (MT), while the rest were treated with water as the control (N). The melatonin treatment (100 mL per pot) was applied once every three days for 30 days, and the total melatonin content was 232.28

mg/kg soil. Twelve hours after the last melatonin application, half of the MT plants and the control plants were exposed to a 48-hour low temperature treatment (2 ± 0.5°C). Therefore, four treatments were established: NT_N, normal temperature + water; NT_MT, normal temperature + 1 mmol/L melatonin; LT_N, low temperature + water; LT_MT, low temperature + 1 mmol/L melatonin. Six pots were included in each treatment.

The root samples were gently rinsed several times with tap water, then washed with sterile water, followed by drying on sterilized filter paper. The last fully expanded leaves samples and roots samples were collected immediately after the low temperature treatment and then were snap frozen in liquid nitrogen and stored at -80°C. The roots were carefully taken out of the pots, shaken to remove loosely adhering soil, and then about 1 mm of soil from the roots was retained as rhizosphere soil (Edwards et al., 2015). The rhizosphere soil samples were carefully collected and screened using a 2.0 mm sterile sieve and snap frozen in liquid nitrogen and stored at -80°C.

Chlorophyll a fluorescence measurement

Three fully expanded leaves from different plants in each treatment were selected for the dark-adapted imaging of maximal photochemical efficiency (F_V/F_m) using a FluorCam (FC 800MF, Photon Systems Instruments, Brno, Czech Republic). Just after the end of low temperature treatment, the chlorophyll a fluorescence (OJIP) transient of the same leaves for chlorophyll a fluorescence imaging was measured using a portable fluorometer (Fluorpen FP100, Photon System Instruments, Drasov, Czech Republic).

Analysis of melatonin metabolism enzyme activity and related metabolites

Concentrations of melatonin and the metabolites related to melatonin synthesis (tryptophan, tryptamine, serotonin, *N*-acetylserotonin) and activities of key enzymes (tryptophan decarboxylase (TDC), tryptamine 5-hydroxylase (T5H), serotonin *N*-acetyltransferase (SNAT), *N*-acetylserotonin *O*-methyltransferase (ASMT), caffeic acid *O*-methyltransferase (COMT)) in roots were detected using enzyme-linked immunosorbent assay (ELISA) kit by an Epoch^{TM} Microplate Spectrophotometer (BioTek Instruments, Inc., Winosky, Vermont, USA). All measurements were determined in triplicate.

Measurement of antioxidant enzyme activities

According to the protocol of Fimognari et al. (2020), the activities of key antioxidant enzymes in roots, i.e., ascorbate peroxidase (APX), catalase (CAT), cell wall peroxidase (cwPOX), dehydroascorbate reductase (DHAR), glutathione reductase (GR), glutathione S-transferase (GST), monodehydroascorbate reductase (MDHAR), peroxidase (POX), superoxide dismutase (SOD), were measured using an Epoch^{TM} Microplate Spectrophotometer (BioTek Instruments, Inc., Winosky, Vermont, USA) with a semi high-throughput 96-well assay format. The enzyme activities were normalized by fresh weight. The unit of antioxidant enzyme activity was nkat g^{-1} FW. All measurements were determined in triplicate.

Measurement of carbohydrate metabolism enzyme activities

According to the protocol of Jammer et al. (2015), the activities of 13 key primary carbohydrate metabolism enzymes in leaf and root samples, i.e., ADP-glucose pyrophosphorylase (AGPase), aldolase (Ald), cytoplasmic invertase (cytInv), cell wall invertase (cwInv), fructokinase (FK), glucose-6-phosphate dehydrogenase (G6PDH), hexokinase (HXK), phosphofructokinase (PFK), phosphoglucoisomerase (PGI), phosphoglucomutase (PGM), sucrose synthase (SuSy), UDP-glucose pyrophorylase (UGPase), vacuolar invertase (vacInv), were determined using an Epoch^{TM} Microplate Spectrophotometer (BioTek Instruments, Inc., Winosky, Vermont, USA) with a semi high-throughput 96-well assay format. The enzyme activity was normalized by fresh weight. The unit of carbohydrate metabolism enzyme activity was nkat g^{-1} FW. All measurements were determined in triplicate.

Concentrations of total soluble sugars, sucrose and reducing sugar

The root samples were harvested and oven-dried to measure the concentrations of total soluble sugar, sucrose and reducing sugar. Concentrations of total soluble sugar and sucrose in dry root samples were measured according to the anthrone reagents method (Fales, 1951). Reducing sugar concentration was measured following our previous methods (Li et al., 2015).

Determination of urease and nitrate reductase activities and total nitrogen concentrations in soil

Fresh soil samples were oven-dried at 37°C and then sieved by passing through a 50-mesh sieve. The activities of soil urease and nitrate reductase were tested by assay kits (Boxbio Science & Technology Co., Ltd., Beijing, China) using an Epoch^{TM} Microplate Spectrophotometer (BioTek Instruments, Inc., Winosky, Vermont, USA) following the user manual at 630 nm and 520 nm. Soil samples were thoroughly mixed, and the representative sub-samples were extracted immediately using 2 M KCl solution (soil solution ratio: 1:5) and shaken for 1 h on a rotary shaker (180 rev min^{-1}), followed by filtration. The concentrations of NH_4^+-N, NO_3^-N and total nitrogen were measured according to the methods of Shi et al. (2012).

DNA extraction, sequencing, and microbial community analysis

Soil samples (0.5 g) were used for microbial genomic DNA extraction with the OMEGA Soil DNA Kit (D5625-01) (Omega Bio-Tek, Norcross, GA, USA) following the manufacturer's instructions for amplification of bacterial 16S V3-V4 and fungal ITS1 regions. The NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and agarose gel electrophoresis were used to measure the quantity and quality of extracted DNAs, respectively. The measurement for each treatment included six biological repeats.

The PCR amplification of the bacterial 16S V3-V4 region was performed using the forward primer 338F (5'- ACTCCTACGGGAGGCAGCA -3') and the reverse primer 806R (5'- CGGACTACHVGGGTWTCTAAT -3'), the forward primer ITS5F (5'- GGAAGTAAAAGTCGTAACAAGG -3') and the reverse primer ITS2R (5'- GCTGCGTTCTTCATCGATGC -3') was for fungal ITS V1 region, simultaneously. Bacteria and fungi microbial genomic DNA had the same PCR components: 5 μL of reaction buffer (5×), 5 μL of GC buffer (5×), 0.25 μL of Fast pfu DNA Polymerase (5U/μL), 2 μL (2.5 mM) of dNTPs, 1 μL (10 uM) of each Forward and Reverse primer, 1 μL of DNA Template, and 9.75 μL of ddH_2O.

The PCR reactions of bacteria microbial genomic DNA were applied using the following program: 5 min of denaturation at 98°C, 25 cycles of 30 s at 98°C, 30s for annealing at 52°C, and 1 min for elongation at 72°C, and a final extension at 72°C for 5 min. The amplification of fungi microbial genomic DNA were conducted using the following protocol: 5 min of denaturation at 98°C, 28 cycles of 30 s at 98°C, 30s for annealing at 52°C, 1 min for elongation at 72°C, and a final extension at 72°C for 5 min. The resulted PCR products were purified with Vazyme VAHTSTM DNA Clean Beads (Vazyme, Nanjing, China) and quantified using the Quant-iT PicoGreen dsDNA Assay Kit (Invitrogen, Carlsbad, CA, USA). The Illumina NovaSeq 6000 (Hayward CA USA) was used for paired-end sequenced (2 × 250) with the purified amplicons pooled in equimolar (Personal Biotechnology Co., Ltd, Shanghai, China).

Sequences were then quality filtered, denoised, merged and chimera removed using the DADA2 plugin (Callahan et al., 2016). Sequence data analyses were mainly performed with QIIME2 and R packages (v3.2.0). Unique reads with 100% similarity based on the representative 16S or ITS1 sequences were clustered into ASVs (amplicon sequence variants). Taxonomy was assigned to ASVs using the classify-sklearn naïve Bayes taxonomy classifier in feature-classifier plugin (Bokulich et al., 2018) against the SILVA Release132 (http://www.arb-silva.de) for bacteria, while UNITE Release 8.0 (https://unite.ut.ee/) for fungi Database (Kõljalg et al., 2013). The sequences from the host were filtered from the bacterial ASV table. ASV-level alpha diversity indices, such as Chao1 richness estimator, observed species, Shannon diversity index and Simpson index were calculated using the ASV table in QIIME2 by Kruskal-Wallis test and visualized as box plots. LEfSe (Linear discriminant analysis effect size) (http://huttenhower.sph.harvard.edu/galaxy/root?tool_id=lefse_upload) was applied to detect biomarkers at multiple taxonomical levels with an LDA score threshold >3.5 from phylum to genus. Microbial functions were predicted by MetaCyc databases (https://metacyc.org/) at level 1 and 2.

Soil metabolomic detection and analysis

Soil samples (50 mg) were extracted with 0.5 mL of acetonitrile: isopropanol: water (3:3:2, v/v/v) mixed solution (-20°C) and then vibrated at 30 Hz for 2 min and ultrasonic for 5 min at room temperature. 0.5 mL of acetonitrile, isopropanol and water (3:3:2, v/v/v) mixed solution (-20°C) were added to the extraction, and then it was ultrasonic for 5 min at room temperature, centrifuged at 10 000 g for 2 min. The supernatant was concentrated to dry by vacuum concentrator. Eighty μL of 20 mg/mL MEOX solution was added for redissolution through vortex vibration for 30 s, and then incubated for 60 min (60°C). 100 μL BSTFA reagent was added into the extraction, and then it was reacted at 70°C for 90 min, 90-100 μL of supernatant were added into the detection bottle after centrifuged at 12 000 g for 3 min. To correct the deviation of analysis results of mixed samples and errors caused by the analyzer itself, quality control (QC) was applied. Twenty μL of samples were mixed into the QC samples, the remaining samples were detected by GC-MS. Gas chromatography was performed to separate the derivatives at a constant flow of 1 mL/min helium. Mass spectrometry was determined by the full-scan method with a range from 75 to 650 (m/z). Metabolites with a VIP value > 1.0 and p-value < 0.05 were selected as the ones significantly affected by the treatments. A correlation heatmap was presented to show the correlation between differential metabolites and biomarkers of bacterial and fungal microbial communities.

Univariate statistical analysis

All data were firstly tested for homogeneity of variance and then subjected to one-way ANOVA to detect significant differences at $P < 0.05$ level. All data were subjected to

Duncan-test to determine statistical differences using the SPSS 22.0 (SPSS Inc., Chicago, IL, USA).

Results

Chlorophyll a fluorescence and carbohydrate metabolism enzyme activities in leaves

The dark-adapted images of Fv/Fm in barley leaves showed that low temperature decreased significantly the Fv/Fm value compared with the normal temperature control (Figures 1A, B). However, Fv/Fm of melatonin treated plants was significantly higher than that of the control plants under low temperature. Low temperature significantly reduced the quantum yield for photosystem II (PS II) electron transport (φ_Eo), trapped energy flux per reaction center (TRo/RC) and electron transport flux per RC (ETo/RC), while increased the absorption flux per RC (ABS/RC) and dissipated energy flux per RC (DIo/RC), in relation to the normal temperature control. Under low temperature, melatonin treated plants had significantly higher φ_Eo, TRo/RC and ETo/RC than the control plants, while the DIo/RC was significantly lower in LT_MT plants compared with LT_N plants.

The activities of key enzymes involved in carbohydrate metabolism in leaves were significantly affected by the interaction of melatonin and low temperature (Figure 1C). In

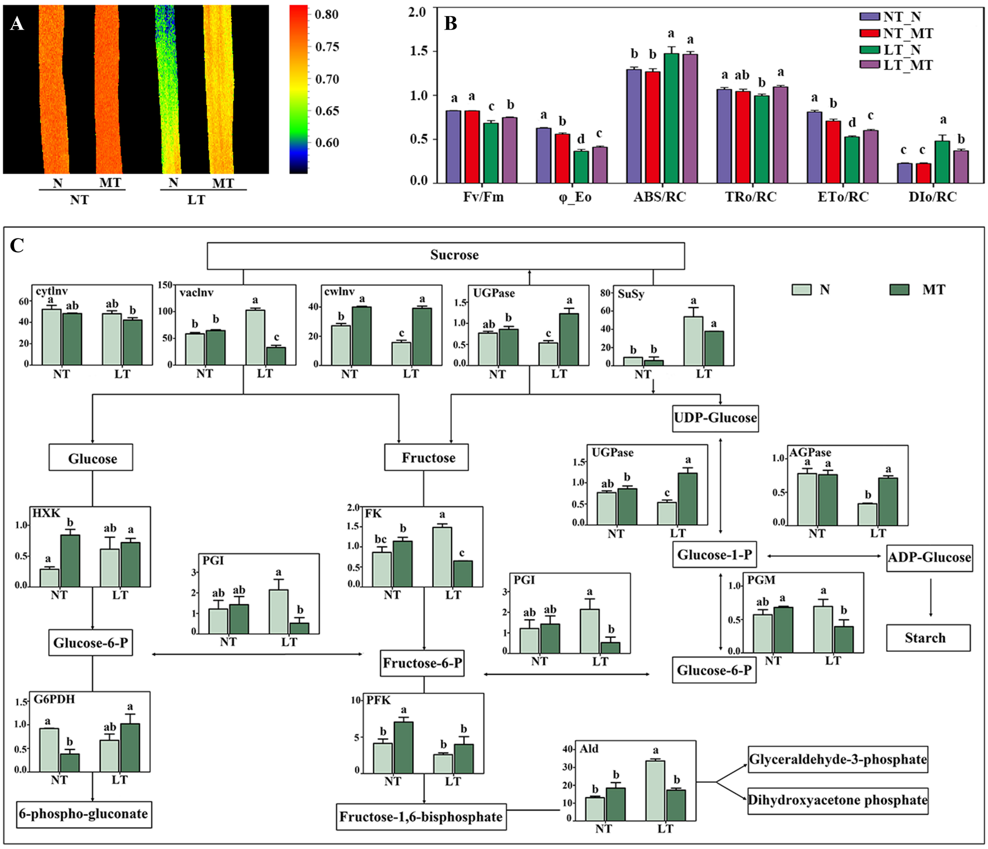


FIGURE 1
Dark-adapted image of maximum quantum efficiency of photosystem II (Fv/Fm, **A**), chlorophyll a fluorescence parameters **(B)**, and the primary carbohydrate metabolism enzyme activities **(C)** of the last fully expanded leaves as affected by melatonin and low temperature in barley. N, the control; MT, melatonin treatment; NT, normal temperature; LT, low temperature. NT_N, normal temperature control; NT_MT, normal temperature + melatonin treatment; LT_N, low temperature control; LT_MT, low temperature + melatonin treatment. Data are presented as mean ± SE (n = 8 in panel **(B)**; n = 3 in panel **(C)**). Different small letters represent significant differences at $P < 0.05$. The unit of carbohydrate metabolism enzyme activity is nkat g^{-1} FW. φ_E_O, quantum yield for PSII electron transport; ABS/RC, absorption flux per RC (reaction center); DI_O/RC, dissipated energy flux per RC (at t=0). AGPase, ADP-glucose pyrophosphorylase; Ald, aldolase; cytInv, cytoplasmic invertase; cwInv, cell wall invertase; ET_O/RC, electron transport flux per RC (at t=0); FK, fructokinase; Fructose-6-P, fructose-6-phosphate; Glucose-1-P, glucose-1-phosphate; Glucose-6-P, glucose-6-phosphate; G6PDH, glucose-6-phosphate dehydrogenase; HXK, hexokinase; PFK, phosphofructokinase; PGI, phosphoglucoisomerase; PGM, phosphoglucomutase; SuSy, sucrose synthase; TR_O/RC, trapped energy flux per RC (at t=0); UGPase, UDP-glucose pyrophorylase; vacInv, vacuolar invertase.

the sucrolytic pathway, low temperature significantly increased the activity of vacInv but decreased the activity of cwInv compared with the normal temperature control. The MT plants had significantly higher cwInv activity while significantly lower vacInv activity than N plants under low temperature. For the pathway of glycolysis, compared with the normal control, low temperature only significantly increased the activities of FK and Ald, while the activities of PGM, PGI, HXK and PFK were not affected. Under low temperature, MT plants possessed significantly lower activities of FK, Ald, PGM and PGI than N plants; however, no significant difference was found in HXK and PFK activities between MT and N plants. LT_N plants showed significantly lower activity of enzyme related to starch biosynthesis (AGPase) and sucrose biosynthesis (UGPase) compared with NT_N plants, while LT_MT plants had significantly higher activities of these two enzymes compared with LT_N plants. For the pentose phosphate pathway, no significant difference was found in G6PDH activity either between NT_N and LT_N plants or between LT_N and LT_MT plants.

Melatonin synthesis and metabolism, ROS and carbohydrate metabolism in roots

Low temperature significantly decreased the activities of TDC, ASMT and COMT, while had no significant effect on T5H and SNAT activities in roots, in relation to the normal temperature control (Figure 2A). Under low temperature, the activities of most tested enzymes were significantly increased in MT roots, including TDC, T5H, ASMT and COMT, while the SNAT activity was significantly decreased, compared with that under control treatment. The concentrations of serotonin and melatonin were significantly decreased while the *N*-Acetylserotonin concentration was significantly increased in the roots of LT_N plants, in relation to NT_N plants. The concentrations of serotonin and melatonin in MT plants was significantly higher, while the *N*-Acetylserotonin concentration was lower than that of N plants under low temperature. In addition, no significant difference was found in the concentrations of tryptophan and tryptamine among these treatments.

The antioxidant enzyme activity was obviously influenced by the interactive effects of melatonin and low temperature in barley roots (Figure 2B). Low temperature significantly increased the activities of SOD, CAT and GR, while reduced significantly the APX and GST activities, compared with the normal temperature control in barley roots. Under low temperature, the activities of APX and DHAR were significantly higher, while activities of SOD, CAT and GR were significantly lower in MT plants compared with N plants.

For the carbohydrate metabolism in roots, low temperature only significantly reduced the activity of vaInv, while had no significant effect on the activities of other enzymes in the sucrolytic pathway (Figure 2C). Under low temperature, MT plants had significantly higher vaInv activity while lower cwInv activity than N plants. For the pathway of glycolysis, low temperature significantly reduced the activities of PGI, PFK, Ald and FK, in relation to the normal temperature control. When exposed to low temperature, the MT plants had significantly higher activities of PGI, PFK, Ald and FK, while lower HXK activity than N plants. In addition, the activities of SuSy, PGM and UGPase were not affected by either melatonin or low temperature. Low temperature significantly decreased the concentration of reducing sugar, and slightly reduced the total soluble sugar concentration in roots (Figure S1). The concentration of the total soluble sugars was significantly higher in MT roots compared with that in N roots under low temperature.

Diversity of bacterial and fungal communities

To investigate the influence of low temperature and rhizospheric melatonin application on rhizosphere microbiota compositions, we compared the alpha diversity and the relative abundance of bacterial and fungal communities among treatments. At a threshold of 100% sequence identity and after filtering out the ASVs of chloroplasts and mitochondria from the bacterial ASVs table, 595,3 and 529,42 ASVs were identified in terms of fungi and bacteria, respectively. For fungal communities, 2516, 2215, 2600 and 1832 ASVs had been enriched in NT_N, NT_MT, LT_N and LT_MT, respectively. In addition, in fungal communities, 482 of the common ASVs were enriched among these treatments and 1203, 966, 1257 and 727 of the unique ASVs were enriched in the rhizosphere of NT_N, NT_MT, LT_N and LT_MT, respectively (Figure S2A). For bacterial communities, 21874, 19194, 22819 and 15913 ASVs were enriched in NT_N, NT_MT, LT_N and LT_MT. Among them, 377,0 of the common ASVs were enriched in all treatments, while 10212, 8341, 11457 and 7625 of the unique ASVs were enriched in NT_N, NT_MT, LT_N and LT_MT, respectively (Figure S2B).

The alpha diversity of microbial communities as affected by low temperature and rhizospheric melatonin application was further tested. For fungal communities, the Shannon index and Simpson index were similar in the rhizosphere of NT_N, NT_MT, LT_N, and LT_MT at the ASV level (Figure 3A). However, significant reductions were found in the alpha diversity index of Chao 1 and observed species in the rhizosphere of LT_MT compared with LT_N. For bacterial communities, no significant difference was found in the alpha diversity between LT_N and NT_N (Figure 3B), while rhizospheric melatonin application significantly decreased the alpha diversity indexes (i.e., Chao 1, observed species, Shannon and Simpson) in bacterial community under low temperature.

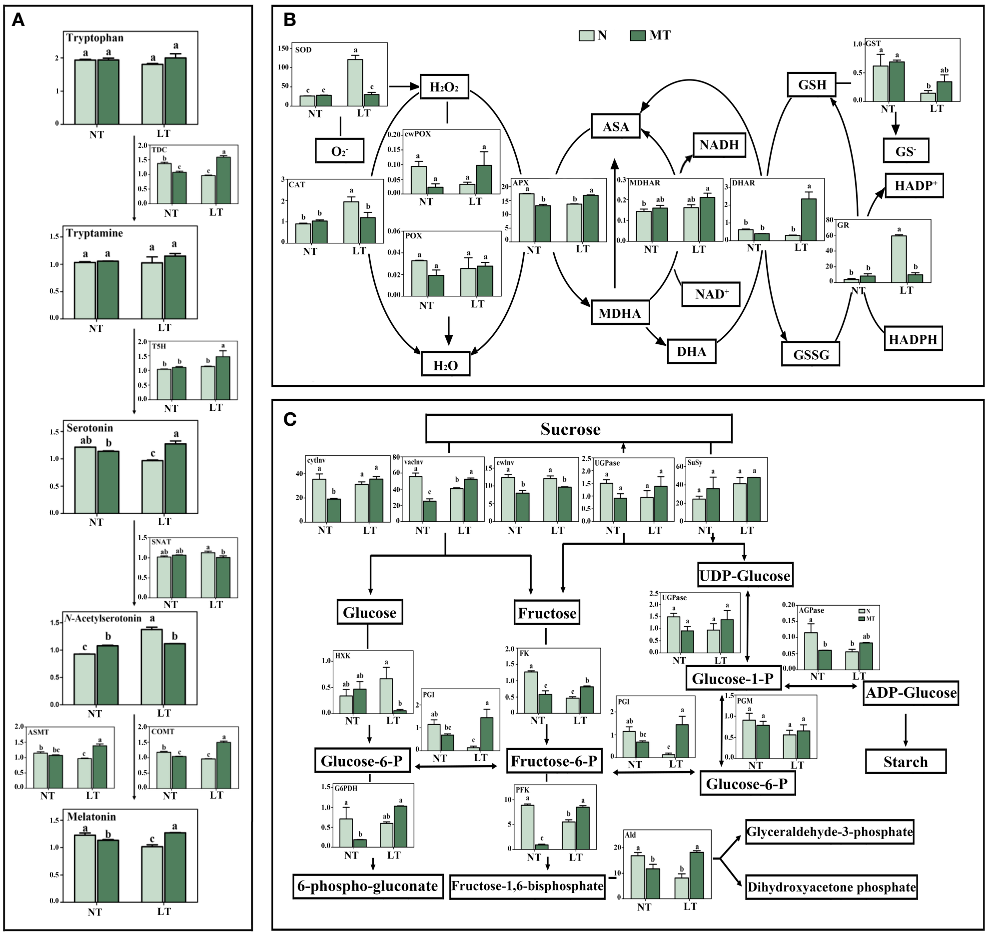


FIGURE 2
Metabolite concentrations and activities of enzymes involved in melatonin synthesis and metabolism **(A)**, activities of antioxidant enzymes **(B)** and activities of the primary carbohydrate metabolism enzymes **(C)** in roots as affected by melatonin and low temperature in barley. N, the control; MT, melatonin treatment; NT, normal temperature; LT, low temperature. Data are presented as mean ± SE (n = 3). Different small letters represent significant differences at $P < 0.05$. The unit for antioxidant enzyme activity and carbohydrate metabolism enzyme activity is nkat g^{-1} FW. AGPase, ADP-glucose pyrophosphorylase; Ald, aldolase; APX, ascorbate peroxidase; ASA, ascorbic acid; ASMT, *N*-acetylserotonin *O*-methyltransferase, U g^{-1} FW; CAT, catalase; COMT, caffeic acid *O*-methyltransferase, U g^{-1} FW; cwPOX, cell wall peroxidase; cwInv, cell wall invertase; cytInv, cytoplasmic invertase; DHA, dehydroascorbic acid; DHAR, dehydroascorbate reductase; FK, fructokinase; Fructose-6-P, fructose-6-phosphate; G6PDH, glucose-6-phosphate dehydrogenase; Glucose-1-P, glucose-1-phosphate; Glucose-6-P, glucose-6-phosphate; GR, glutathione reductase; GSH, reduced glutathione; GSSG, oxidized glutathione; GST, glutathione S-transferase; HXK, hexokinase; MDHA, monodehydroascorbate; MDHAR, monodehydroascorbate reductase; Melatonin, μg g^{-1} FW; *N*-Acetylserotonin, μg g^{-1} FW; PFK, phosphofructokinase; PGM, phosphoglucomutase; PGI, phosphoglucoisomerase; POX, peroxidase; Serotonin, μg g^{-1} FW; SNAT, Serotonin *N*-acetyltransferase, U g^{-1} FW; SOD, superoxide dismutase; SuSy, sucrose synthase; T5H, Tryptamine 5-hydroxylase, U g^{-1} FW; TDC, Tryptophan decarboxylase, U mg^{-1} FW; Tryptamine, ng g^{-1} FW; Tryptophan, ng g^{-1} FW; UGPase, UDP-glucose pyrophorylase; vacInv, vacuolar invertase.

In this study, the fungal community consisted of 14 phyla, 39 classes, 87 orders, 193 families, 363 genera and 480 species, and the top 10 phyla of fungal community across treatments accounted for 69.51%, 81.85%, 51.60% and 90.03% of the total sequences in NT_N, NT_MT, LT_N and LT_MT, respectively (Figure 3C). Ascomycota, Mortierellomycota and Basidiomycota were the dominant fungi with a relative abundance > 1% (at least in one treatment) in fungal community. The proportion of Ascomycota was significantly higher in LT_MT compared with LT_N, and it was the only strain whose abundance increased under low temperature and melatonin among the dominant fungi. In addition, the proportion of Ascomycota was not significantly affected by low temperature. Compared with NT_N, the relative abundance of Mortierellomycota was significantly lower in LT_N. Nonetheless, no significant difference was observed in terms of the proportion of Mortierellomycota in LT_N, in relation to that in LT_MT. For the percentage of Basidiomycota, no significant difference was

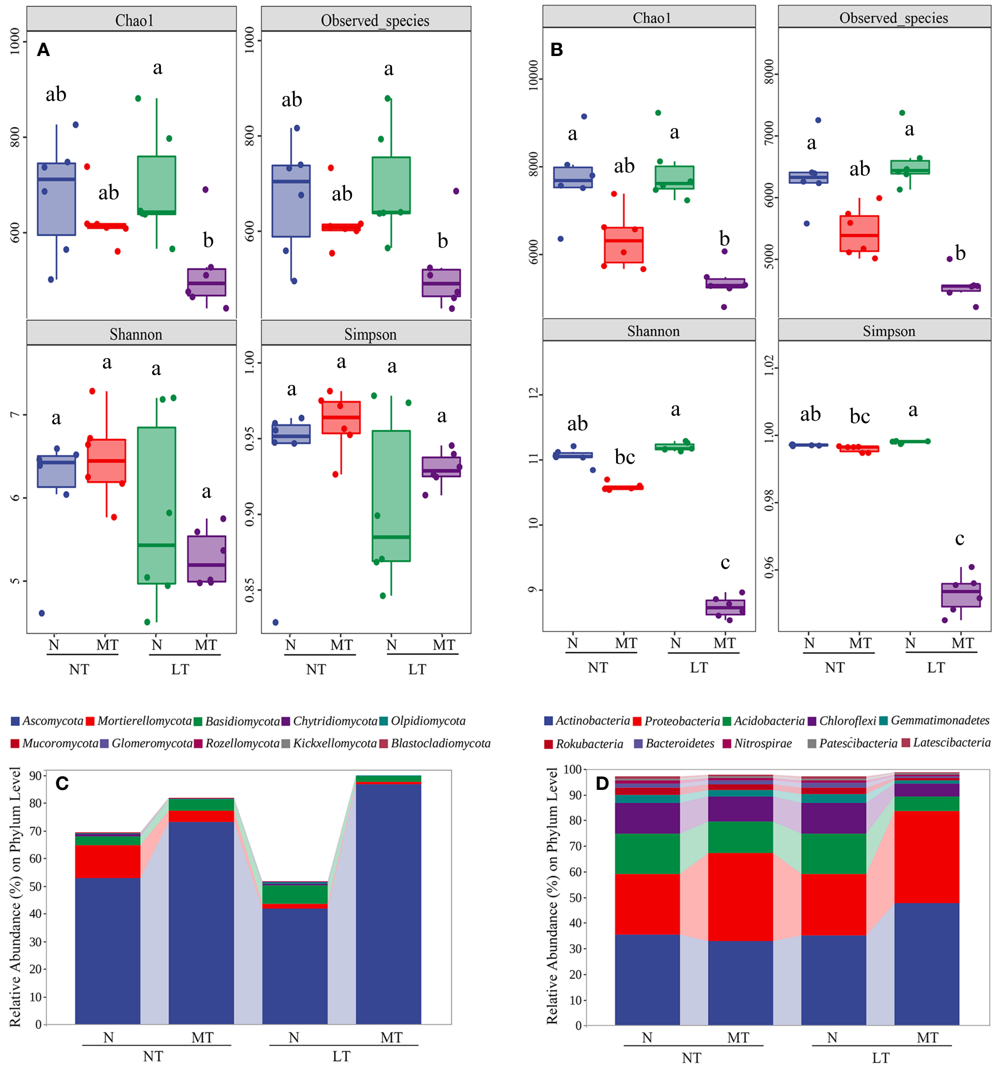


FIGURE 3
The index of Chao 1, observed species, Shannon and Simpson of fungal **(A)** and bacterial **(B)** microbiota in the rhizosphere at the ASV level and the relative abundance of fungal **(C)** and bacterial **(D)** community at the phylum level as affected by melatonin and low temperature (n = 6). N, the control; MT, melatonin treatment; NT, normal temperature; LT, low temperature.

found between NT_N and LT_N, while that of MT plants was significantly lower than that of the control plants under low temperature.

The community of bacteria consisted of 41 phyla, 112 classes, 262 orders, 420 families, 812 genera and 369 species. The top 10 phyla of bacterial community across treatments accounted for 97.05%, 97.87%, 97.07% and 98.60% of the total sequences in NT_N, NT_MT, LT_N and LT_MT, respectively (Figure 3D). Among them, 8 dominant bacteria were found in the top 10 phyla of bacterial community. Actinobacteria and Proteobacteria were the bacteria whose proportion showed an increase trend under low temperature in LT_MT compared with LT_N in dominant bacteria. The other dominant bacteria were Acidobacteria, Chloroflexi, Gemmatimonadetes, Rokubacteria, Bacteroidetes and Nitrospirae, and the percentages of all these six bacteria were significantly lower in LT_MT compared with those in LT_N. The other two bacteria in the top 10 phyla were Patescibacteria and Latescibacteria. All the above bacteria showed no significant difference between NT_N and LT_N, except for Latescibacteria whose percentage was significantly reduced by low temperature.

Under low temperature, *Azotobacter* was not found in the rhizosphere of either NT_N or LT_N (0%), but it was significantly enriched in LT_MT rhizosphere (0.70%) after the melatonin treatment. The percentage of *Azoarcus* was significantly increased in LT_MT rhizosphere (0.51%)

compared with that in LT_N (0%). The relative abundance of *Gemmobacter*, *Dechloromonas* and *Ensifer* were significantly increased by melatonin treatment under low temperature. However, all the above five bacteria, which were belong to Proteobacteria, showed no significant difference between NT_N and LT_N. The relative abundances of *Nitrospira* and *Rubrobacter* were significantly lower in LT_MT, compared with LT_N.

Biomarkers of fungal and bacterial community

Linear discriminant analysis effect size (LEfSe) analysis was applied to identify the microbes as biomarkers with LDA scores (> 3.5) from the level of phylum to genus in fungal (Figure 4A) and bacterial (Figure 4B) communities. These biomarkers showed significant variations in the relative abundances of the core community and were accompanied by considerable changes in response to environmental disturbances. A total of 38 fungi clades exhibited significant variations in NT_N (2 phyla, 1 class, 2 orders, 1 family and 1 genus), NT_MT (3 classes, 3 orders, 4 families and 3 genera), LT_N (1 phylum, 2 orders, 2 families and 2 genera) and LT_MT (1 phylum, 1 class, 1 order, 2 families and 6 genera).

In total, 85 bacteria clades exhibited significant variations in the rhizosphere of NT_N (3 phyla, 5 classes, 8 orders, 9 families and 8 genera), NT_MT (1 class, 2 orders, 2 families and 1 genus), LT_N (3 phyla, 10 classes, 9 orders, 7 families and 6 genera) and LT_MT (2 phyla, 2 classes, 2 orders, 2 families and 3 genera). The bacteria dominant Acidobacteria, Rokubacteria and Nitrospirae at phylum level were significantly abundant in NT_N, and the biomarkers of Nitrospira at class level, Nitrospirales at order level, *Nitrospiraceae* at family level and *Nitrospira* at genus level belonging to Nitrospirae were also observed in NT_N rhizosphere. Alphaproteobacteria at class, Rhizobiales at order, *Rhodocyclaceae* at family and *Azotobacter* at genus were significantly abundant in NT_MT rhizosphere as biomarkers. The proportions of Bacteroidetes, Gemmatimonadetes and Chloroflexi at phylum level, and *Rubrobacter* at genus level were significantly enriched in LT_N. The bacteria dominant Proteobacteria and Actinobacteria at phylum level were significantly abundant in LT_MT rhizosphere. Within Actinobacteria, Gammaproteobacteria at class, Pseudomonadales at order and *Pseudomonadaceae* at family were significantly abundant in the NT_MT rhizosphere.

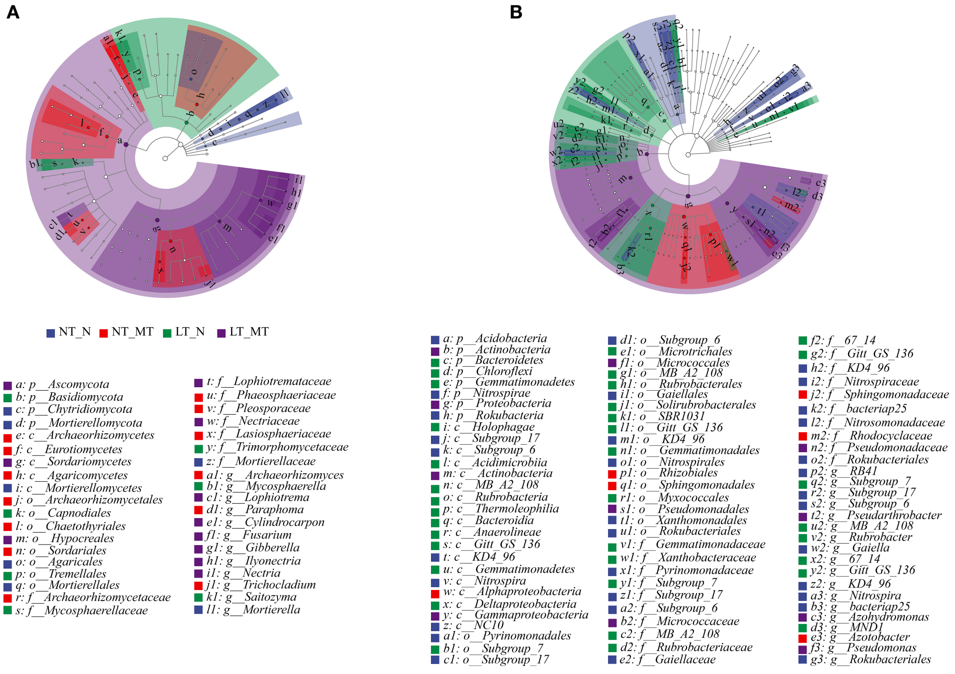


FIGURE 4
Linear discriminant analysis effect size (LEfSe) of the fungal **(A)** and bacterial **(B)** taxa with a LDA score > 3.5 under low temperature and melatonin treatment. Cladograms indicate the phylogenetic distribution of microbial lineages. Circles represent phylogenetic levels from phylum to genus (n = 6). NT_N, normal temperature control; NT_MT, normal temperature + melatonin treatment; LT_N, low temperature control; LT_MT, low temperature + melatonin treatment.

Prediction of functional composition in the microbial community

The activities of urease and nitrate reductase were significantly increased in the rhizosphere soil by rhizospheric melatonin application regardless of temperature treatments (Figure 5A). The concentrations of NH_4^+-N and NO_3^-N were significantly increased by melatonin treatment at normal temperature. In addition, no significant difference was found in the total nitrogen concentration among these treatments.

The prediction of functional composition by FAPROTAX indicated that 7 pathways related to the nitrogen cycle were observed among the 8 most abundant pathways (Figure 5B). Low temperature had no effect on the relative abundance of ASVs in any pathway. However, the relative abundance of ASVs related to the nitrogen cycle was significantly increased (i.e., ureolysis, nitrogen respiration, nitrate respiration and nitrogen fixation), while that in the pathways related to nitrite oxidation and nitrification was decreased in LT_MT compared with that in LT_N.

By predicting the functional composition of the microbial community with the sequencing of bacteria and fungi using PICRUSt2, it was found that the major fungal community was enriched in the biosynthesis pathway, degradation processes, generation of precursor metabol, glycan pathways and metabolic clusters (Figure 5C); while the bacterial community was enriched in the biosynthesis pathway, degradation processes, detoxification, generation of precursor metabol, glycan pathways, macromolecule modification and metabolic clusters (Figure 5D) under these treatments in the level 1 of the MetaCyc database. The biosynthesis pathways were significantly enriched under low temperature in fungal community, while the pathway of degradation was not affected. Nonetheless, for bacterial community, no significant different was observed in LT_N compared with NT_N in the above two pathways. Rhizospheric melatonin application significantly decreased the reads in the fungal community under low temperature in the pathways of biosynthesis and degradation, whereas it was significantly decreased the reads in the pathway of biosynthesis while increased the reads in the pathway of degradation in the bacterial community. In addition, the reads related to the pathways of amino acid biosynthesis was significantly increased in LT_N compared with NT_N in the fungi microbial community. Under low temperature, the reads were significantly enriched in the pathway of amino acid biosynthesis in LT_N, compared with LT_MT, in level 2 in both fungal (Figure 5E) and bacterial (Figure 5F) communities; however, an opposite trend was found in the pathway of amino acid degradation.

Correlation between metabolites and biomarkers of microbial communities in rhizosphere soil

The soil metabolites pool, which composed of both plant-secreted metabolites and exogenous metabolites from the microbial community, was significantly changed by low temperature and melatonin (Figure 6). Using GC-MS based non-target metabolomics, a total of 104 metabolites were identified and semi-quantified in soil samples. Furthermore, using a statistical threshold of $P < 0.05$ and VIP (variable importance in the projection) > 1, 63 differential metabolites were found in the group of LT_N vs LT_MT. For pathway analysis of the identified differential metabolites using the KEGG database, the $P < 0.05$ and IF (impact factor) > 0.04 were set as threshold values. The TOP 5 pathways, including 11 specific metabolites were divided into two categories: 2 pathways related to amino acid synthesis and metabolism (glycine, serine and threonine metabolism, biosynthesis of amino acids) and 3 carbon metabolism related pathways (glyoxylate and dicarboxylate metabolism, carbon metabolism and galactose metabolism). The metabolites could be annotated into different pathways: L-serine, glycine, L-threonine and 1,3-diaminopropane were annotated into the pathway of glycine, serine and threonine metabolism ($P = 0.01$, IF = 0.43). The amino acids biosynthesis pathway ($P = 0.01$, IF = 0.08) including L-valine, L-threonine, L-serine, glycine, citrate and alanine. Glycolate, citrate, L-serine and glycine belonged to the glyoxylate and dicarboxylate metabolism pathway ($P = 0.01$, IF = 0.07). Citrate, L-serine, glycine, alanine and glycolate were annotated into the pathway of carbon metabolism ($P = 0.03$, IF = 0.05). The alpha-D-galactosyl-(1->3)-1D-myo-inositol, D-glucose 1-phosphate and D-Glucose belonged to the galactose metabolism pathway ($P = 0.03$, IF = 0.05).

The heatmap presented the relationship between biomarkers of fungal community and significantly varied metabolites (Figure 6A). The dominant phylum Ascomycota showed significantly negative correlations with L-threonine, 1,3-diaminopropane, alanine, glycolate, alpha-D-galactosyl-(1->3)-1D-myo-inositol, D-glucose 1-phosphate and D-glucose. The biomarkers of Nitrospira, Nitrospirales, *Nitrospiraceae*, *Nitrospira* and Nitrospirae all showed significantly positive correlations with L-threonine, 1,3-diaminopropane, citrate, alanine, glycolate, D-glucose 1-phosphate and D-glucose (Figure 6B). The Chloroflexi, Gemmatimonadetes and Bacteroidetes, Alphaproteobacteria, Rhizobiales showed significantly positive correlation with glycine, L-threonine, 1,3-diaminopropane, citrate, alanine, glycolate, alpha-D-galactosyl-(1->3)-1D-myo-inositol, D-glucose 1-phosphate and D-glucose.

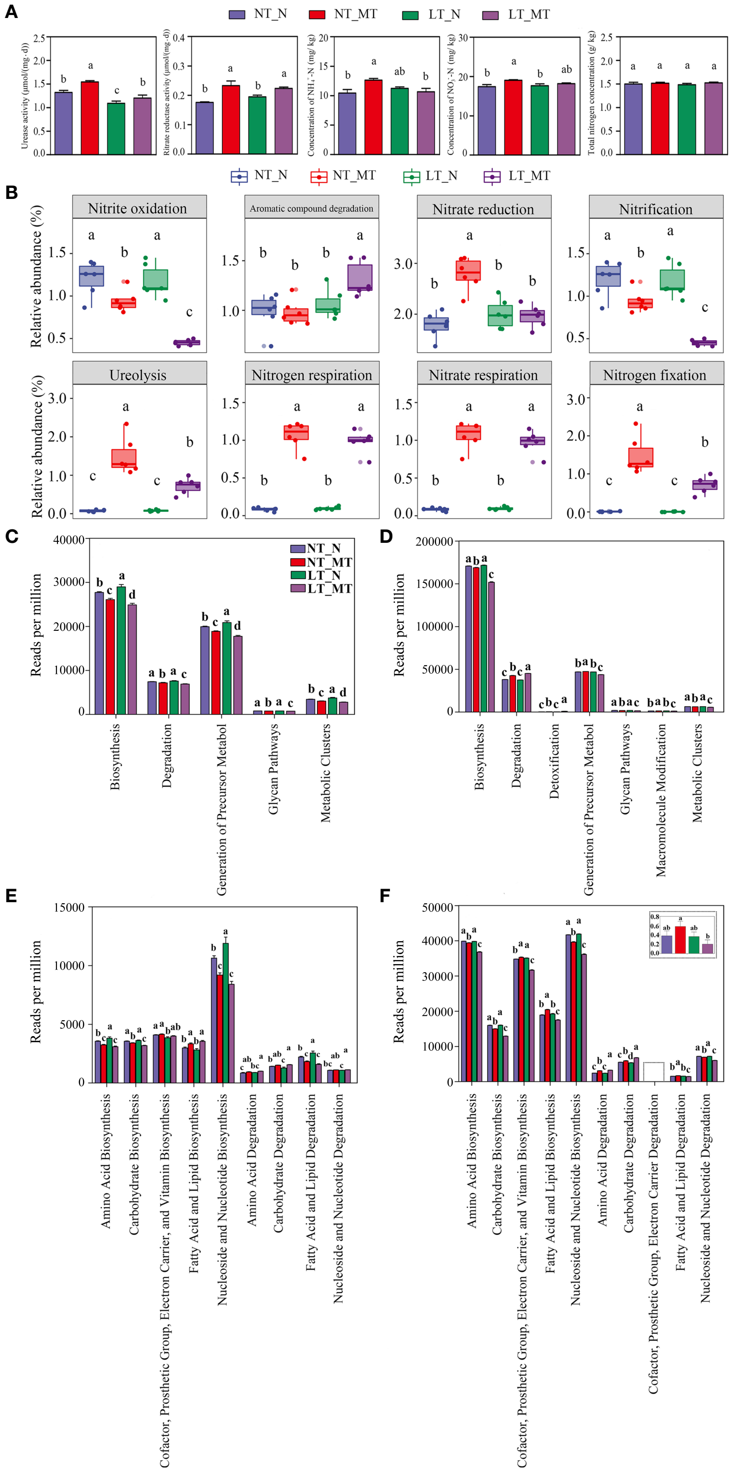


FIGURE 5
Activities of urease and nitrate reductase and concentrations of NH_4^+-N, NO_3^-N and total nitrogen in rhizosphere soil and relative abundance of rhizosphere microorganisms with different functions under low temperature and melatonin treatment. Activities of urease and nitrate reductase and concentrations of NH_4^+-N, NO_3^-N and total nitrogen in rhizosphere soil among different treatments **(A)**. Prediction of the functional composition in the microbial community according to FAPROTAX database **(B)**. Fungal **(C, E)** and bacterial **(D, F)** microbiota at the level 1 and the level 2 according to MetaCyc database, respectively. NT_N, normal temperature control; NT_MT, normal temperature + melatonin treatment; LT_N, low temperature control; LT_MT, low temperature + melatonin treatment. Data are presented as mean ± SE (n = 6). Different small letters represent significant differences at $P < 0.05$.

In addition, Proteobacteria, Gammaproteobacteria, Pseudomonadales and *Pseudomonadaceae* showed significantly negative correlations with those metabolites. *Rubrobacter* significantly positive correlations with all metabolites except for 1,3-diaminopropane.

Discussion

Rhizospheric melatonin application enhanced the low temperature tolerance by maintaining a better photosynthetic carbon assimilation

Photosynthesis is a highly sensitive process to low temperatures (Tan et al., 2008). In the present study, low temperature reduced the quantum yield of PS II, while rhizospheric melatonin application alleviated this damage induced by low temperature. φ_Eo reflects the highest quantum yield for PS II electron transport, while TRo/RC and ETo/RC reflect the trapped energy flux per reaction center and the electron transport flux per RC, respectively (Chen et al., 2014). Here, these parameters were all significantly enhanced by rhizospheric melatonin application, indicating that melatonin helped the plant to maintain a higher photosynthetic efficiency by alleviating the damage to the photosynthetic apparatus under low temperature.

Carbohydrate synthesis and catabolism are closely related to abiotic stress tolerance in plants (Jammer et al., 2015). Here, the carbohydrate metabolism in both leaves and roots was affected by the rhizospheric melatonin application. For leaves, in the sucrolytic pathway, the melatonin treated plants had a lower vacInv activity while a higher cwInv activity than the control plants under low temperature, which was contrary to the trends in roots. As a soluble acidic invertase, vacInv determines the sucrose amount stored in vacuole and its remobilization for

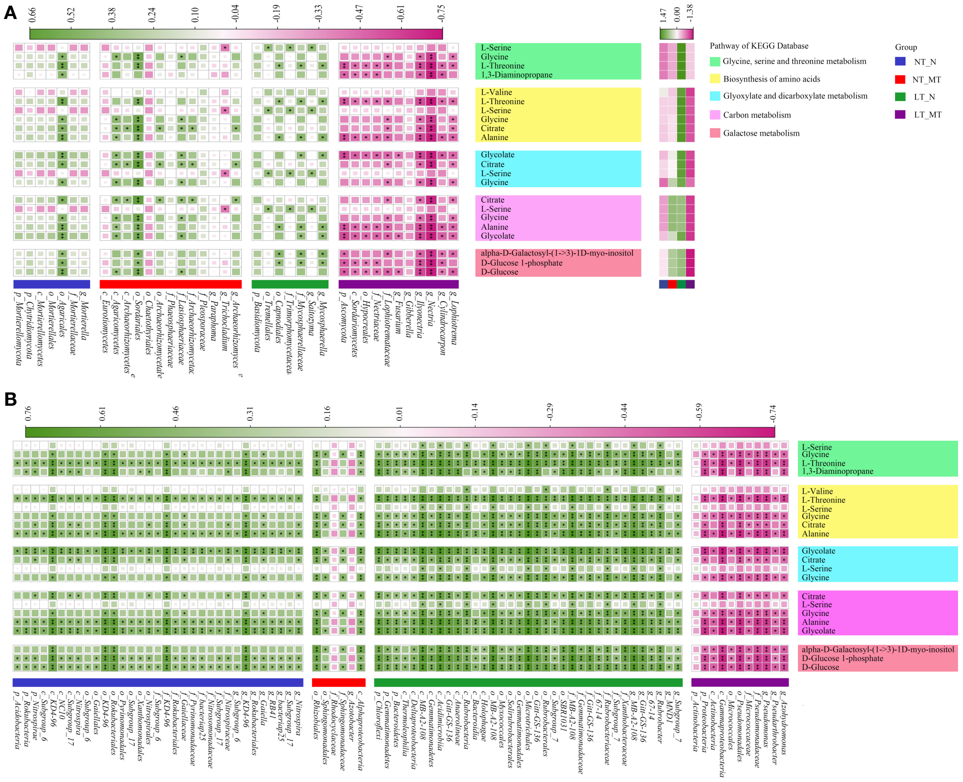


FIGURE 6
Correlations between biomarker taxon and differential metabolites in rhizosphere for fungal **(A)** and bacterial **(B)** under low temperature and melatonin treatment. The spearman rank correlation coefficients and the corresponding *P*-values were calculated based on the analyses of the microbiota whose LDA score > 3.5 with phylogenetic levels from phylum to genus and the metabolites enriched in the top 5 functions of KEGG pathway in rhizosphere. The proportion of the square and the darkness of the color in each box corresponds to the r^2 value according to the legend. NT_N, normal temperature control; NT_MT, normal temperature + melatonin treatment; LT_N, low temperature control; LT_MT, low temperature + melatonin treatment. * and ** indicate significance at $P < 0.05$ and $P < 0.01$. Heatmap of metabolite concentration under low temperature and melatonin treatment is shown in the right part of panel **(A)**. The difference of abundance for metabolites among these treatments is deviation standardization with Z-score and converted to a color scale. Increase and decrease in abundance is indicated in the colored bar with pink and green.

metabolism (Roitsch and González, 2004); whereas cwInv maintains the extracellular sucrose gradient by breaking down sucrose (Jammer et al., 2015). Thus, the changes of these two invertases may result in the fluctuation of sucrose concentration. For the pathway of glycolysis, lower activities of FK, PGI and Ald in MT leaves in relation to the control plants under low temperature indicated that the conversions of fructose and glucose-6-phosphate to fructose-6-phosphate and fructose-1,6-bisphosphate to glyceraldehyde-3-phosphate and dihydroxyacetone phosphate were both depressed. However, in relation to those in control, the significantly enhanced activities of PGI, PFK, Ald and FK in MT roots suggested that fructose metabolism and glycolysis flux were regulated by melatonin (Dhatt et al., 2019), which might contribute to the enhanced low temperature tolerance in barley. This was also proved by the higher levels of total soluble sugars in roots of melatonin treated barley.

In plants, melatonin has a common biosynthetic pathway from tryptophan through sequential enzymatic steps, including TDC, T5H, SNAT and COMT/ASMT (Nawaz et al., 2016). Rhizospheric melatonin application significantly increased the activities of all these five enzymes except for SNAT under low temperature, indicating that rhizospheric melatonin application enhanced the endogenous melatonin synthesis, which was consistent with results of Sun et al. (2020). In agreement with the enzyme activities, the serotonin and endogenous melatonin levels could be related to the melatonin induced low temperature tolerance, since serotonin and melatonin are of importance for stress defense and plant growth (Li et al., 2016; Wan et al., 2018). Besides the direct effects of rhizospheric melatonin application, it is hard to rule out the possibility of rhizosphere microorganisms for enhancing the stress tolerance in plants (Pieterse et al., 2014).

Rhizospherically applied melatonin altered the diversity of microbial community related to nitrogen cycling

The alpha diversity refers to the indicators for richness and diversity of fungal and bacterial species, including Chao 1, observed species, Shannon and Simpson. The indexes of Chao 1 and observed species both represent richness (Chao, 1984), while Shannon and Simpson represent the diversity of microorganisms (Shannon, 1948; Simpson, 1997). Here, two indexes (Chao 1 and observed species) for fungal communities and four indexes (Chao 1, observed species, Shannon and Simpson) for bacterial communities all showed significant differences among treatments, which implied that rhizospherically applied melatonin changed the richness of fungal communities and altered both richness and diversity of bacterial communities under low temperature. Similarly, exogenous melatonin alters the structure of soil bacteria and has a significant effect on bacterial alpha diversity under abiotic stress conditions (Madigan et al., 2019). In addition, exogenous melatonin reduces the Shannon diversity of bacterial community and reprograms the rhizosphere microbial community to modulate the responses of barley to abiotic stress (Ye et al., 2022), which is consistent with our findings. However, the release of secondary metabolism compounds from plants into the soil as root exudates may also have an indirect effect on rhizosphere microbial diversity (Wolfe and Klironomos, 2005; Trivedi et al., 2021).

Also, the compositions of fungal and bacterial communities were modulated by rhizospherically applied melatonin regardless of temperature regimes, where Ascomycota, Mortierellomycota and Basidiomycota were the most dominant phyla in the rhizosphere microorganisms. As the most common and diverse community of eukaryotes, Ascomycota is involved in the decomposition of organic substrates (e.g., dead leaves, wood chips and faeces), and it is the dominant fungal community in organically improved soils (Guo et al., 2018). The significantly higher relative abundance of Ascomycota in LT_MT compared with LT_N indicated that melatonin could help to decompose organic substrates. Meanwhile, Basidiomycota has also been proved to be the main decomposer that plays an important role in transformation of soil nutrients and degrade lignocellulose and organic matter (Yelle et al., 2008). The significant difference in the relative abundance of Basidiomycota between LT_MT and LT_N suggested that melatonin had an important effect on nutrient metabolism in the rhizosphere of barley plants.

Besides fungal communities, some significantly altered bacterial communities with functioning in nitrogen fixation and nutrient cycling were also observed in this study. Under low temperature, MT treatment showed a significantly higher relative abundance of Actinobacteria and Proteobacteria compared with the control. As the dominant bacterium with the highest relative abundance, Actinobacteria is the soil-dwelling organisms with an important role in the turnover of organic matter, nutrient recycling and plant growth (Trujillo et al., 2015). Meanwhile, Actinobacteria is a nitrogen-fixing microorganism that has received wide attentions (Verma et al., 2009). In addition to being involved in nitrogen cycling processes, Actinobacteria from special and extreme habitats probably contain novel taxa and compounds for enhancing their host tolerance to environmental stress (Mesa et al., 2017; Qin et al., 2018). For instance, Actinobacteria act as important players in inhibiting the root growth under infection of pathogens (Bhatti et al., 2017) and is associated with regulating plant abiotic stress response (Xu et al., 2021). For another dominant bacterium, Proteobacteria plays a vital role in nitrogen fixation in the biosphere as well as in the carbon, sulfur and nitrogen cycles (Kersters et al., 2006). It was also reported that Proteobacteria has various functions, including protein and amino acid metabolism, carbohydrate metabolism and energy metabolism (Jiang et al., 2021). The significantly increased relative abundances of Actinobacteria and

Proteobacteria indicated that rhizospherically applied melatonin regulated the nutrient recycling, nitrogen-fixing and amino acid metabolism, which might enhance the plant performance under low temperature. Acidobacteria participates in nitrogen metabolism and exopolysaccharide production (Kuramae and de Assis Costa, 2019). The significant changes in the relative abundance of Acidobacteria also proved that melatonin induced changes in the fungal and bacterial communities were closely related to the nitrogen metabolism.

The relative abundances of some other microorganisms associated with the nitrogen-cycling were also significantly changed by melatonin under low temperature. Nitrite oxidation is the main biochemical pathway that produces nitrate, and this process is catalyzed by nitrite oxidoreductase, which is encoded by aerobic nitrite-oxidizing bacteria (Daims et al., 2016), including the dominant phyla Chloroflexi and Nitrospirae in this study. The favorable effects of *Azotobacter* have been reported to provide nutrients to cereals and enhance plant growth (Sohal et al., 1998; Baghaie and Aghili, 2021). The genus *Azoarcus* can secrete auxin (Rasul et al., 1998) and promote plant growth (Mehnaz et al., 1998; Raittz et al., 2021). *Dechloromonas* includes genes for dozens of metabolisms, such as nitrogen fixation protein, nitrogen regulatory protein, has the ability to metabolize nitrogen, including nitrogen fixation, denitrification and dissimilatory nitrate reduction (Zhang et al., 2021). The significantly increased in the relative abundance of *Azotobacter*, *Azoarcus* and *Dechloromonas* in MT treatment further proved that melatonin induced changes in microorganism under low temperature were closely related to the nitrogen metabolism, which was beneficial to the plant growth. The common bacterium genus *Nitrospira* was restricted to oxidize nitrite to nitrate in the pathway of nitrification (Stein and Klotz, 2017). The altered compositions in the nitrogen-cycling related bacterial and fungal communities suggested that rhizospherically applied melatonin might actively coordinate the microbial community to modulate soil nutrients for optimal plant growth under low temperature.

Nitrogen-transforming microorganisms are generally classified according to one of the processes, such as nitrification, denitrification, nitrogen fixation, et al. (Kuypers et al., 2018). Notably, it has been observed that some pathways were related to the nitrogen cycle according to the FAPROTAX database in bacterial communities. The significant decrease in the relative abundance of nitrite oxidation and nitrification while the significant increase in that of ureolysis, nitrogen respiration, nitrate respiration and nitrogen fixation were observed regardless of temperature regimes, which could be ascribed to the melatonin application. The activity of soil urease and nitrate reductase were significantly enhanced by melatonin, indicating that melatonin promoted the nitrogen cycling in soil. The concentrations of NH_4^+-N and NO_3^-N were not affected by melatonin under low temperature; that might be due to the complexity of nitrogen metabolism changes induced by melatonin in the barley rhizosphere. It has been well known that diverse microorganisms can fix dinitrogen gas and denitrify simultaneously (Stein and Klotz, 2017). Furthermore, by predicting the functional composition with the reads of bacterial and fungal communities using the MetaCyc database in level 2, it was found that the reads related to amino acid biosynthesis was significantly decreased, while that related to amino acid degradation was significantly increased by rhizospherically applied melatonin, which also proved this result.

Melatonin induced changes of metabolites in synthesis and metabolism of amino acids pathway in response to the low temperature tolerance

To verify whether the changes of microbial diversity affect the rhizosphere micro-environment of barley plants, metabolites in rhizosphere soil were detected in this study. The pathway enrichment analysis with the KEGG database was conducted to elucidate the specific changes in rhizosphere metabolic processes. The metabolism of glycine, serine and threonine and biosynthesis of amino acids were the top two altered pathways in the rhizosphere soil, which were both amino acid synthesis and metabolic related pathways. Most of the biomarkers of bacteria and fungi showed significantly positive correlations with the altered metabolites included in these two pathways. However, all the biomarkers showed significantly negative correlations with the metabolites related to amino acid synthesis and metabolism in response to low temperature and melatonin treatments. Soil amino acids can be rapidly decomposed by the microorganisms within a few hours and are an important part of soil nitrogen cycling (Ma et al., 2021). This suggested that rhizosphere microorganisms might accelerate the decomposition of amino acids in the soil to regulate the soil nitrogen cycle in this case. Modulations in certain metabolic pathways might be a key strategy for nitrogen-cycling related microbial communities to help plants adapt to environmental stress.

Conclusion

Rhizospheric melatonin application promoted the low temperature tolerance in barley, as exemplified by higher photosynthetic carbon assimilation and better redox homeostasis. The melatonin treatment obviously changed in the diversity of microbial community in rhizosphere of barley, especially the species and relative abundance of nitrogen cycling-related microorganisms, which was also related to the changes in rhizosphere soil metabolites in the pathways of amino acid synthesis and metabolism. The altered rhizospheric microbial status were associated with the promotion of the performance of both roots and shoots in barley exposed to low temperature, which might be one of the main reasons for modulate the response of barley to low temperature. Thus, the rhizospheric melatonin application induced low temperature tolerance in barley may be associated with the nitrogen-cycling related rhizosphere microorganisms. These results have important implications for understanding the role of rhizospheric melatonin in regulation of plants to improve low temperature resistance.

Data availability statement

The original contributions presented in the study are publicly available. This data can be found here: NCBI, PRJNA861116.

Author contributions

MJ: Conceptualization, Methodology, Writing - original draft. FY: Visualization, Software. FL: Conceptualization, Methodology. MB: Software, Validation. XL: Conceptualization, Methodology, Writing - review & editing. All authors contributed to the article and approved the submitted version.

Funding

This research was funded by the Strategic Priority Research Program of the Chinese Academy of Sciences (XDA28020400), National Natural Science Fund for Excellent Young Scholars (31922064), CAS Pioneer Hundred Talents Program (C08Y194), the Science & Technology Development Program of Jilin Province (20190201118JC; 20210402036GH) and Danmarks Frie Forskningsfond (0217-00084B).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2022.998861/full#supplementary-material

References

Arnao, M. B., and Hernández-Ruiz, J. (2009). Protective effect of melatonin against chlorophyll degradation during the senescence of barley leaves. *J. Pineal Res.* 46, 58–63. doi: 10.1111/j.1600-079X.2008.00625.x

Baghaie, A. H., and Aghili, F. (2021). Soil zn fertilization and inoculation with arbuscular mycorrhizal fungus and *Azotobacter chroococcum* bacteria affect the cd concentration and zn bioavailability in bread wheat grown in a cd-spiked soil. *Soil Sediment Contam.* 30, 819–837. doi: 10.1080/15320383.2021.1893647

Bano, S., Wu, X., and Zhang, X. (2021). Towards sustainable agriculture: rhizosphere microbiome engineering. *Appl. Microbiol. Biot.* 105, 7141–7160. doi: 10.1007/s00253-021-11555-w

Bhatti, A. A., Haq, S., and Bhat, R. A. (2017). Actinomycetes benefaction role in soil and plant health. *Microb. Pathog.* 111, 458–467. doi: 10.1016/j.micpath.2017.09.036

Bokulich, N. A., Dillon, M. R., Zhang, Y., Rideout, J. R., Bolyen, E., Li, H., et al. (2018). q2-longitudinal: longitudinal and paired-sample analyses of microbiome data. *Msystems* 3, e00219–e00218. doi: 10.1128/mSystems.00219-18

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., and Holmes, S. P. (2016). DADA2: High-resolution sample inference from illumina amplicon data. *Nat. Methods* 13 581–583. doi: 10.1038/NMETH.3869

Chao, A. (1984). Nonparametric estimation of the number of classes in a population. *Scand. J. Stat.* 11, 265–270. doi: 10.2307/4615964

Chen, S., Strasser, R. J., and Qiang, S. (2014). *In vivo* assessment of effect of phytotoxin tenuazonic acid on PSII reaction centers. *Plant Physiol. Bioch.* 84, 10–21. doi: 10.1016/j.plaphy.2014.09.004

Daims, H., Lüecker, S., and Wagner, M. (2016). A new perspective on microbes formerly known as nitrite-oxidizing bacteria. *Trends Microbiol.* 24, 699–712. doi: 10.1016/j.tim.2016.05.004

Dhatt, B. K., Abshire, N., Paul, P., Hasanthika, K., Sandhu, J., Zhang, Q., et al. (2019). Metabolic dynamics of developing rice seeds under high night-time temperature stress. *Front. Plant Sci.* 10 1443. doi: 10.3389/fpls.2019.01443

Ding, J., Sun, H., Liang, A., Liu, J., Song, L., Lv, M., et al. (2021). Testosterone amendment alters metabolite profiles of the soil microbial community. *Environ. pollut.* 272, 115928. doi: 10.1016/j.envpol.2020.115928

Edwards, J., Johnson, C., Santos-Medellín, C., Lurie, E., Podishetty, N. K., Bhatnagar, S., et al. (2015). Structure, variation, and assembly of the root-associated microbiomes of rice. *PNAS.* 112, E911–E920. doi: 10.1073/pnas.1414592112

Egamberdieva, D., Li, L., Lindström, K., and Räsänen, L. A. (2016). A synergistic interaction between salt-tolerant *Pseudomonas* and *Mesorhizobium* strains improves growth and symbiotic performance of liquorice (*Glycyrrhiza uralensis* fish.) under salt stress. *Appl. Microbiol. Biol.* 100, 2829–2841. doi: 10.1007/s00253-015-7147-3

Eisenhauer, N., Scheu, S., and Jousset, A. (2012). Bacterial diversity stabilizes community productivity. *PLoS One* 7, e34517. doi: 10.1371/journal.pone.0034517

Fales, F. W. (1951). The assimilation and degradation of carbohydrates by yeast cells. *J. Biol. Chem.* 193, 113–124. doi: 10.1515/bchm2.1951.286.1-6.270

Fan, J., Hu, Z., Xie, Y., Chan, Z., Chen, K., Amombo, E., et al. (2015). Alleviation of cold damage to photosystem II and metabolisms by melatonin in bermudagrass. *Front. Plant Sci.* 6. doi: 10.3389/fpls.2015.00925

Fimognari, L., Dölker, R., Kaselyte, G., Jensen, C. N. G., Akhtar, S. S., Großkinsky, D.K., et al. (2020). Simple semi-high throughput determination of activity signatures of key antioxidant enzymes for physiological phenotyping. *Plant Methods* 16, 42. doi: 10.1186/s13007-020-00583-8

Geng, L., Shao, G., Raymond, B., Wang, M., Sun, X., Shu, C., et al. (2018). Subterranean infestation by *Holotrichia parallela* larvae is associated with changes in the peanut (*Arachis hypogaea* l.) rhizosphere microbiome. *Microbiol. Res.* 211, 13–20. doi: 10.1016/j.micres.2018.02.008

Goh, C. H., Vallejos, D. F. V., Nicotra, A. B., and Mathesius, U. (2013). The impact of beneficial plant-associated microbes on plant phenotypic plasticity. *J. Chem. Ecol.* 39, 826–839. doi: 10.1007/s10886-013-0326-8

Guo, J., Liu, W., Zhu, C., Luo, G., Kong, Y., Ling, N., et al. (2018). Bacterial rather than fungal community composition is associated with microbial activities and nutrient-use efficiencies in a paddy soil with short-term organic amendments. *Plant Soil* 424, 335–349. doi: 10.1007/s11104-017-3547-8

Jammer, A., Gasperl, A., Luschin-Ebengreuth, N., Heyneke, E., Chu, H., Cantero-Navarro, E., et al. (2015). Simple and robust determination of the activity signature of key carbohydrate metabolism enzymes for physiological phenotyping in model and crop plants. *J. Exp. Bot.* 66, 5531–5542. doi: 10.1093/jxb/erv228

Jiang, M., Wang, Z., Li, X., Liu, S., Song, F., and Liu, F. (2021). Relationship between endophytic microbial diversity and grain quality in wheat exposed to multi-generational CO$_2$ elevation. *Sci. Total Environ.* 776, 146029. doi: 10.1016/j.scitotenv.2021.146029

Kersters, K., Vos, P. D., Gillis, M., Swings, J., Vandamme, P., and Stackebrandt, E. (2006). "Introduction to the proteobacteria," in *The prokaryotes, proteobacteria: alpha and beta subclasses*. Eds. M. Dworkin, S. Falkow, E. Rosenberg, K. H. Schleifer and E. Stackebrandt (New York: Springer), 3–37.

Koide, R. T., Fernandez, C., and Petprakob, K. (2011). General principles in the community ecology of ectomycorrhizal fungi. *Ann. For. Sci.* 68, 45–55. doi: 10.1007/s13595-010-0006-6

Kõljalg, U., Nilsson, R. H., Abarenkov, K., Tedersoo, L., Taylor, A. F. S., Bahram, M., et al. (2013). Towards a unified paradigm for sequence-based identification of fungi. *Mol. Ecol.* 22, 5271–5277. doi: 10.1111/mec.12481

Kuramae, E. E., and de Assis Costa, O. Y. (2019). "Acidobacteria," in *Encyclopedia of microbiology (Fourth edition)*. Ed. T. M. Schmidt (Oxford: Academic Press), 1–8.

Kuypers, M. M. M., Marchant, H. K., and Kartal, B. (2018). The microbial nitrogen-cycling network. *Nat. Rev. Microbiol.* 16, 263–276. doi: 10.1038/nrmicro.2018.9

Kuzyakov, Y., and Razavi, B. S. (2019). Rhizosphere size and shape: Temporal dynamics and spatial stationarity. *Soil Biol. Biochem.* 135, 343–360. doi: 10.1016/j.soilbio.2019.05.011

Li, X., Cai, J., Liu, F., Dai, T., Cao, W., and Jiang, D. (2014a). Physiological, proteomic and transcriptional responses of wheat to combination of drought or waterlogging with late spring low temperature. *Funct. Plant Biol.* 41, 690–703. doi: 10.1071/FP13306

Li, X., Cai, J., Liu, F., Dai, T., Cao, W., and Jiang, D. (2014b). Cold priming drives the sub-cellular antioxidant systems to protect photosynthetic electron transport against subsequent low temperature stress in winter wheat. *Plant Physiol. Bioch.* 82, 34–43. doi: 10.1016/j.plaphy.2014.05.005

Li, X., Cai, J., Liu, F., Zhou, Q., Dai, T., Cao, W., et al. (2015). Wheat plants exposed to winter warming are more susceptible to low temperature stress in the spring. *Plant Growth Regul.* 77, 11–19. doi: 10.1007/s10725-015-0029-y

Li, S., Guo, J., Wang, T., Gong, L., Liu, F., Brestic, M., et al. (2021). Melatonin reduces nanoplastic uptake, translocation, and toxicity in wheat. *J. Pineal Res.* 71, e12761. doi: 10.1111/jpi.12761

Li, X., Tan, D., Jiang, D., and Liu, F. (2016). Melatonin enhances cold tolerance in drought-primed wild-type and abscisic acid-deficient mutant barley. *J. Pineal Res.* 61, 328–339. doi: 10.1111/jpi.12350

Liu, H., Brettell, L. E., Qiu, Z., and Singh, B. K. (2020). Microbiome-mediated stress resistance in plants. *Trends Plant Sci.* 25, 733–743. doi: 10.1016/j.tplants.2020.03.014

Madigan, A. P., Egidi, E., Bedon, F., Franks, A. E., and Plummer, K. M. (2019). Bacterial and fungal communities are differentially modified by melatonin in agricultural soils under abiotic stress. *Front. Microbiol.* 10 2616. doi: 10.3389/fmicb.2019.02616

Ma, Q. X., Kuzyakov, Y., Pan, W. K., Tang, S., Chadwick, D. R., Wen, Y., et al. (2021). Substrate control of sulphur utilisation and microbial stoichiometry in soil: Results of ^{13}C, ^{15}N, ^{14}C, and ^{35}S quad labelling. *Isme J.* 15, 3148–3158. doi: 10.1038/s41396-021-00999-7

Mehnaz, S., Mirza, M. S., Hassan, U., and Malik, K. A. (1998). "Detection of inoculated plant growth-promoting rhizobacteria in the rhizosphere of rice," in *Developments in plant and soil sciences*. Eds. K. A. Malik, M. S. Mirza and J. K. Ladha (Kluwer Academic, London), 75–83.

Mendes, R., Garbeva, P., and Raaijmakers, J. M. (2013). The rhizosphere microbiome: significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. *FEMS Microbiol. Rev.* 37, 634–663. doi: 10.1111/1574-6976.12028

Mesa, V., Navazas, A., González-Gil, R., González, A., Weyens, N., Lauga, B., et al. (2017). Use of endophytic and rhizosphere bacteria to improve phytoremediation of arsenic-contaminated industrial soils by autochthonous *Betula celtiberica*. *Appl. Environ. Microb.* 83, e03411–e03416. doi: 10.1128/AEM.03411-16

Nawaz, M. A., Huang, Y., Bie, Z., Ahmed, W., Reiter, R. J., Niu, M., et al. (2016). Melatonin: current status and future perspectives in plant science. *Front. Plant Sci.* 6. 1230 doi: 10.3389/fpls.2015.01230

Philippot, L., Raaijmakers, J. M., Lemanceau, P., and van der Putten, W. H. (2013). Going back to the roots: the microbial ecology of the rhizosphere. *Nat. Rev. Microbiol.* 11, 789–799. doi: 10.1038/nrmicro3109

Pieterse, C. M. J., Zamioudis, C., Berendsen, R. L., Weller, D. M., Van Wees, S. C. M., and Bakker, P. A. H. M. (2014). Induced systemic resistance by beneficial microbes. *Annu. Rev. Phytopathol.* 52, 347–375. doi: 10.1146/annurev-phyto-082712-102340

Purahong, W., and Krüeger, D. (2012). A better understanding of functional roles of fungi in the decomposition process: using precursor rRNA containing ITS regions as a marker for the active fungal community. *Ann. Foest Sci.* 69, 659–662. doi: 10.1007/s13595-012-0210-7

Qin, S., Feng, W., Zhang, Y., Wang, T., Xiong, Y., and Xing, K. (2018). Diversity of bacterial microbiota of coastal halophyte *Limonium sinense* and amelioration of salinity stress damage by symbiotic plant growth-promoting actinobacterium *Glutamicibacter halophytocola* KLBMP 5180. *Appl. Environ. Microb.* 84, e01533–e01518. doi: 10.1128/AEM.01533-18

Raittz, R. T., Pierri, C. R. D., Maluk, M., Batista, M. B., Carmona, M., Junghare, M., et al. (2021). Comparative genomics provides insights into the taxonomy of *Azoarcus* and reveals separate origins of *Nif* genes in the proposed *Azoarcus* and *Aromatoleum* genera. *Genes-Basel.* 12, 71. doi: 10.3390/genes12010071

Rasul, G., Mirza, M. S., Latif, F., and Malik, K. A. (1998). "Identification of plant growth hormones produced by bacterial isolates from rice, wheat and kallar grass," in *Developments in plant and soil sciences*. Eds. K. A. Malik, M. S. Mirza and J. K. Ladha (Kluwer Academic, London), 25–37.

Roitsch, T., and González, M. C. (2004). Function and regulation of plant invertases: sweet sensations. *Trends Plant Sci.* 9, 606–613. doi: 10.1016/j.tplants.2004.10.009

Shannon, C. E. (1948). A mathematical theory of communication. *Bell System Tech. J.* 27, 379–423. doi: 10.1002/j.1538-7305.1948.tb00917.x

Shi, Z., Li, D., Qi, J., Cai, J., Jiang, D., Cao, W., et al. (2012). Effects of nitrogen applications on soil nitrogen balance and nitrogen utilization of winter wheat in a rice - wheat rotation. *Field Crop Res.* 127, 241–247. doi: 10.1016/j.fcr.2011.11.025

Simpson, E. H. (1997). Measurement of diversity. *J. Cardiothor Vasc. An.* 11, 812–812. doi: 10.1136/thx.27.2.261

Sohal, P. K., Gupta, R. P., and Pandher, M. S. (1998). "Effect of inoculation of azotobacter and PSM on fertilizer economy, plant growth and yield of winter maize," in *Developments in plant and soil sciences*. Eds. K. A. Malik, M. S. Mirza and J. K. Ladha (Kluwer Academic, London), 271–273.

Stein, L. Y., and Klotz, M. G. (2017). The nitrogen cycle. *Curr. Biol.* 26, R94–R98. doi: 10.1016/j.cub.2015.12.021

Sun, L., Song, F., Guo, J., Zhu, X., Liu, S., Liu, F., et al. (2020). Nano-ZnO-induced drought tolerance is associated with melatonin synthesis and metabolism in maize. *Int. J. Mol. Sci.* 21, 782. doi: 10.3390/ijms21030782

Tan, D., Hardeland, R., Manchester, L. C., Korkmaz, A., Ma, S., Rosales-Corral, S., et al. (2012). Functional roles of melatonin in plants, and perspectives in nutritional and agricultural science. *J. Exp. Bot.* 63, 577–597. doi: 10.1093/jxb/err256

Tan, W., Liu, J., Dai, T., Jing, Q., Cao, W., and Jiang, D. (2008). Alterations in photosynthesis and antioxidant enzyme activity in winter wheat subjected to post-anthesis water-logging. *Photosynthetica.* 46, 21–27. doi: 10.1007/s11099-008-0005-0

Tan, D., and Reiter, R. J. (2019). Mitochondria: The birth place, battle ground and the site of melatonin metabolism in cells. *Melatonin Res.* 2, 44–66. doi: 10.32794/mr11250011

Toju, H., Peay, K. G., and Yamamichi, M. (2018). Core microbiomes for sustainable agroecosystems. *Nat. Plants* 4, 247–257. doi: 10.1038/s41477-018-0139-4

Trivedi, P., Leach, J. E., Tringe, S. G., Sa, T., and Singh, B. K. (2021). Plan-microbiome interactions: from community assembly to plant health. *Nat. Rev. Microbiol.* 19, 72. doi: 10.1038/s41579-020-0412-1

Trujillo, M. E., Riesco, R., Benito, P., and Carro, L. (2015). Endophytic observed species and the interaction of *Micromonospora* and nitrogen fixing plants. *Front. Microbiol.* 6. doi: 10.3389/fmicb.2015.01341

Verma, V. C., Gond, S. K., Kumar, A., Mishra, A., Kharwar, R. N., and Gange, A. C. (2009). Endophytic actinomycetes from *Azadirachta indica* a. juss.: solation, diversity, and anti-microbial activity. *Microbl Ecol.* 57, 749–756. doi: 10.1007/s00248-008-9450-3

Wan, J., Zhang, P., Wang, R., Sun, L., Ju, Q., and Xu, J. (2018). Comparative physiological responses and transcriptome analysis reveal the roles of melatonin and serotonin in regulating growth and metabolism in *Arabidopsis*. *BMC Plant Biol.* 18, 362. doi: 10.1186/s12870-018-1548-2

Withers, E., Hill, P. W., Chadwick, D. R., and Jones, D. L. (2020). Use of untargeted metabolomics for assessing soil quality and microbial function. *Soil Biol. Biochem.* 143, 107758. doi: 10.1016/j.soilbio.2020.107758

Wolfe, B. E., and Klironomos, J. N. (2005). Breaking new ground: soil communities and exotic plant invasion. *Bioscience.* 55, 477–487. doi: 10.1641/00063568(2005)055[0477:BNGSCA]2.0.CO;2

Xu, L., Dong, Z., Chiniquy, D., Pierroz, G., Deng, S., Gao, C., et al. (2021). Genome-resolved metagenomics reveals role of iron metabolism in drought-induced rhizosphere microbiome dynamics. *Nat. Commun.* 12, 3209. doi: 10.1038/s41467-021-23553-7

Xu, L., Naylor, D., Dong, Z., Simmons, T., Pierroz, G., Hixson, K. K., et al. (2018). Drought delays development of the sorghum root microbiome and enriches for monoderm bacteria. *PNAS.* 115, E4592. doi: 10.1073/pnas.1717308115

Ye, F., Jiang, M., Zhang, P., Liu, L., Liu, S., Zhao, C., et al. (2022). Exogenous melatonin reprograms the rhizosphere microbial community to modulate the responses of barley to drought stress. *Int. J. Mol. Sci.* 23, 9665. doi: 10.3390/ijms23179665

Yelle, D. J., Ralph, J., Lu, F., and Hammel, K. E. (2008). Evidence for cleavage of lignin by a brown rot basidiomycete. *Environ. Microbiol.* 10, 1844–1849. doi: 10.1111/j.1462-2920.2008.01605.x

Yousefi, S., Kartoolinejad, D., Bahmani, M., and Naghdi, R. (2017). Effect of *Azospirillum lipoferum* and *Azotobacter chroococcum* on germination and early growth of hopbush shrub (*Dodonaea viscosa* l.) under salinity stress. *J. Sustain Forest.* 36, 107–120. doi: 10.1080/10549811.2016.1256220

Zhang, S., Amanze, C., Sun, C., Zou, K., Fu, S., Deng, Y., et al. (2021). Evolutionary, genomic, and biogeographic characterization of two novel xenobiotics-degrading strains affiliated with *Dechloromonas*. *Heliyon.* 7, e07181. doi: 10.1016/j.heliyon.2021.e07181

Zhao, H., Su, T., Huo, L., Wei, H., Jiang, Y., Xu, L., et al. (2015). Unveiling the mechanism of melatonin impacts on maize seedling growth: sugar metabolism as a case. *J. Pineal Res.* 59, 255–266. doi: 10.1111/jpi.12258

frontiers | Frontiers in Plant Science

TYPE Original Research
PUBLISHED 17 October 2022
DOI 10.3389/fpls.2022.1012186

OPEN ACCESS

EDITED BY
Safina Naz,
Bahauddin Zakariya University,
Pakistan

REVIEWED BY
Miguel Rebollo-Hernanz,
University of Illinois at
Urbana-Champaign, United States
El-Hadji Malick Cisse,
Hainan University, China

*CORRESPONDENCE
Ji-dao Du
djdbynd@163.com
Yan-li Du
dyl0305@sina.cn
Pengyu Jia
jiapengyu@126.com

†These authors have contributed
equally to this work

SPECIALTY SECTION
This article was submitted to
Plant Abiotic Stress,
a section of the journal
Frontiers in Plant Science

RECEIVED 05 August 2022
ACCEPTED 22 September 2022
PUBLISHED 17 October 2022

CITATION
Zhang Q, Qin B, Wang G-d,
Zhang W-j, Li M, Yin Z-g, Yuan X,
Sun H-y, Du J-d, Du Y-l and
Jia P (2022) Exogenous melatonin
enhances cell wall response to
salt stress in common bean
(*Phaseolus vulgaris*) and the
development of the associated
predictive molecular markers.
Front. Plant Sci. 13:1012186.
doi: 10.3389/fpls.2022.1012186

Exogenous melatonin enhances cell wall response to salt stress in common bean (*Phaseolus vulgaris*) and the development of the associated predictive molecular markers

Qi Zhang[1†], Bin Qin[1†], Guang-da Wang[1†], Wen-jing Zhang[1], Ming Li[1], Zhen-gong Yin[2], Xiankai Yuan[1], Hao-yue Sun[3], Ji-dao Du[1,4*], Yan-li Du[1,4*] and Pengyu Jia[1,4*]

[1]College of Agriculture, Herlongjiang Bayi Agricultural University, Daqing, China, [2]Crop Resources Institute, Heilongjiang Academy of Agricultural Sciences, Harbin, China, [3]Qiqihar Branch, Heilongjiang Academy of Agricultural Sciences, Qiqihaer, China, [4]National Coarse Cereals Engineering Research Center, Herlongjiang Bayi Agricultural University, Daqing, China

Common bean (*Phaseolus vulgaris*) is an important food crop; however, its production is affected by salt stress. Salt stress can inhibit seed germination, promote senescence, and modify cell wall biosynthesis, assembly, and architecture. Melatonin, an indole heterocycle, has been demonstrated to greatly impact cell wall structure, composition, and regulation in plants under stress. However, the molecular basis for such assumptions is still unclear. In this study, a common bean variety, "Naihua" was treated with water (W), 70 mmol/L NaCl solution (S), and 100 μmol/L melatonin supplemented with salt solution (M+S) to determine the response of common bean to exogenous melatonin and explore regulatory mechanism of melatonin against salt stress. The results showed that exogenous melatonin treatment alleviated salt stress-induced growth inhibition of the common bean by increasing the length, surface area, volume, and diameter of common bean sprouts. Moreover, RNA sequencing (RNA-seq) and real-time quantitative PCR (qRT-PCR) indicated that the cell wall regulation pathway was involved in the salt stress tolerance of the common bean enhanced by melatonin. Screening of 120 germplasm resources revealed that melatonin treatment improved the salt tolerance of more than 65% of the common bean germplasm materials. Melatonin also up-regulated cell wall pathway genes by at least 46%. Furthermore, we analyzed the response of the common bean germplasm materials to melatonin treatment under salt stress using the key genes associated with the synthesis of the common bean cell wall as the molecular markers. The results showed that two pairs of markers were significantly associated with melatonin, and these could be used as candidate markers to predict whether common bean respond to exogenous melatonin and then enhance salt tolerance at the sprouting stage. This study shows that cell wall can respond to exogenous melatonin and enhance the salt tolerance of common bean. The makers identified in this study can be used to select common bean varieties that can respond to melatonin under stress. Overall, the study found that cell wall could response melatonin and enhance the salt tolerance and developed the makers for predicting varieties fit for melatonin under stress in common bean, which may be applied in the selection or development of common bean varieties with abiotic stress tolerance.

KEYWORDS

common bean, melatonin, RNA-seq, cell wall, makers, salt tolerance, regulation

Introduction

Soil salinization has become a global problem (Shabala, 2013), affecting 7% (more than 900 million hectares) of the available land worldwide. Salt stress is a limiting factor in crop production and is caused by excessive accumulation of NaCl (Wang et al., 2018). High concentrations of sodium ions (Na^+) can reduce the osmotic potential of cells, leading to plant metabolic disorders (Fang et al., 2021). Salt stress affects plants from the seed germination stage through maturation and continues until the plant senescence. The sprouting stage has been reported to be the most sensitive to salt stress (Zhang et al., 2020). Therefore, there is an urgent need to improve the salt tolerance of plants, especially at the sprouting stage.

Plants have developed various strategies for dealing with salt stress, including the expression of salt stress-responsive genes (in ion transport, osmotic homeostasis, and toxicity resolution) and altering their physiological structures (such as lipids and cell wall) to adapt to salt stress (Zhu, 2002; Gong, 2021). The plant cell wall is a protective barrier that determines the size and shape of cells through the mechanical control of cell expansion (Carpita and Gibeaut, 1993), thus playing an integral role in salt stress tolerance (Endler et al., 2015). The cell wall is the first line of defense against salt stress (Van Zelm et al., 2020). Salt stress dehydrates plant cells, causing cellular stress (Monniaux and Hay, 2016). Overexpressing cell wall-related genes in transgenic *Arabidopsis* enhanced salt tolerance (Liu et al., 2016; Chun et al., 2019). Recent studies have shown that β-1,4-galactan (a cell wall component), whose synthesis is catalyzed by Galactan Synthase 1 (GALS1), also plays a role in salt stress tolerance. RNA-seq analysis showed that salt stress affects cell wall biosynthesis pathways (Zhang et al., 2021b). However, the relationship between cell wall biosynthesis regulation and salt stress tolerance is still unclear.

Melatonin (MT) was first discovered in the pineal gland of cattle by the physician Lerner (1959). Since its discovery, MT has been demonstrated to regulate plant growth and development processes, such as seed germination, root growth, leaf senescence, and abiotic stress responses (Arnao and Hernández-Ruiz, 2019). Moreover, MT has been shown to enhance plant stress tolerance in various ways. For example, melatonin can induce antioxidant activity, inhibit peroxidative metabolism and up-regulate ion homeostasis-related genes, such as *NHX1* and *AKT1* (Rodriguez et al., 2004; Li et al., 2012). Some studies also showed that melatonin could interact with other hormones associated with salt stress resistance in plants and stimulate the expression of defensive transcription factors (Iuchi et al., 2001; Zhao et al., 2017). Recently, special focus has been directed to the regulation of cell wall by exogenous melatonin. Melatonin content positively correlated with cell wall strength in various herbaceous peony (*Paeonia lactiflora)*, suggesting a connection between the cell wall and melatonin (Zhao et al., 2022). Exogenous melatonin could regulate cell wall biosynthesis, increase the strength of the cell wall and enhance the ability to capture ions, limiting ion levels in the cytoplasm to mitigate the toxic effects (Cao et al., 2019; Sun et al., 2021). Melatonin has been widely used in field crops such as wheat (Sun et al., 2020), soybean (Zhang et al., 2019), and cotton (Li et al., 2019); however, there are few reports about its application in the common bean.

Common bean (*Phaseolus vulgaris*), an important edible bean, is an annual legume grown in the temperate and subtropical regions. About 8,000 years ago, the common bean was cultivated only in Peru and Mexico, but it is currently grown worldwide. In 2010, the common bean planting area was about 32 million hectares, with an output of 25.42 million tons (Ganesan and Xu, 2017). However, environmental stresses, such as salt stress, have affected the growth of common bean (Popelka et al., 2004; Beaver and Osorno, 2009), necessitating its improvement for salt stress tolerance. In this study, common bean was treated with water, salt stress, and exogenous melatonin under salt stress to explore the regulatory mechanism of melatonin against salt stress. In addition, we screened 120 germplasm of common bean for salt stress tolerance. Also, the molecular markers of common bean were developed, and the valid markers associated with melatonin traits were identified. This study provides theoretical insights and application value for improving the salt tolerance of common bean at the sprouting stage through exogenous melatonin application.

Materials and methods

Plant materials

The common bean (*Phaseolus vulgaris*) variety 'Naihua' was used in this study, which was a salt-sensitive variety tested by laboratory and grown in local grown conventional variety. The validity analysis of 120 common bean germplasm varieties is listed in Table S1. All the germplasm materials were provided by the National Cereals Engineering Technology Research Center (NCETRE, Daqing, P.R. China).

Plant culture conditions and treatments

The common bean seeds of similar sizes were selected and sterilized using NaClO for 5 min. The seeds were rinsed thrice with sterile distilled water. Filter papers were placed into Petri dishes and soaked with different treatment solutions to form germination beds. The seeds were then placed in the Petri dishes (20 seeds per plate) and transferred to an incubator set at 25 °C without light. Three treatments were set including distilled water (W), salt stress (S) and exogenous melatonin under salt stress (M +S). The salt stress concentration was set at 70 mmol/L (Zhang et al., 2020); The concentration of exogenous melatonin solution was 100 μmol/L. 2.3228g melatonin (WM=232.28, Coolaber, Beijing, China) was dissolved using 10mL absolute ethanol and then fixed to 1L to make the storage solution and store in the dark at 4°C. 100μL of storage solution was added in to 999.9mL 70mmol/L NaCl soultion to make the M+S treatment solution. Each treatment had five replicates. Samples were collected from the seedlings on the fifth day after the treatment. Germination indicators (such as germination rate and vitality index) were captured as described previously (Zhang et al., 2021c). The bean sprouts from the different treatments were scanned using an Epson V750 root system scanner. Morphological indicators were analyzed by WINRHIZOPRO-2004a, and images were analyzed using Image J software.

RNA-seq analysis

The radicles of "Naihua" seedlings were selected in three biological replicates from the different treatments for RNA-Seq. Briefly, 0.5 g of the samples were used for total RNA extraction using the Versatile Plant RNA Extraction Kit (CW0581, CWBIO, Beijing, P.R. China). The RNA quality was determined by 1% agarose gel electrophoresis and NanoDrop spectrophotometer (NanoDrop^{TM} OneC, Thermo, Massachusetts, U.S.A). RNA samples with good qualities were sent to Novogene (Beijing, P.R. China) for sequencing. Novomagic platform (https://magic.novogene.com/) was used to analyze the obtained RNA-Seq, using the "PhaVulg1_0" genome from the Ensembl database as the reference. Thereafter, DEseq (Kvam et al., 2012) was used to analyze the differentially expressed genes (DEGs) between different sample groups using the value of fold change (FC) and false discovery rate (FDR) as the screening criteria under the settings FC≥2 ($\log_2$FC≥1) and FDR<0.01. The enrichment of the DEGs was subsequently analyzed based on Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) databases.

Real-time polymerase chain reaction analysis

The radicles were selected in three biological replicates from the different treatments for qRT-PCR analysis. Total RNA was extracted from the samples using Plant RNA Extraction Kit (CW0559, CWBIO, Beijing, China). The obtained RNA was used to synthesize single-strand cDNA using the *Evo M-MLV* RT Premix (AG11706, Accurate Biology, Hunan, China). The synthesized cDNA products were diluted 10-fold and then used for qRT-PCR, which was performed on a Light Cycler 480II system (Roche, Roche Diagnostics, Basel, Switzerland) using Universal qPCR SYBR Green Master Mix (11184E, Yeasen, Shanghai, China). *Pvactin11* was used as the reference gene for qRT-PCR assay (Borges et al., 2012). The qRT-PCR primers were designed by Primer Premier 5.0 software and are listed in Table S2. The relative expression levels of the biological and technological replicates were calculated using the delta-delta Ct ($2^{-\Delta Ct}$) method (Zhang et al., 2021c).

Construction and selection of molecular markers

The Perl script-based MISA (MIcroSAtellite) software was used to search for the genome-wide MISA sites, using the "PhaVulg1_0" genome from the Ensembl database as the reference (Peng and Lapitan, 2005). The markers were automatically designed by the Linux system-based Primer Premier software, using the design thresholds described by Qu and Liu (2013). The designed makers were then named accordingly using a Perl script. The locations of DEGs enriched in the cell wall-related GO terms and KEGG pathway were selected. The start site before and end site after 2000 bp of the DEGs were marked as the retrieval sites for screening the molecular markers.

Genomic DNA was extracted from the bean samples using the Plant Genomic DNA Extraction Kit (CW0531, CWBIO, Beijing, P.R. China). The DNA quality and quantity were determined using 1% agarose gel electrophoresis and NanoDrop spectrophotometer (NanoDropTM OneC, Thermo, Massachusetts, U.S.A). The samples were then subjected to PCR analysis using *EasyTaq*® DNA Polymerase for PAGE (AP112, Trans, Beijing, P.R. China) on a T100™ PCR machine (Hercules, California, U.S.A). Thereafter, the PCR products were separated on 9% (w/v) denaturing polyacrylamide gel (Trigiano and Caetano-Anolles, 1998).

Statistical analysis

Analysis of variance (ANOVA) was performed at $P<0.05$ significance level on the SPSS19.0 software to determine if there were significant differences between various treatments. Figures were generated using GraphPad Prism software, while the data of molecular makers were subjected to single-marker analysis (Prasad et al., 2003).

Results

Phenotypic characteristics of the bean sprouts under different treatments

The germination and phenotype indicators of the common bean were evaluated at the sprouting stage under three treatments (Figure 1). The germination results showed that although there was no significant difference in the germination rate among the three treatments, salt stress significantly decreased the vitality index, but this was significantly alleviated by exogenous melatonin ($P<0.05$). Salt stress inhibited the growth of common bean by significantly reducing ($P<0.05$) the length, surface area, volume, and diameter of common bean sprouts compared with CK (W). However, the exogenous melatonin alleviated the effects of salt stress on the common bean sprouts, as shown by the significant increase ($P<0.05$) in length, surface area, volume, and diameter of common bean sprouts under the M+S treatment. Collectively, these results illustrate that exogenous melatonin can enhance the phenotype of bean sprouts under salt stress.

The quality assessments of the RNA-Seq samples are shown in Table S3. The Q20 values of each sample were all greater than 94.93%, while the Q30 values were all greater than 88.21%. Moreover, the sequencing error rates of all samples were less than 0.02%, indicating that the samples were suitable for subsequent analysis. The raw data generated by RNA-Seq analysis have been uploaded to the NCBI (National Center for Biotechnology Information) database, with the accession number PRJNA603150.

A total of 217 DEGs were identified between S and M+S treatments based on log2FC≥1 and FDR<0.01, among which 150 genes were up-regulated (Table S4) while 67 were down-regulated (Table S5). We subjected these 217 DEGs to GO and KEGG enrichment analysis and found that five GO terms were significantly enriched (*Corrected P_Value<0.05*), among which three terms (GO:0009664, GO:0071669, and GO:0005199) were related to cell wall biosynthesis (Table 1). Therefore, four DEGs (*Phvul.004G098300*, *Phvul.007G002400*, *Phvul.007G099700*, and *Phvul.008G003200*) enriched in these cell wall-related GO terms were selected for qRT-PCR analysis, which the detailed information had been shown in Table S6. The results showed a significant change in the relative expression of these four DEGs, further illustrating the accuracy of the RNA-Seq data and the possible involvement of the cell wall-related terms in response to exogenous melatonin application (Figure 2).

In KEGG analysis, the "Plant-pathogen interaction (pvu04626)" pathway was the most enriched, with an enrichment P-value of 0.000. Cyclic nucleotide-gated channel and calcium-binding protein, which regulate nitric oxide (NO) for cell wall reinforcement, were the enriched points of this pathway (Figure 3A). Similarly, we selected four DEGs (*Phvul.001G005200*, *Phvul.002G329300*, *Phvul.004G107700*, and *Phvul.008G036200*) enriched in this pathway for qRT-PCR analysis, which the detailed information had been shown in Table S7. The results showed a significant change in the relative expression of these four genes, illustrating the accuracy of the RNA-Seq data. This suggested that the cell wall might be the response point to exogenous melatonin application (Figures 3B–E). The results of GO and KEGG analysis suggest that the cell wall may respond to melatonin and enhance salt stress in common bean.

Regulation of melatonin and cell wall-related responses in the germplasm resources

To determine whether exogenous melatonin enhances the salt tolerance in common bean, we analyzed its change rate at the sprouting stage of the common bean under salt stress by measuring the length and diameter of the sprouts. The change rate of melatonin under salt stress ranged from -0.0739 to 2.5988, as calculated from the length of the sprouts (Table S8). We found that 79 germplasm resource materials were positively regulated ($P<0.05$), while five were negatively regulated by melatonin. The remaining 41 materials showed insignificant regulation by melatonin. The positive regulation rate of melatonin by the measured sprout length was 65.83%, while the change rate of melatonin under salt stress ranged from -0.3348 to 1.5485, as calculated from the diameter of the sprouts (Table S9). Furthermore, 85 materials were positively regulated ($P<0.05$). The positive regulation rate of melatonin by the measured sprout

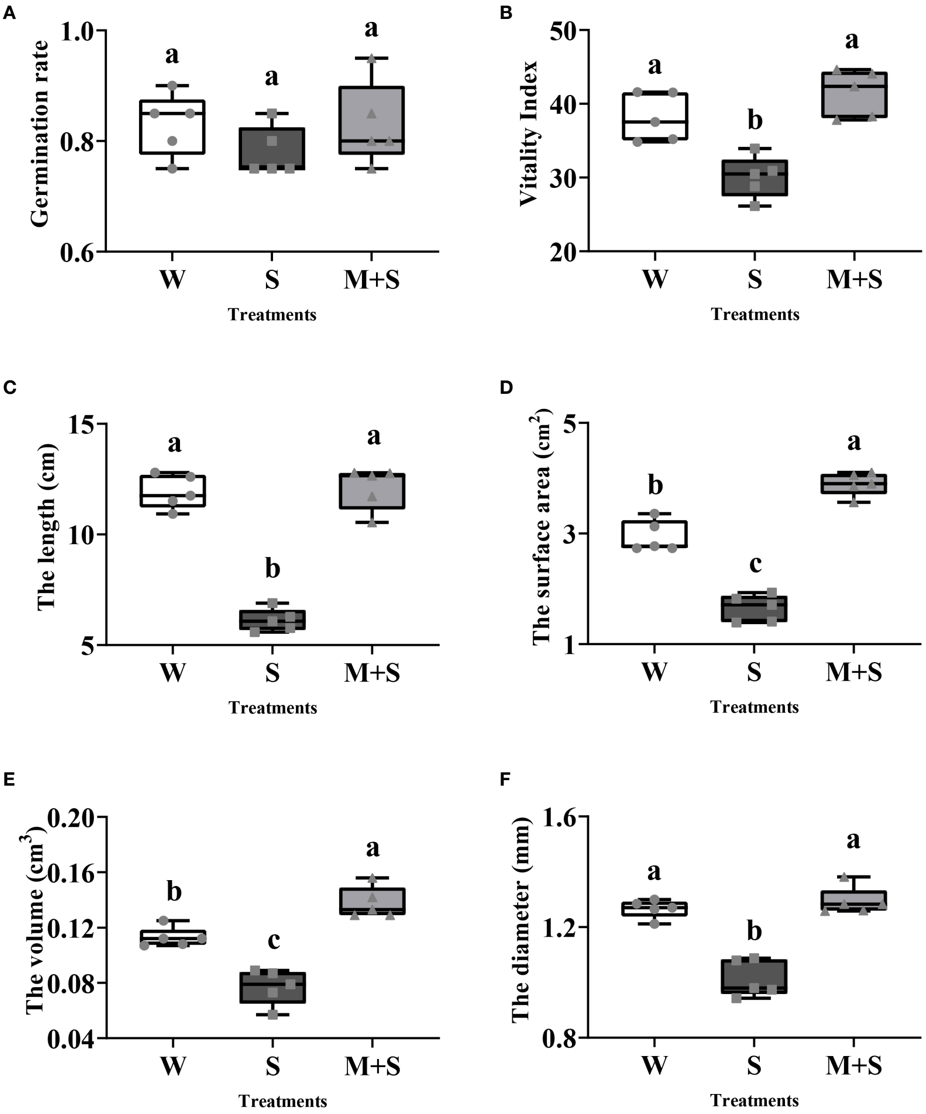


FIGURE 1
Phenotypic analysis of the common bean under W (water), S (salt), and M+S (melatonin with salt) treatments at the sprouting stage. Different lowercase letters in the same column indicate significant differences between the treatments (*P<0.05*). Each gray point represents the mean value of the technical replicates. **(A)** Germination rate; **(B)** Vitality index; **(C)** Length; **(D)** Surface area; **(E)** Volume; **(F)** Diameter.

diameter was 70.83% (Figure 4). Altogether, these findings showed that the positive regulation rate of melatonin was more than 65%, suggesting that most varieties of common bean could have a positive response to melatonin.

To determine whether the cell wall is a regulatory pathway for melatonin processing, we selected 47 materials responding positively to the exogenous melatonin under salt stress for qRT-PCR analysis. S treatment served as the control. Four cell wall-related DEGs in GO terms and four DEGs enriched in the "Plant-pathogen interaction (pvu04626)" pathway by KEGG were selected for the analysis.

In the cell wall-related GO terms, the max $\log_2$FC value of *Phvul.004G098300* was 6.160 while the values of 35 materials were greater than one, with a positive response rate of 74.5%; The $\log_2$FC values of 38 materials were greater than one in the expression of *Phvul.007G002400*, with a positive response rate of 80.9%; Moreover, the expression of *Phvul.007G099700* showed that 33 materials had $\log_2$FC values greater than one and a positive response rate of 70.2%; while in *Phvul.008G003200*, the positive response rate with $\log_2$FC values greater than one was 74.5% (Figure 5).

In KEGG, the $\log_2$FC value of *Phvul.001G005200* ranged from 0.067 to 5.787: $\log_2$FC value of 22 materials was greater

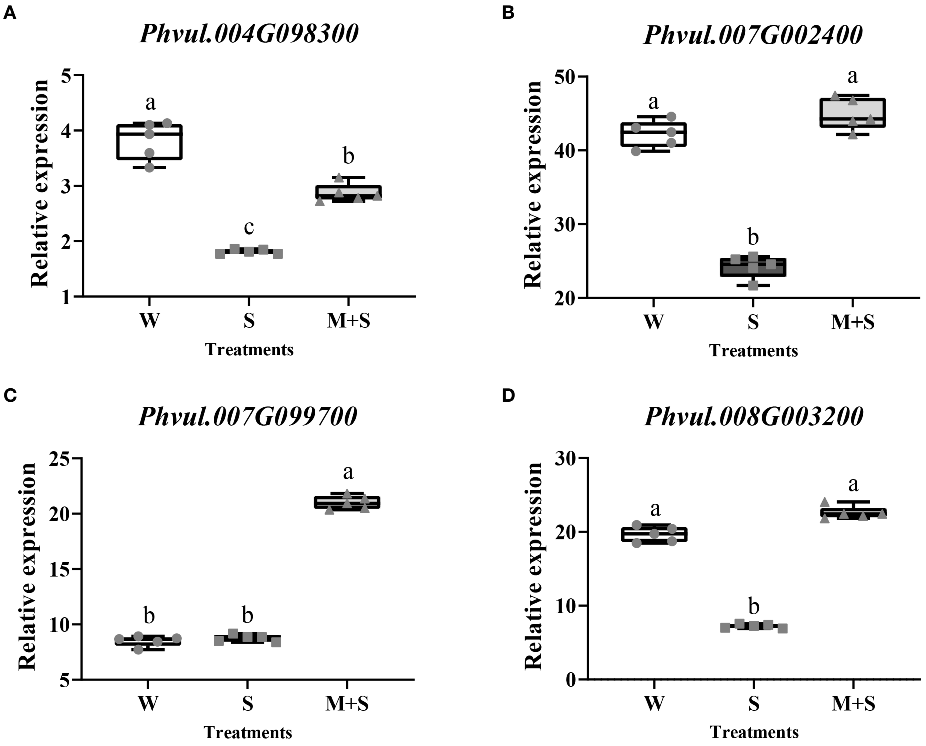


FIGURE 2
Gene expression analysis of the cell wall-related GO terms enriched in the common bean under W (water: CK), S (salt), and M+S (melatonin with salt) treatments at sprouting stage. Different lowercase letters in the same column indicate significant differences between the treatments (P<0.05). **(A)** Expression analysis of the *Phvul.004G098300*. **(B)** Expression analysis of the *Phvul.007G002400*. **(C)** Expression analysis of the *Phvul.007G099700*. **(D)** Expression analysis of the *Phvul.008G003200*.

than one, and the positive response rate was 46.8%; $\log_2$FC value of 33 materials of *Phvul.002G329300* was greater than one with a positive response rate of 70.2%, the positive response rate of *Phvul.004G107700* was also 70.2%; $\log_2$FC value of 31 materials of *Phvul.008G036200* was greater than one, with the positive response rate of 66.0% (Figure 6). The expression data of the DEGs enriched in GO and KEGG showed that various DEGs had different positive response rates. More than half of the genes responded to melatonin. Altogether, these results indicate that the cell wall is an essential pathway in response to melatonin and enhances the salt tolerance of common bean at the sprouting stage.

Construction and selection of markers

MISA locus was identified, and the markers were developed based on the common bean reference genome (PhaVulg1_0) from the ensemble database. A total of 135,015 molecular markers were developed, and linkage group 3 (LG3) contained the largest number of molecular markers (15,471). Additionally, eight LGs had more than 10,000 molecular markers (Table S10). The locations of DEGs in cell wall-related GO terms and the "Plant-pathogen interaction" pathway were selected as conditions using the 2000 bp interval adjacent to the DEGs. We screened 25 pairs of

TABLE 1 The GO enrichment analysis of DEGs.

GO_ID	Description	Term_type	Corrected P_Value
GO:0009664	Plant-type cell wall organization	Biological_process	0.0011
GO:0071669	Plant-type cell wall organization or biogenesis	Biological_process	0.0011
GO:0005199	Structural constituent of cell wall	Molecular_function	0.0050
GO:0030623	U5 snRNA binding	Molecular_function	0.0145
GO:0017069	SnRNA binding	Molecular_function	0.0156

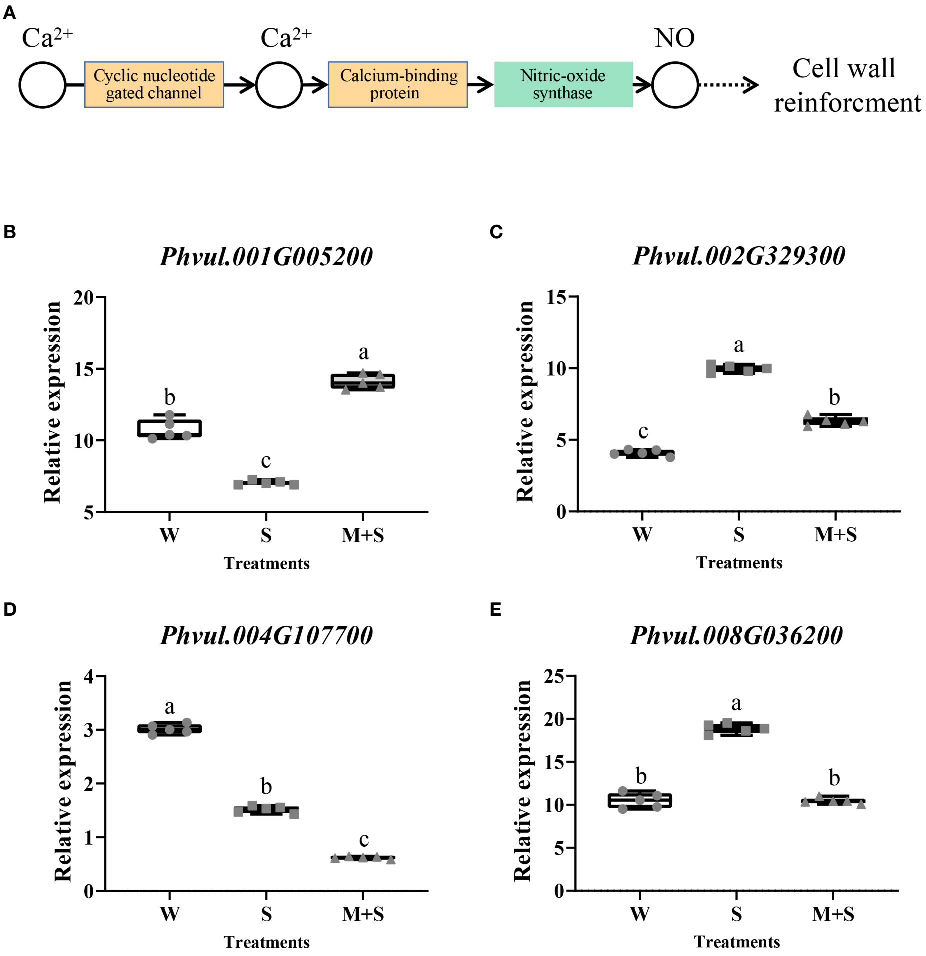


FIGURE 3
Analysis of the "Plant-pathogen interaction (pvu04626)" pathway and its related genes enriched in the KEGG pathway of the common bean under W (water: CK), S (salt), and M+S (melatonin with salt) treatments. Different lowercase letters in the same column indicate significant differences between the treatments (P<0.05). **(A)** "Plant-pathogen interaction" pathway; the brown part represents the enriched DEGs points. **(B)** Expression analysis of *Phvul.004G098300*. **(C)** Expression analysis of *Phvul.007G002400*. **(D)** Expression analysis of *Phvul.007G099700*. **(E)** Expression analysis of *Phvul.008G003200*.

markers (Table S11) and found ten makers with polymorphisms that could be used for trait association analysis in response to exogenous melatonin. Furthermore, we selected 40 materials with or without positive melatonin regulation for association analysis and recorded the data as melatonin-responsive or non-melatonin-responsive phenotypes. The PCR-Acrylamide analysis of the ten makers was also recorded using different codes. The results showed that two markers: *Phvul.001G005200 (1)* and *Phvul.007G099700(2)*, had a significant association in response to melatonin ($P<0.05$), and could be used to predict whether common bean materials interact with exogenous melatonin under salt stress (Table 2).

Discussion

Salt stress can cause excessive accumulation of ions, increase osmotic stress, stimulate overproduction of reactive oxygen species (ROS) and cause toxic effects in plants, thus inhibiting plant growth (Ubbens et al., 2018; Zhao et al., 2021). In this study, phenotypic indicators (such as length, surface area, volume, and diameter) revealed that salt stress inhibited the sprouting of common bean. Similarly, salt stress significantly inhibited primary radicle elongation ($P<0.05$) in maize (*Zea Mays*) at the sprouting stage (Li et al., 2021a). In common bean (*P. vulgaris*), the length, surface area, volume, and lateral root were reduced under salt stress compared with the control

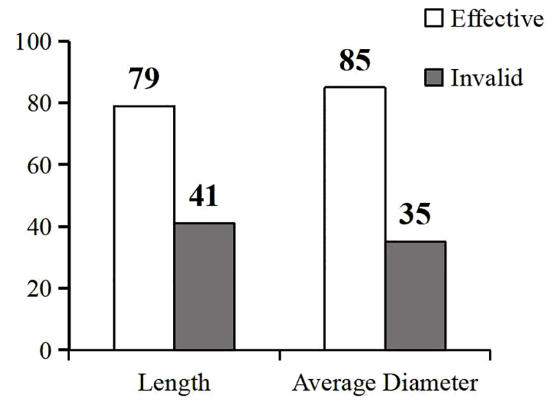


FIGURE 4
Validity analysis of exogenous melatonin in the common bean germplasm resources under salt stress. The gray columns represent the valid resources, while the white columns are the invalid resources.

treatment (Zhang et al., 2021b). Salt stress also restricted the growth of Chinese Cabbage (*Brassica rapa*) by reducing the fresh weight and leaf area (Li et al., 2021b). Exogenous application of melatonin can be implemented to enhance plant tolerance to abiotic stress (Sun et al., 2021). Exogenous melatonin enhanced salt tolerance of *Arabidopsis thaliana* (Chen et al., 2017), orange (*Citrus aurantium*) (Kostopoulou et al., 2015), rice (*Oryza sativa*) (Li et al., 2017b), sweet potato (*Ipomoea batatas*) (Yu et al., 2018), tomato (*Solanum lycopersicum*) (Zhou et al., 2016), and watermelon (*Citrullus lanatus*) (Li et al., 2017a). In this study, phenotypic indicators of common bean seedlings were assessed after the seedlings were exposed to M+S treatment. The results showed that exogenous melatonin alleviated the growth inhibition caused by salt stress at the sprouting stage. This was consistent with previous studies on other plants (Zhan et al., 2019).

In this study, three GO terms related to the cell wall were identified by RNA-Seq, including plant-type cell wall tissue (GO:0009664), plant-type cell wall tissue, or related biomass synthesis (GO:0071669), and structural components of cell walls (GO:0005199) (Table 1). The "Plant-pathogen interaction" pathway (pvu04626) was the most enriched in KEGG analysis. The enriched DEGs suggested its role in the cell wall reinforcement (Figure 3A). Notably, qRT-PCR analysis of cell wall-related genes showed similar trends. Collectively, these results show that exogenous melatonin regulates salt stress by interacting with the genes related to the cell wall pathway. The cell wall has also been demonstrated to participate in salt stress (Endler et al., 2015). Under salt stress, the cell wall acts as the first barrier, which senses and initiates defense responses to increase plant salt tolerance (Zhao et al., 2021). Cell wall biosynthesis, especially remodeling under stress, is a common response to environmental changes (Endler et al., 2015). Several genes related to cell wall are regulated under stress (Zhao et al., 2020). The degree to which the cell wall responds to stress may affect the salt tolerance of plants (Zhang et al., 2021a). Various components of the cell wall may play a part in salt stress. For example, mutation in the genes involved in cellulose synthesis in the cell wall affects salt tolerance (Zhu et al., 2010). Also, proteins related to cellulose synthesis have been shown to accumulate in compartments in response to salt stress (Endler et al., 2015). The pectin content (cell wall composition) of two salt-extreme soya bean cultivars revealed the influence of cell wall on salt tolerance of roots (An et al., 2014). Lignin is thought to be closely related to salt stress. Some enzymes (such as phenylalaninammonialyase) in the lignin biosynthetic pathway were altered after salt treatment (Guo and Wang, 2009; Li et al., 2010). Increasing the strength of the cell wall could increase its ability to capture ions (Zhong and Ye, 2007) and reduce damage to the cell (Sun et al., 2020). The strengthening of the cell wall could increase the compactness of cellulose and change the physicochemical properties (such as pectin) of the cell wall components, which could intercept or adsorb more ions into the cell (Le Gall et al., 2015). Several studies have shown that exogenous melatonin can regulate cell wall tolerance to abiotic stress in plants. In cucumber, cell wall promoted copper ions (Cu^{2+}) chelation, thus reducing their concentration in the cytoplasm and mitigating their harmful effects (Cao et al., 2019). Xie et al. (2020) reported that DEGs were significantly enriched in the GO terms of cell wall biogenesis (GO:0071554) in rice treated with exogenous melatonin under salt stress treatment. Additionally, exogenous melatonin enhanced stress tolerance in radish, and the cell wall-related terms, including cell wall macromolecule catabolic and cell wall macromolecule metabolic processes, were among the enriched pathways (Xu et al., 2020). Exogenous melatonin also increased antioxidant enzymes to regulate ROS and altered cell wall polysaccharides to mitigate Al^{3+} toxicity in wheat (Sun et al., 2020). In watermelon, exogenous melatonin regulated the expression of redox reaction and cell wall-related genes to enhance the tolerance of Cu^{2+} (Hu et al., 2020). Altogether, these studies indicate that exogenous melatonin regulates the cell wall to enhance plant tolerance to abiotic stress.

Although it has been reported that exogenous melatonin enhances abiotic stress tolerance in various crops, its effects vary in different crop varieties (Sun et al., 2020). Han et al. (2022) reported genotypic differences in the effect of exogenous melatonin on salt tolerance in common bean. Specifically, exogenous melatonin significantly enhanced the salt tolerance of some common bean varieties at the sprouting stage, except for the variety Xuliyabai. Similarly, not all common bean germplasm materials responded to melatonin treatment in the present study. Melatonin enhanced salt tolerance in some germplasm materials but did not affect some. The effective response rate to melatonin treatment was 65.83% by sprout length and 70.83% by sprout diameter, all above 65%. We found that most materials responded to exogenous melatonin, which enhanced their salt

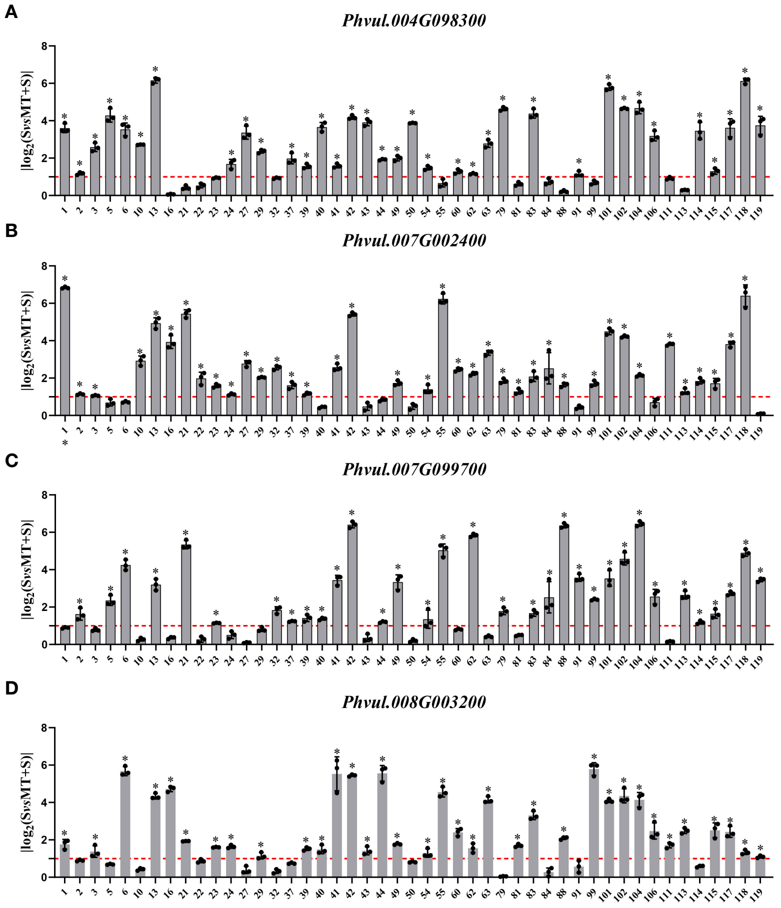


FIGURE 5
Effects of melatonin on the expression of genes associated with the cell wall-related GO terms in common bean germplasm resources under salt stress. The horizontal axis represents the number of resources, while the vertical axis shows the $\log_2$(fold change) values. Log_2(fold change) value 1 (red line) is the critical value showing changes in the expression, while the columns higher than the red line represent the expression level with a significant change ($P<0.05$). * represents genes with significant different expression levels between S (salt) and M+S (melatonin and salt) treatments. **(A)** Expression analysis of *Phvul.004G098300*. **(B)** Expression analysis of *Phvul.007G002400*. **(C)** Expression analysis of *Phvul.007G099700*. **(D)** Expression analysis of *Phvul.008G003200*.

tolerance at the sprouting stage. Regulatory mechanisms by which exogenous melatonin confers salt tolerance in plants differ with plant species and genotypes (Sun et al., 2021). In this study, cell wall-related DEGs were found in “Naihua” common bean variety (GZ-YD014) through RNA-Seq, and 47 positive materials were used to evaluate the regulatory mechanisms associated with these DEGs. The results showed that the regulation rate of all cell wall-related DEGs was more than 46%, indicating that cell walls could interact with exogenous melatonin to enhance common bean salt tolerance at the sprouting stage.

Different varieties of plants exhibit different characteristics (such as plant height and tolerance to abiotic stress). Therefore, it is necessary to develop different types of identification and predictive analytics for practical applications, such as cell membrane stability (CMS) technique and prediction of molecular markers (Pih et al., 1997; Farooq and Azam, 2006). Molecular markers have co-dominance, high reproducibility, high polymorphism, low development cost, and rapidity (Biswas et al., 2020). These features make molecular markers ideal for genetic diversity surveys (Biswas et al., 2018), population structure analysis (Miyatake et al., 2019), genotyping (Chen et al., 2015), linkage mapping (Ambawat et al., 2016), and plant breeding studies. Molecular markers were utilized to identify the leaf- and seed-related traits in perilla (*Perilla frut escens*) (Lim et al., 2021). Also, Gllc 527 marker was used for marker-assisted selection of rust resistance in lentil (*Lens culinaris* Medikus) (Dikshit et al., 2016). However, there are a few reports on the markers associated with plant response to exogenous melatonin. In this study, two pairs of markers were found to be significantly associated with common bean response to exogenous melatonin. These two markers could be used as

TABLE 2 The molecular markers associated with exogenous melatonin response under salt stress.

The name of SSR makers	P-value	Significance
Phvul.001G005200 (1)	0.048	*
Phvul.001G067400 (1)	0.788	NS
Phvul.002G329300 (2)	0.567	NS
Phvul.003G110200 (2)	0.803	NS
Phvul.004G107700 (1)	0.465	NS
Phvul.005G026000 (2)	0.215	NS
Phvul.007G002400 (2)	0.413	NS
Phvul.007G084600 (2)	0.803	NS
Phvul.007G099700 (2)	0.034	*
Phvul.008G031800 (1)	0.917	NS

NS represents no significant difference between the two treatments, and * represents a significant difference between treatments (P<0.05).

candidate markers to determine whether a particular common bean variety can respond to melatonin at the sprouting stage. This study provides useful data for screening of common bean varieties positively responding to exogenous melatonin.

Conclusion

Exogenous melatonin increased the length, surface area, volume, and diameter of common bean sprouts under salt stress. GO, KEGG, RNA-Seq, and qRT-PCR analyses showed that the cell wall pathway was significantly enriched. Additionally, more than 65% of the germplasm materials were positively regulated by melatonin, as was shown by the length and diameter of common bean sprouts. Since the regulation rate of all cell wall-related DEGs was more than 46%, we suggest that

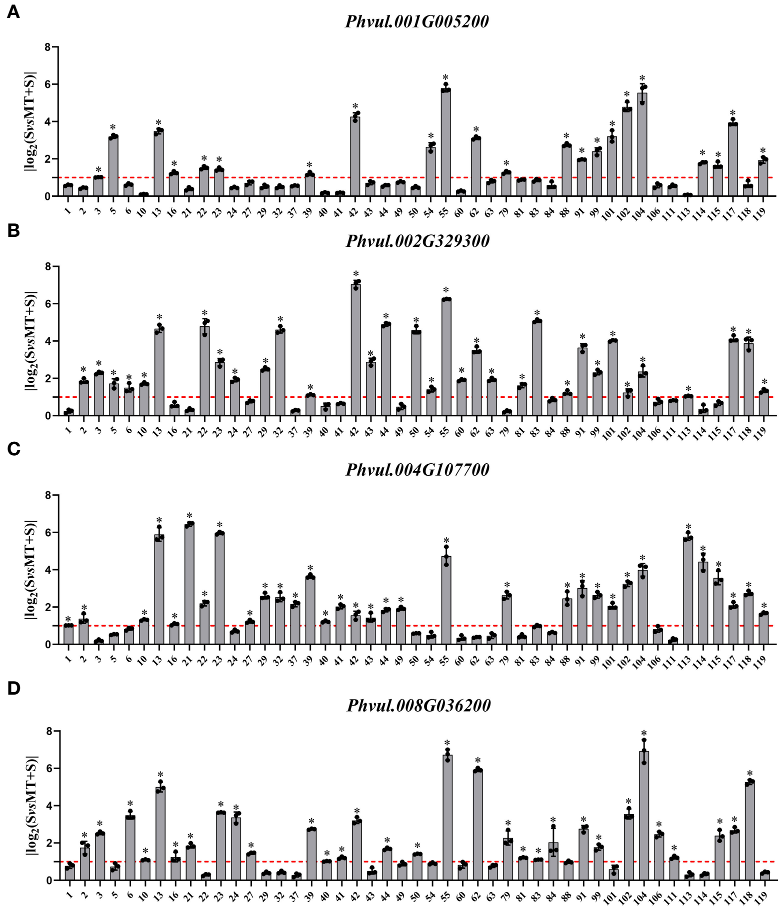


FIGURE 6
Effects of exogenous melatonin on the expression of genes associated with the “Plant-pathogen interaction (pvu04626)” pathway-related KEGG terms in common bean germplasm resources under salt stress. The horizontal axis represents the number of resources, while the vertical axis shows the $\log_2$(fold change) values. Log_2(fold change) value 1 (red line) is the critical value showing changes in the expression, while the columns higher than the red line represent the expression level with a significant change ($P<0.05$). * represents genes with significant different expression levels between S (salt) and M+S (melatonin and salt) treatments. **(A)** Expression analysis of *Phvul.001G005200*. **(B)** Expression analysis of *Phvul.002G329300*. **(C)** Expression analysis of *Phvul.004G107700*. **(D)** Expression analysis of *Phvul.008G036200*.

cell walls might interact with exogenous melatonin to enhance the salt tolerance of the common bean at the sprouting stage. Furthermore, two pairs of makers were found to be associated with melatonin, which could be used as candidate markers for predicting how varieties of common bean respond to melatonin.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

Author contributions

QZ: Data curation and writing original draft. BQ and G-dW: Data curation. W-jZ, XY and ML: Conceptualization and methodology. Z-gY and H-yS: Software; QZ and Y-lD: Formal data analysis and preparation of materials; J-dD, Y-lD and PJ: Conceptualization, data curation, revised the manuscript and funding acquisition. All authors contributed to the article and approved the submitted version.

Acknowledgments

This study was financially supported by the National Key Research and Development Program (2020YFD1001402), the Research Project of Heilongjiang Bayi Agricultural University (XDB2011-02) and University Nursing Program for Young Scholars with Creative Talents in Heilongjiang Province (UNPYSCT-2018086).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2022.1012186/full#supplementary-material

References

Ambawat, S., Senapathy, S., Hash, C. T., Nepolean, T., Rajaram, V., Eshwar, K., et al. (2016). Qtl mapping of pearl millet rust resistance using an integrated dart- and ssr-based linkage map. *Euphytica* 2, 461–476. doi: 10.1007/s10681-016-1671-9

An, P., Li, X., Zheng, Y., Matsuura, A., Abe, J., Eneji, A. E., et al. (2014). Effects of nacl on root growth and cell wall composition of two soya bean cultivars with contrasting salt tolerance. *J. Agron. Crop Sci.* 3, 212–218. doi: 10.1111/jac.12060

Arnao, M. B., and Hernández-Ruiz, J. (2019). Melatonin: A new plant hormone and/or a plant master regulator? *Trends Plant Sci.* 1, 38–48. doi: 10.1016/j.tplants.2018.10.010

Beaver, J. S., and Osorno, J. M. (2009). Achievements and limitations of contemporary common bean breeding using conventional and molecular approaches. *Euphytica* 2, 145–175. doi: 10.1007/s10681-009-9911-x

Biswas, M. K., Darbar, J. N., Borrell, J. S., Bagchi, M., Biswas, D., Nuraga, G. W., et al. (2020). The landscape of microsatellites in the enset (Ensete ventricosum) genome and web-based marker resource development. *Sci. Rep.* 1, 15312. doi: 10.1038/s41598-020-71984-x

Biswas, M. K., Nath, U. K., Howlader, J., Bagchi, M., Natarajan, S., Kayum, Md A., et al. (2018). Exploration and exploitation of novel ssr markers for candidate transcription factor genes in lilium species. *Genes* 2, 97. doi: 10.3390/genes9020097

Borges, A., Tsai, S. M., and Caldas, D. G. (2012). Validation of reference genes for rt-qpcr normalization in common bean during biotic and abiotic stresses. *Plant Cell Rep.* 5, 827–838. doi: 10.1007/s00299-011-1204-x

Cao, Y. Y., Qi, C. D., Li, S., Wang, Z., Wang, X., Wang, J., et al. (2019). Melatonin alleviates copper toxicity *Via* improving copper sequestration and ros scavenging in cucumber. *Plant Cell Physiol.* 3, 562–574. doi: 10.1093/pcp/pcy226

Carpita, N. C., and Gibeaut, D. M. (1993). Structural models of primary cell walls in flowering plants: Consistency of molecular structure with the physical properties of the walls during growth. *Plant J.* 1, 1–30. doi: 10.1111/j.1365-313x.1993.tb00007.x

Chen, H., Qiao, L., Wang, L., Wang, S., Blair, M. W., and Cheng, X. (2015). Assessment of genetic diversity and population structure of mung bean (Vigna radiata) germplasm using est-based and genomic ssr markers. *Gene* 2, 175–183. doi: 10.1016/j.gene.2015.04.043

Chen, Z., Xie, Y., Gu, Q., Zhao, G., Zhang, Y., Cui, W., et al. (2017). The atrbohf-dependent regulation of ros signaling is required for melatonin-induced salinity tolerance in arabidopsis. *Free Radical Biol. Med.* 465–77. doi: 10.1016/j.freeradbiomed.2017.04.009

Chun, H. J., Baek, D., Cho, H. M., Lee, S. H., Jin, B. J., Yun, D.-J., et al. (2019). Lignin biosynthesis genes play critical roles in the adaptation of arabidopsis plants to high-salt stress. *Plant Signaling Behav.* 8, 1625697. doi: 10.1080/15592324.2019.1625697

Dikshit, H. K., Singh, A., Singh, D., Aski, M., Jain, N., Hegde, V. S., et al. (2016). Tagging and mapping of ssr marker for rust resistance gene in lentil (Lens culinaris medikus subsp. culinaris). *Indian J. Exp. Biol.* 54(6), 394–399. https://pubmed.ncbi.nlm.nih.gov/27468466/

Endler, A., Kesten, C., Schneider, R., Zhang, Y., Ivakov, A., Froehlich, A., et al. (2015). A mechanism for sustained cellulose synthesis during salt stress. *Cell* 6, 1353–1364. doi: 10.1016/j.cell.2015.08.028

Fang, S., Hou, X., and Liang, X. (2021). Response mechanisms of plants under saline-alkali stress. *Front. Plant Sci.* 12, 667458. doi: 10.3389/fpls.2021.667458

Farooq, S., and Azam, F. (2006). The use of cell membrane stability (Cms) technique to screen for salt tolerant wheat varieties. *J. Plant Physiol.* 6, 629–637. doi: 10.1016/j.jplph.2005.06.006

Ganesan, K., and Xu, B. (2017). Polyphenol-rich dry common beans (Phaseolus vulgaris l.) and their health benefits. *Int. J. Mol. Sci.* 11, 2331. doi: 10.3390/ijms18112331

Gong, Z. (2021). Plant abiotic stress: New insights into the factors that activate and modulate plant responses. *J. Integr. Plant Biol.* 3, 429–430. doi: 10.1111/jipb.13079

Guo, J., and Wang, M. H. (2009). Characterization of the phenylalanine ammonia-lyase gene (Slpal5) from tomato (Solanum lycopersicum l.). *Mol. Biol. Rep.* 6, 1579–1585. doi: 10.1007/s11033-008-9354-9

Han, Y., Gao, Y., Li, M., Du, Y., Zhang, Y., Zhang, W., et al. (2022). The molecular events underpinning cultivar differences in melatonin counteracting salt damage in phaseolus vulgaris. *Funct. Plant Biol.* 2, 201–217. doi: 10.1071/fp21126

Hu, Z., Fu, Q., Zheng, J., Zhang, A., and Wang, H. (2020). Transcriptomic and metabolomic analyses reveal that melatonin promotes melon root development under copper stress by inhibiting jasmonic acid biosynthesis. *Hortic. Res.* 1, 79. doi: 10.1038/s41438-020-0293-5

Iuchi, S., Kobayashi, M., Taji, T., Naramoto, M., Seki, M., Kato, T., et al. (2001). Regulation of drought tolerance by gene manipulation of 9-Cis-Epoxycarotenoid dioxygenase, a key enzyme in abscisic acid biosynthesis in arabidopsis. *Plant J.* 4, 325–333. doi: 10.1046/j.1365-313x.2001.01096.x

Kostopoulou, Z., Therios, I., Roumeliotis, E., Kanellis, A. K., and Molassiotis, A. (2015). Melatonin combined with ascorbic acid provides salt adaptation in citrus aurantium l. seedlings. *Plant Physiol. Biochem.* 86, 155–165. doi: 10.1016/j.plaphy.2014.11.021

Kvam, V. M., Liu, P., and Si, Y. (2012). A comparison of statistical methods for detecting differentially expressed genes from rna-seq data. *Am. J. Bot.* 2, 248–256. doi: 10.3732/ajb.1100340

Le Gall, H., Philippe, F., Domon, J. M., Gillet, F., Pelloux, J., and Rayon, C. (2015). Cell wall metabolism in response to abiotic stress. *Plants* 1, 112–166. doi: 10.3390/plants4010112

Lerner, AB, Case, JD, Mori, W, and Wright, MR. (1959). Melatonin in peripheral nerve. *Nature*. 183, 1821. doi: 10.1038/1831821a0

Li, C., He, Q., Zhang, F., Yu, J., Li, C., Zhao, T., et al. (2019). Melatonin enhances cotton immunity to verticillium wilt via manipulating lignin and gossypol biosynthesis. *Plant J.* 4, 784–800. doi: 10.1111/tpj.14477

Li, J., Li, Y., Tian, Y., Qu, M., Zhang, W., and Gao, L. (2017a). Melatonin has the potential to alleviate cinnamic acid stress in cucumber seedlings. *Front. Plant Sci.* 8, 1193. doi: 10.3389/fpls.2017.01193

Lim, S. E., Sa, K. J., and Lee, J. K. (2021). Bulk segregant analysis identifies ssr markers associated with leaf- and seed-related traits in perilla crop (Perilla frutescens l.). *Genes Genomics* 4, 323–332. doi: 10.1007/s13258-021-01056-5

Liu, Z., Schneider, R., Kesten, C., Zhang, Y., Somssich, M., Zhang, Y., et al. (2016). Cellulose-microtubule uncoupling proteins prevent lateral displacement of microtubules during cellulose synthesis in arabidopsis. *Dev. Cell* 3, 305–315. doi: 10.1016/j.devcel.2016.06.032

Li, C., Wang, P., Wei, Z., Liang, D., Liu, C., Yin, L., et al. (2012). The mitigation effects of exogenous melatonin on salinity-induced stress in malus hupehensis. *J. Pineal Res.* 3, 298–306. doi: 10.1111/j.1600-079X.2012.00999.x

Li, X. J., Yang, M. F., Chen, H., Qu, L. Q., Chen, F., and Shen, S. H. (2010). Abscisic acid pretreatment enhances salt tolerance of rice seedlings: Proteomic evidence. *Biochim. Biophys. Acta* 4, 929–940. doi: 10.1016/j.bbapap.2010.01.004

Li, P.-c., Yang, X.-y., Wang, H.-m., Ting, P. A. N., YANG, J.-y., Wang, Y.-y., et al. (2021b). Metabolic responses to combined water deficit and salt stress in maize primary roots. *J. Integr. Agric.* 12, 683891. doi: 10.1016/S2095-3119(20)63242-7

Li, X., Yu, B., Cui, Y., and Yin, Y. (2017b). Melatonin application confers enhanced salt tolerance by regulating na+ and cl− accumulation in rice. *Plant Growth Regul.* 3, 441–454. doi: 10.1007/s10725-017-0310-3

Li, N., Zhang, Z., Chen, Z., Cao, B., and Xu, K. (2021a). Comparative transcriptome analysis of two contrasting Chinese cabbage (Brassica rapa l.) genotypes reveals that ion homeostasis is a crucial biological pathway involved in the rapid adaptive response to salt stress. *Front. Plant Sci.* 12, 683891. doi: 10.3389/fpls.2021.683891

Miyatake, K., Shinmura, Y., Matsunaga, H., Fukuoka, H., and Saito, T. (2019). Construction of a core collection of eggplant (Solanum melongena l.) based on genome-wide snp and ssr genotypes. *Breed Sci.* 3, 498–502. doi: 10.1270/jsbbs.18202

Monniaux, M., and Hay, A. (2016). Cells, walls, and endless forms. *Curr. Opin. Plant Biol.* 34, 114–121. doi: 10.1016/j.pbi.2016.10.010

Peng, J. H., and Lapitan, N. L. (2005). Characterization of est-derived microsatellites in the wheat genome and development of essr markers. *Funct. Integr. Genomics* 2, 80–96. doi: 10.1007/s10142-004-0128-8

Pih, K. T., Jang, H. J., Kang, S. G., Piao, H. L., and Hwang, I. (1997). Isolation of molecular markers for salt stress responses in arabidopsis thaliana. *Mol. Cells* 4, 567–571. doi: 10.1016/S0966-842X(97)01108-6

Popelka, J.C., Terryn, N., and Higgins, T. J. V. (2004). Gene technology for grain legumes: Can it contribute to the food challenge in developing countries? *J. Plant Sci.* 2, 195–206. doi: 10.1016/j.plantsci.2004.03.027

Prasad, M., Kumar, N., Kulwal, P. L., Röder, M. S., Balyan, H. S., Dhaliwal, H. S., et al. (2003). Qtl analysis for grain protein content using ssr markers and validation studies using Nils in bread wheat. *Theor. Appl. Genet.* 4, 659–667. doi: 10.1007/s00122-002-1114-y

Qu, J., and Liu, J. (2013). A genome-wide analysis of simple sequence repeats in maize and the development of polymorphism markers from next-generation sequence data. *BMC Res. Notes* 403, 403. doi: 10.1186/1756-0500-6-403

Rodriguez, C., Mayo, J. C., Sainz, R. M., Antolín, I., Herrera, F., Martín, V., et al. (2004). Regulation of antioxidant enzymes: A significant role for melatonin. *J. Pineal Res.* 1, 1–9. doi: 10.1046/j.1600-079x.2003.00092.x

Shabala, S. (2013). Learning from halophytes: Physiological basis and strategies to improve abiotic stress tolerance in crops. *Ann. Bot.* 7, 1209–1221. doi: 10.1093/aob/mct205

Sun, C., Liu, L., Wang, L., Li, B., Jin, C., and Lin, X. (2021). Melatonin: A master regulator of plant development and stress responses. *J. Integr. Plant Biol.* 1, 126–145. doi: 10.1111/jipb.12993

Sun, C., Lv, T., Huang, L., Liu, X., Jin, C., and Lin, X. (2020). Melatonin ameliorates aluminum toxicity through enhancing aluminum exclusion and reestablishing redox homeostasis in roots of wheat. *J. Pineal Res.* 4, e12642. doi: 10.1111/jpi.12642

Trigiano, R. N., and Caetano-Anolles, G. (1998). Laboratory exercises on DNA amplification fingerprinting for evaluating the molecular diversity of horticultural species. *HortTechnology* 3, 413–423. doi: 10.21273/HORTTECH.8.3.413

Ubbens, J., Cieslak, M., Prusinkiewicz, P., and Stavness, I. (2018). The use of plant models in deep learning: An application to leaf counting in rosette plants. *Plant Methods* 6, eCollection. doi: 10.1186/s13007-018-0273-z

Van Zelm, E., Zhang, Y., and Testerink, C. (2020). Salt tolerance mechanisms of plants. *Annu. Rev. Plant Biol.* 71, 403–433. doi: 10.1146/annurev-arplant-050718-100005

Wang, H., Takano, T., and Liu, S. (2018). Screening and evaluation of saline–alkaline tolerant germplasm of rice (Oryza sativa l.) in soda saline–alkali soil. *Agronomy* 10, 205. doi: 10.3390/agronomy8100205

Xie, Z., Wang, J., Wang, W., Wang, Y., Xu, J., Li, Z., et al. (2020). Integrated analysis of the transcriptome and metabolome revealed the molecular mechanisms underlying the enhanced salt tolerance of rice due to the application of exogenous melatonin. *Front. Plant Sci.* 11, 618680. doi: 10.3389/fpls.2020.618680

Xu, L., Zhang, F., Tang, M., Wang, Y., Dong, J., Ying, J., et al. (2020). Melatonin confers cadmium tolerance by modulating critical heavy metal chelators and transporters in radish plants. *J. Pineal Res.* 1, e12659. doi: 10.1111/jpi.12659

Yu, Y., Wang, A., Li, X., Kou, M., Wang, W., Chen, X., et al. (2018). Melatonin-stimulated triacylglycerol breakdown and energy turnover under salinity stress contributes to the maintenance of plasma membrane h(+)-atpase activity and K (+)/Na(+) homeostasis in sweet potato. *Front. Plant Sci.* 256. doi: 10.3389/fpls.2018.00256

Zhang, B., Gao, Y., Zhang, L., and Zhou, Y. (2021a). The plant cell wall: Biosynthesis, construction, and functions. *J. Integr. Plant Biol.* 1, 251–272. doi: 10.1111/jipb.13055

Zhang, M., He, S., Zhan, Y., Qin, B., Jin, X., Wang, M., et al. (2019). Exogenous melatonin reduces the inhibitory effect of osmotic stress on photosynthesis in soybean. *PloS One* 12, e0226542. doi: 10.1371/journal.pone.0226542

Zhang, Q., Li, M., Xia, C. Y., Zhang, W. J., Yin, Z. G., Zhang, Y. L., et al. (2021b). Transcriptome-based analysis of salt-related genes during the sprout stage of common bean (Phaseolus vulgaris) under salt stress conditions. *Biotechnol. Biotechnol. Equip.* 1, 1086–1098. doi: 10.1080/13102818.2021.1954091

Zhang, Q., Zhang, W.-j., Yin, Z.-g., Li, W.-j., Xia, C.-Y., Sun, H.-Y., et al. (2021c). Genome-wide identification reveals the potential functions of the bzip gene family in common bean (Phaseolus vulgaris) in response to salt stress during the sprouting stage. *J. Plant Growth Regul.*, 1–16. doi: 10.1007/s00344-021-10497-x

Zhang, Q., Zhang, W. J., Yin, Z. G., Li, W. J., Zhao, H. H., Zhang, S., et al. (2020). Genome- and transcriptome-wide identification of C3hs in common bean (Phaseolus vulgaris l.) and structural and expression-based analyses of their functions during the sprout stage under salt-stress conditions. *Front. Genet.* 11, 564607. doi: 10.3389/fgene.2020.564607

Zhan, H., Nie, X., Zhang, T., Li, S., Wang, X., Du, X., et al. (2019). Melatonin: A small molecule but important for salt stress tolerance in plants. *Int. J. Mol. Sci.* 3, 709. doi: 10.3390/ijms20030709

Zhao, D., Luan, Y., Shi, W., Tang, Y., Huang, X., and Tao, J. (2022). Melatonin enhances stem strength by increasing the lignin content and secondary cell wall thickness in herbaceous peony. *J. Exp. Bot* 18, erac165. doi: 10.1093/jxb/erac165

Zhao, S., Zhang, Q., Liu, M., Zhou, H., Ma, C., and Wang, P. (2021). Regulation of plant responses to salt stress. *Int. J. Mol. Sci.* 9, 4609. doi: 10.3390/ijms22094609

Zhao, C., Zhang, H., Song, C., Zhu, J. K., and Shabala, S. (2020). Mechanisms of plant responses and adaptation to soil salinity. *Innovation* 1, 100017. doi: 10.1016/j.xinn.2020.100017

Zhao, H., Zhang, K., Zhou, X., Xi, L., Wang, Y., Xu, H., et al. (2017). Melatonin alleviates chilling stress in cucumber seedlings by up-regulation of Cszat12 and modulation of polyamine and abscisic acid metabolism. *Sci. Rep.* 1, 4998. doi: 10.1038/s41598-017-05267-3

Zhong, R., and Ye, Z. H. (2007). Regulation of cell wall biosynthesis. *Curr. Opin. Plant Biol.* 6, 564–572. doi: 10.1016/j.pbi.2007.09.001

Zhou, X., Zhao, H., Cao, K., Hu, L., Du, T., Baluška, F., et al. (2016). Beneficial roles of melatonin on redox regulation of photosynthetic electron transport and synthesis of D1 protein in tomato seedlings under salt stress. *Front. Plant Sci.* 7, 1823. doi: 10.3389/fpls.2016.01823

Zhu, J. K. (2002). Salt and drought stress signal transduction in plants. *Annu. Rev. Plant Biol.* 53, 247–273. doi: 10.1146/annurev.arplant.53.091401.143329

Zhu, J., Lee, B. H., Dellinger, M., Cui, X., Zhang, C., Wu, S., et al. (2010). A cellulose synthase-like protein is required for osmotic stress tolerance in arabidopsis. *Plant J.* 1, 128–140. doi: 10.1111/j.1365-313X.2010.04227.x

frontiers | Frontiers in Plant Science

TYPE Original Research
PUBLISHED 05 December 2022
DOI 10.3389/fpls.2022.1069143

OPEN ACCESS

EDITED BY
Vijay Gahlaut,
Institute of Himalayan Bioresource Technology (CSIR), India

REVIEWED BY
Anil Kumar Singh,
Academy of Scientific and Innovative Research (AcSIR), India
Vijay K. Singh,
Massachusetts General Hospital and Harvard Medical School, United States

*CORRESPONDENCE
Gudipalli Padmaja
gudipallipadmaja@gmail.com

†These authors have contributed equally to this work

SPECIALTY SECTION
This article was submitted to Plant Abiotic Stress, a section of the journal Frontiers in Plant Science

RECEIVED 13 October 2022
ACCEPTED 17 November 2022
PUBLISHED 05 December 2022

CITATION
Shreya S, Supriya L and Padmaja G (2022) Melatonin induces drought tolerance by modulating lipoxygenase expression, redox homeostasis and photosynthetic efficiency in *Arachis hypogaea* L. *Front. Plant Sci.* 13:1069143.
doi: 10.3389/fpls.2022.1069143

Melatonin induces drought tolerance by modulating lipoxygenase expression, redox homeostasis and photosynthetic efficiency in *Arachis hypogaea* L.

Sharma Shreya†, Laha Supriya† and Gudipalli Padmaja*

Department of Plant Sciences, School of Life Sciences, University of Hyderabad, Hyderabad, TG, India

Melatonin (N-acetyl-5-hydroxy tryptamine), a multipotent biomolecule is well known for its ability to confer tolerance to several abiotic and biotic stresses. The regulation of melatonin-mediated drought tolerance in drought-distinguished varieties can be different due to discriminating redox levels. The present study was focused on assessing the effects of melatonin priming against polyethylene glycol (PEG)-induced stress with respect to the antioxidant system, photosynthetic parameters, lipoxygenase expression, JA and ABA levels in drought-sensitive (Kadiri-7) and drought-tolerant (Kadiri-9) varieties. Exogenous melatonin alleviated the drought stress effects in sensitive variety (Kadiri-7) by increasing the endogenous melatonin content with an improved antioxidant system and photosynthetic attributes. The primed stressed plants of the sensitive variety exhibited reduced expression and activity of the chlorophyll degrading enzymes (Chl-deg PRX, pheophytinase and chlorophyllase) with a concomitant increase in chlorophyll content in comparison to unprimed controls. Interestingly, melatonin priming stimulated higher expression and activity of lipoxygenase (LOX) as well as enhanced the expression of genes involved in the synthesis of jasmonic acid (JA) including its content in drought stressed plants of the sensitive variety. The expression of *NCED3* (involved in ABA-biosynthesis) was upregulated while *CYP707A2* (ABA-degradation) was downregulated which corresponded with higher ABA levels. Contrastingly, priming caused a decrease in endogenous melatonin content under drought stress in tolerant variety (Kadiri-9) which might be due to feedback inhibition of its synthesis to maintain intracellular redox balance and regulate better plant metabolism. Furthermore, the higher endogenous melatonin content along with improved antioxidant system, photosynthetic efficiency and LOX expression associated with the increased levels of JA and ABA in unprimed stressed plants of the tolerant variety (Kadiri-9) is pointing towards the effectiveness of melatonin in mediating drought stress tolerance. Overall, exogenous melatonin alleviated the adverse effects of drought stress in sensitive variety while having no add-on effect on drought stress responses of tolerant variety which is inherently equipped to withstand the given duration of drought stress treatment.

KEYWORDS

antioxidants, drought stress, groundnut, lipoxygenase, melatonin, photosynthesis

1 Introduction

The non-availability of water to plants is a major concern for mankind and also for the whole ecosystem. Global temperature rise and anthropogenic activities including industrialization, deforestation and urbanization affect rainfall patterns (Warner and Afifi, 2014) leading to water scarcity. Drought stress, one of the most limiting factors in agriculture, affects the yield and quality of the crops (Stagnari et al., 2016). Groundnut, an economically important legume crop has attained global importance due to its edible seeds and oil-producing capability (Stalker, 1997). India, the second largest producer of groundnut in the world is estimated to experience a fall in production in 2022 (https://www.statista.com/statistics/769471/india-groundnut-production-volume). Among the various factors that affect its production, abiotic stress especially aridity is a major factor responsible for the decline in its yield worldwide (Carvalho et al., 2017). Therefore, improving the ability of groundnut to withstand drought stress is a primary concern for researchers.

Drought stress adversely affects the growth and development of groundnut plants by interfering with various morphological, physiological and biochemical processes. Lower leaf water potential, turgor pressure with decreased stomatal conductance, photosynthetic and transpiration rate under drought affects the pod development (Reddy et al., 2003). In addition, exposure to drought stress results in the production of reactive oxygen intermediates (ROIs) from photorespiration, photosynthetic apparatus and mitochondrial respiration. The over-accumulation of reactive oxygen species (ROS) in the cells causes oxidative damage by disruption of cellular integrity, inactivation of enzymes and protein oxidation (Mittler, 2002). Plants have developed several defense systems to cope with this oxidative condition *viz.*, non-enzymatic and enzymatic antioxidant pathways (Jung, 2004; Ajithkumar and Panneerselvam, 2014), which scavenge the ROS molecules to maintain cellular redox homeostasis. It is well known that environmental stresses also lead to an increase in malondialdehyde (MDA) which is a lipid peroxidation product, and compatible solutes such as proline and glutamate, which are used as stress markers in plants (Loggini et al., 1999; Fu and Huang, 2001; Ajithkumar and Panneerselvam, 2014).

Various approaches *viz.*, conventional breeding, molecular breeding and genetic manipulation using recombinant DNA technologies have been used to develop drought tolerant varieties in different crop plants. In addition to the above, seed priming with natural and synthetic compounds has proved to be beneficial in improving drought stress tolerance (Jisha et al., 2013). Recent studies suggest that priming of seeds conferred better stress tolerance against adverse environmental conditions by retaining stress memory thereby enabling protection against oxidative stress (Jisha et al., 2013; Ibrahim, 2016; Farooq et al., 2019). Groundnut seeds primed with gibberellic acid exhibited higher antioxidant activity, proline, lesser malondialdehyde and higher chlorophyll content under salt stress (Erbil, 2021). Zinc and iron priming significantly enhanced the yield of field-grown groundnut (Khan et al., 2017). Seed priming with several agents (gibberellic acid, hydrogen peroxide, salicylic acid, ascorbic acid and so on) improved the salinity tolerance of the groundnut plants (Pal et al., 2017).

Melatonin, an amphipathic molecule was first discovered by Lerner et al. (1958) as a skin-lightening agent in animals. It has broadened its horizons to the plant arena due to its ubiquitous nature. In 1993, melatonin was discovered to be a powerful antioxidant and free radical scavenger (Tan, 1993). Melatonin is known to scavenge ROS and reactive nitrogen species (RNS) which are detrimental to the plant system. Exogenous melatonin was found to improve several abiotic stress responses *viz.*, salinity, drought, high/low temperature and so on (Debnath et al., 2019). Various studies have also shown a significant role of melatonin in promoting physiological processes under abiotic stresses. Melatonin exhibits a wide variety of effects including enhancement of germination to delayed senescence of plants. Zhang et al. (2020) reported that melatonin improved the germination parameters of seeds and promoted the radicle growth by increasing the antioxidant enzyme activity, balancing cellular osmotic potential and reducing lipid membrane peroxidation against PEG-induced water stress in soybean. Furthermore, transcriptome analysis of melatonin treated *Arabidopsis* revealed altered expression of several defense-related genes indicating its key role in plant defense against various environmental stresses (Weeda et al., 2014).

Transgenic plants overexpressing melatonin biosynthesis-related genes have been reported to exhibit delayed leaf senescence (Huangfu et al., 2022).

Phyto-oxylipins, are the products of unsaturated fatty acid metabolism, and its derivatives (*viz.*, JA) are well known to actively participate in plant defense mechanisms (Blée, 2002). Lipoxygenases (LOXs) are a group of enzymes that catalyze the oxylipin formation from polyunsaturated fatty acids (linoleic and linolenic acid) in plants (Blée, 2002; Porta and Rocha-Sosa, 2002). They play important roles in plant development and tolerance to abiotic and biotic stresses (Chen et al., 2022; Mou et al., 2022). LOXs potentially participate in signaling caused by stressor effects and are considered as a stress biomarkers against biotic and abiotic stresses (Singh et al., 2022). Lim et al. (2015) reported that *CaLOX*-overexpressing *Arabidopsis* plants exhibited tolerance phenotypes to drought and high salt stresses *via* rapid scavenging of ROS and by inducing high expression of ABA (abscisic acid) and stress-responsive marker genes. Similarly, the overexpression of *MdLOX* enhanced the salt tolerance in apple calli, and its heterologous expression increased ROS scavenging capacity in Arabidopsis (Chen et al., 2022). Hou et al. (2015) found that *DkLOX3* overexpression in Arabidopsis caused leaf senescence with more lipid peroxidation and ROS accumulation under normal conditions, whereas exposure of such overexpressing lines to high salinity and drought caused a decrease in the accumulation of ROS. It was thus suggested that the participation of LOX in defense pathways is versatile and complicated.

The relationship between melatonin, LOX, JA and ABA is still elusive due to contrasting reports. Luo et al. (2022) reported that alleviation of the alkaline stress by exogenous melatonin in rice seedlings was associated with a decrease in LOX activity and malondialdehyde content. Similarly, the study of Hu et al. (2020) revealed that the protective effects of exogenous melatonin on lateral root formation in response to copper stress in melon seedlings were due to decreased ROS damage as a consequence of reduced expression of LOX-related genes and JA levels. Alharbi et al. (2021) observed that melatonin application alleviated the salt-stress-induced decline in growth by decreasing ROS levels, lipid peroxidation, electrolyte leakage and lipoxygenase activity in seedlings of *Glycine max* (L.). Intriguingly, Radogna et al. (2009) showed the pro-radical effects of melatonin mediated by LOX as they were prevented by a set of LOX inhibitors. Recently, Guo et al. (2022) showed that melatonin induced the accumulation of hydrogen peroxide (H_2O_2), accompanied by upregulation of melon respiratory burst oxidase homolog D (*CmRBOHD*) and $(Ca^{2+})_{cyt}$ signaling to offset ABA action to delay leaf senescence in melon (*Cucumis melo* L.).

LOX performs a crucial role in the synthesis of jasmonates, which are involved in diverse physiological processes including plants' stress tolerance (Ahmad et al., 2016). LOX-induced JA in turn activates MYC2, a positive regulatory transcription factor for the expression of the anti-oxidant defense genes (Ruan et al., 2019). Xing et al. (2020) showed that *CmLOX10* positively regulates drought tolerance through JA-mediated stomatal closure in oriental melon (*Cucumis melo* var. makuwa Makino). There are also reports showing that exogenous application of JA imparts drought tolerance in sensitive cultivar of wheat (Abeed et al., 2021) and different Brassica species (Alam et al., 2014). Jasmonic acid (JA) was found to enhance the ABA accumulation under drought stress (de Ollas et al., 2015). In general, the level of ABA usually increases during abiotic stress conditions and elevated ABA can enhance plant adaptation to various abiotic stresses (Tuteja, 2007). Li et al. (2016) reported that the early drought priming induced an increase in endogenous melatonin production, in the drought-primed plants than in the nonprimed plants when exposed to cold stress in barley. It was suggested that the interplay of melatonin and ABA helps the plants to maintain better water status. The study of Xing et al. (2019) demonstrated that the improvement in drought tolerance was due to lipoxygenase-mediated elevation in ABA content. On the contrary, melatonin treatment lowered the ABA content thus contributing to improved stomatal performance (Li et al., 2015), germination (Li et al., 2021) and drought tolerance (Ahmad et al., 2022).

Despite several reports demonstrating the potential of melatonin as a phyto-protectant against drought stress in a range of crops and the regulatory mechanisms by which it induces drought tolerance, limited information is available on its role in *Arachis hypogaea* L., where drought stress greatly affects its productivity. More research is also needed to shed light on how melatonin regulates LOX expression and phytohormonal changes during induction of drought tolerance in plants. Therefore, the current work is focused on assessing the mechanistic effects of melatonin priming on antioxidant systems, photosynthetic rate and lipoxygenase expression including JA and ABA levels in drought-distinguished varieties of groundnut.

2 Materials and methods

2.1 Plant materials and seed priming

Groundnut (*Arachis hypogaea* L.) seeds of two varieties *viz.* Kadiri-7 (K-7; drought-sensitive) and Kadiri-9 (K-9; drought-tolerant) were procured from Agricultural Research Station Kadiri, Anantapur, Andhra Pradesh, India. The pods were sun-dried for 4 h followed by a brief surface sterilization with 70% (v/v) ethanol for 3 min and then washed with autoclaved double distilled water thrice. The seeds were then imbibed with the working solutions of melatonin (Catalog No. M5250, Sigma Aldrich, U.S.A.) of varying concentrations (5 μM, 10 μM, 25 μM, 50 μM and 100 μM) and kept in dark for 24 h. A batch of hydro-primed seeds served as controls. Seeds were dried overnight at room temperature in the dark for back-drying. The overnight dried seeds were evenly placed on moist sterile germination paper to germinate for 4 days.

2.2 Growth conditions

The germinated seedlings of equal length were placed in 15 ml tubes filled with sterilized Hoagland's nutrient (0.5X) solution. The tubes were covered with parafilm and a small perforation was made to allow the seedlings to stand upright, while the radicle was submerged into the media. The seedlings were allowed to grow in culture room at 25 ± 2°C temperature, 65 ± 2% relative humidity and illuminated under light provided by white fluorescent tube lights (65 μmol photons $m^{-2}s^{-1}$) under 16:8-h light and dark photoperiod. The nutrient solution was replenished every 3 days during the experimental period. Drought stress was induced on phenotypically uniform 20-day-old seedlings (three fully grown leaves and with a fourth emerging leaf) using an autoclaved 10% PEG-6000 (Polyethylene glycol) solution. Plants were grouped into four treatments *viz*., unprimed plants without PEG stress (C), unprimed plants with PEG treatment (S), primed plants without PEG treatment (PC) and primed plants with PEG treatment (PS). The unprimed plants without PEG stress (C) and primed plants without PEG treatment (PC) were raised in Hoagland's nutrient (0.5X) solution while unprimed plants with PEG treatment (S) and primed plants with PEG treatment (PS) were subjected to drought stress with PEG-6000 for 4 days until the appearance of wilting symptoms. The fully grown third leaves were collected from the seedlings of different treatments and used for various experiments, which have been carried out with three independent batches of plants, with triplicates per treatment in each batch of plants.

2.3 Determination of endogenous melatonin content

The melatonin content in leaves of both varieties was determined according to Arnao and Hernández-Ruiz (2009) with minor modifications. The fresh leaves (100 mg) were cut into small pieces and placed into vials containing 1 ml chloroform followed by overnight shaking at 4°C in the dark. The solvent was evaporated at 4°C under N_2 gas and the remnant was dissolved in 100 µl acetonitrile followed by a 0.2-micron polyvinylidene fluoride (PVDF) membrane filtration. The melatonin content in the two varieties was quantified using the Shimadzu High-Performance Liquid Chromatography (Kyoto, Japan) and a C-18 column (Phenomenex KINETEX 250 mm X 4.6 mm). An excitation wavelength of 280 nm and the isocratic mobile phase consisting of water and acetonitrile (50:50) at a flow rate of 0.2 L/min were used for detection. Pure melatonin (Sigma Aldrich, U.S.A.) was used as a standard.

2.4 Measurement of ROS

Histochemical techniques were employed to visualize reactive oxygen species *viz*., superoxide ion (O_2^-) and H_2O_2 in the leaves of control and treated seedlings by nitrobluetetrazolium (NBT) and diaminobenzidine (DAB) staining solution respectively, according to Ramel et al. (2009).

To quantify the superoxide radical content, 100 mg of fresh leaf samples were macerated in 65 mM potassium phosphate buffer (pH 7.8) followed by centrifugation at 5000 rpm for 15 min. The collected supernatant was mixed with 10 mM hydroxylamine hydrochloride and 65 mM phosphate buffer (pH 7.8) in equal ratio and incubated for 20 min at 25°C under dark conditions. Then, 3.0 mM α-naphthylamine and 8.5 mM sulphanilamide were added to the mixture followed by incubation for 20 min. The absorbance was recorded at 530 nm (Elstner and Heupel, 1976).

The content of hydrogen peroxide was quantified following the protocol of Alexieva et al. (2001). Briefly, 100 mg fresh leaf tissue was homogenized in 0.1% trichloroacetic acid and centrifuged at 12,000 rpm for 15 min. The supernatant was mixed with 1 M potassium iodide and 10 mM potassium phosphate buffer (pH 7) in equal ratio and incubated for 1 h under dark conditions. H_2O_2 concentration was determined by measuring the absorbance of the mixture at 390 nm and the values were calculated using a standard curve.

2.5 Antioxidant enzyme assay

To extract crude enzyme for antioxidant enzyme assays, 100-150 mg of the fresh leaf tissue was ground to a fine powder using liquid nitrogen. The tissue was macerated in 3% polyvinylpyrollidone (PVP), 1 mM phenylmethyl sulfonyl flouride (PMSF) and 0.1 mM ethylenediaminetetraacetic acid (EDTA) in ice-cold 50 mM phosphate buffer (pH 7.0). The extracts were centrifuged at 4°C for 30 min at 10000 rpm. The supernatants were used to perform antioxidant enzyme assays (Gogorcena et al., 1997). Superoxide dismutase activity was estimated based on the reduction of nitroblue tetrazolium by light in the presence or absence of protein by following the methodology of Beauchamp and Fridovich (1971). Catalase activity was detected based on the catalytic breakdown of H_2O_2 according to Patterson et al. (1984). Ascorbate peroxidase activity was estimated by the amount of ascorbate (AsA) oxidized using the protocol devised by Nakano and Asada (1981). Peroxidase activity was estimated according to the method of Lin and Kao (1999).

2.6 Measurement of malondialdehyde content and electrolytic leakage

The MDA content was estimated as described by Heath and Packer (1968). Briefly, fresh leaf tissues were homogenized by adding 0.1% (w/v) trichloroacetic acid (TCA) followed by centrifugation at 15,000 *g* at 4°C for 10 min. The supernatants were collected, mixed with 0.5% thiobarbituric acid, diluted in

20% TCA in a ratio of 1:3 and incubated in the water bath at 95°C for 30 min. The reaction was terminated by incubating on ice. The absorbance was measured at 532 and 600 nm. OD_{600} value was subtracted from OD_{532} nm and MDA content was determined using the Lambert-Beer law with an extinction coefficient (ϵM) = 155 mM^{-1} cm^{-1}.

Electrolyte leakage was estimated according to Dionisio-Sese and Tobita (1998) by immersing leaf discs in deionized water in a test tube and heated for 2 h at 32°C and the conductivity was measured (EC_a). Subsequently, the leaf-containing tubes were heated at 120°C for 15 min followed by cooling at room temperature. The conductivity was measured (ECb) and electrolyte leakage was calculated using the formula as described below:

Electrolyte Leakage (%) = $(EC_a/EC_b) \times 100$.

2.7 Measurement of proline content

Proline content was determined according to Bates et al. (1973). Briefly, 200 mg of leaf samples were ground using liquid nitrogen and homogenized in 3% sulfosalicylic acid followed by centrifugation at 12,000 *g* for 10 min. One ml of homogenate was taken and mixed with 1 ml of acid-ninhydrin and 1 ml of glacial acetic acid in a test tube for 1 h in a water bath at 100°C and then kept on ice to terminate the reaction. Then, 2 ml toluene was added to the reaction mixture with vigorous mixing and left at room temperature for 30 min until the separation of the two phases. The optical density of the chromophore-containing toluene (upper phase) was measured spectrophotometrically at 520 nm. The proline concentration was determined from a standard curve using D-Proline (Sigma-Aldrich, U.S.A.).

2.8 Assessment of photosynthetic parameters

2.8.1 Determination of chlorophyll-a fluorescence parameters and chlorophyll content

The concentration of chlorophyll-a was estimated in fully expanded third leaves using the DUAL PAM-100 (Waltz, Germany). The yield of photosystem II (Y(II)), electron transport rate (ETR), photochemical quenching (qP), non-photochemical quenching (NPQ), regulated heat dissipation Y (NPQ) and non-regulated dissipation Y(NO) were estimated at different photosynthetically active radiation (PAR) values. Prior to the measurement of chlorophyll-a fluorescence parameters, the leaf was dark adapted for 15-20 min. The chlorophyll content was estimated according to Lichtenthaler and Wellburn (1983) with some modifications. The leaf discs from each sample were placed in 80% acetone for efficient leaching of pigment followed by its spectrophotometric analysis at 663 nm and 645 nm.

2.8.2 Activity assay of chlorophyll degrading enzymes

The partially extracted chlorophyll was used to determine the activity of the chlorophyll degrading enzymes as per the protocol of Iriyama et al. (1974). The chlorophyll degrading peroxidase activity was estimated at 668 nm according to Aiamla-or et al. (2010) with minor modifications. Chlorophyllase activity was detected at 665 nm using the methodology described by Fang et al. (1998). Pheophytinase was determined spectrophotometrically at 667 nm according to Kaewsuksaeng et al. (2011) with minor modifications.

2.9 Determination of lipoxygenase activity

LOX activity was assayed according to Surrey (1964) with some modifications. To partially purify LOX, the crude protein extract was precipitated using 45% ammonium sulfate and then the assays were performed. The reaction mixture consisting of 20 mM borate buffer (pH-6.0), 0.25% linoleic acid, 0.25% tween-20 and 100 µg of partially purified enzyme extract in a total volume of 1.5 ml was incubated at 25°C for 5 min and the reaction was terminated by the addition of 2 ml of absolute alcohol. The cocktail was centrifuged and the absorbance of the supernatant was measured at 234 nm.

For in-gel activity staining, native gel electrophoresis was carried out according to the procedure described by Aanangi et al. (2016) with minor modifications. The partially purified protein (100 µg) was loaded into the wells of the gel and was allowed to run at 4°C. The gel was then rinsed with 100 mM phosphate buffer (pH - 6.8) and incubated in 250 µM linoleic acid solution. The reaction was carried out for 45 min at room temperature. The gel was later rinsed with the phosphate buffer followed by incubation with 0.05% o-dianisidine dihydrochloride solution at room temperature. Then the gel was visualized and photographed under a white-light illuminator.

2.10 Quantification of JA and ABA

The JA and ABA levels in the leaves were estimated using Liquid Chromatography-Mass Spectrometry/Mass-Spectrometry (LC-MS/MS) analysis. About 50 mg leaf tissue was homogenized in 500 µl solution of propanol, water and conc. HCl (2: 1: 0.002 vol/vol). The samples were vigorously shaken on a shaker at 4°C for 30 min. One ml dichloromethane was added to each sample followed by shaking for 30 min at 4°C. The mix was then centrifuged at 10000 rpm at 4°C. The lower phase was filtered and used for quantitative analysis of JA and ABA (Pan et al., 2010). The analysis was performed in Agilent Q-TOF LC/MS 6520 series system (Agilent Technologies, U.S.A.) with ZORBAX RX-C_{18} column (4.6×150 mm, 5 µm, Agilent) at 24°C. The mobile phase used was water with 0.1%

formic acid (solution A) and methanol with 0.1% formic acid (solution B) on a gradient elution mode with 2 µl injection volume and 0.4 ml/min flow rate. The gradient elution program used was: 1% B at 0 min to 11 min, 40% B up to next 2 min, 70% B for next 2 min, 99% B for next 1 min and then again 1% B for another 4 min. The detected mass range was 100-2000 m/z.

2.11 RNA isolation, cDNA synthesis, and quantitative real-time PCR

Total RNA was extracted from different samples of two varieties by CTAB-ammonium acetate method of Zhao et al. (2012). RNA integrity was analyzed by gel electrophoresis and Nanodrop-2000 UV-vis spectrophotometer (Thermo Fisher Scientific, U.S.A.). The OD 260/280 readings were obtained by spectrophotometry to assess the purity and concentration. Two microgram RNA was converted into cDNA using PrimeScript 1st strand synthesis kit (Takara Bio Inc., Japan) following the manufacturer's protocol. The real-time PCR (Polymerase Chain Reaction) was carried out on Mastercycler Realplex (Eppendorf, Germany) following the thermocycler condition of 95°C for 2 min (initial denaturation), 40 cycles of amplification (95°C for 15 sec, annealing temperature for 20 sec and 72°C for 30 sec). The reaction was terminated and the melting curve was analyzed to confirm whether the amplicon product is of a single reaction. *Actin4*, a housekeeping gene, was used as an internal control for normalization purposes and the relative fold-change in expression was estimated using the $2^{-\Delta\Delta CT}$ method as described by Livak and Schmittgen (2001).

2.12 Protein extraction and quantification of LOX by immunoblot

About 100 mg frozen leaf tissue was homogenized in an extraction buffer containing 50 mM Tris–HCl pH 8.0, 150 mM sodium chloride, 1 mM phenylmethylsulfonyl fluoride, and 10 mM iodoacetamide with protease inhibitor cocktail (Chung et al., 2009). The mix was centrifuged at 12000 rpm at 4°C for 30 min. Two hundred microgram of protein was loaded into each well of 12% polyacrylamide gel and then the gel was blotted onto the nitrocellulose membrane. The membrane was probed with a primary antibody of lipoxygenase (AS06 128, Agrisera, Sweden) with the dilution of 1:750. For equal loading control, anti-histone-H3 (AS10 710, Agrisera, Sweden) was used as primary antibody in the dilution of 1:2000. HRP-conjugated anti-rabbit antibody was used as a secondary antibody.

2.13 Statistical analysis

The data obtained are mean values of three independent experiments with three replicates per treatment in each experiment, which were subjected to one-way analysis of variance (ANOVA). The error bars shown in the graphs depict the standard deviation of mean values. One-way ANOVA was performed for the results using the software SigmaPlot (12.0 version). The significance of differences between treatments was computed using Duncan's multiple range test (DMRT) ($P< 0.05$).

3 Results

3.1 Effect of melatonin on drought stress tolerance in sensitive and tolerant varieties

The sensitive variety of groundnut showed wilting whereas the tolerant variety remained healthy under PEG-induced drought stress for 4 days. The effectiveness of melatonin in improving the drought tolerance was examined in Kadiri-7 (K-7) and Kadiri-9 (K-9) varieties of groundnut that differ in their tolerance levels. Of the different concentrations of melatonin used for seed priming, 100 µM was effective in improving the plant growth in the sensitive variety (K-7) under drought stress (Figure 1A). The plant height, leaf area and root length of the sensitive variety increased in 100 µM melatonin primed plants as compared to other concentrations tested under drought stress conditions (Supplementary Figure 1). Melatonin at higher concentrations (125 and 150 µM) led to a significant decrease in plant height and leaf area while root length remained unchanged in primed stressed plants as compared to unprimed stressed plants in K-7 variety. Surprisingly, there was no significant effect of melatonin (5-100 µM) on the phenotype of the tolerant variety, K-9, under control and stressed conditions (Figure 1B; Supplementary Figure 1). Thus, exogenous melatonin priming had a distinct effect in ameliorating drought stress tolerance in the sensitive variety while no add-an effect was observed in tolerant variety after 4 days exposure to drought stress.

3.2 Endogenous melatonin content in melatonin primed and unprimed stressed plants

Drought stress caused a decrease in melatonin content by 27.3% in K-7. Exogenous melatonin priming caused a marked increase in endogenous melatonin content in K-7 under stress as compared to unprimed stressed plants. However, unprimed plants of K-9 showed 41.16% increased endogenous melatonin content under drought stress compared to its control whereas priming decreased the content by 14.8% compared to unprimed stressed plants (Figure 2).

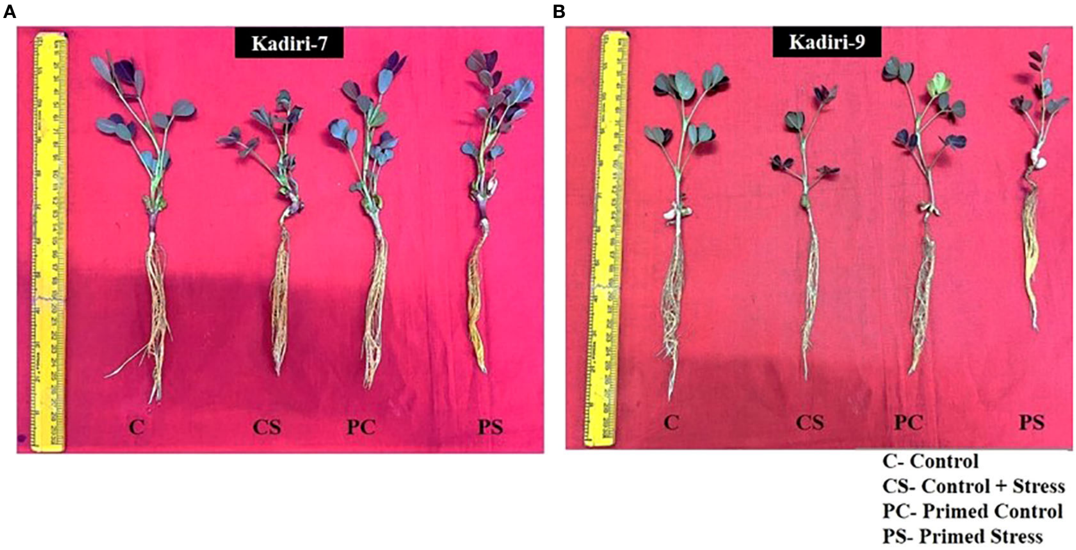


FIGURE 1
Phenotypic changes of melatonin-primed and unprimed plants of drought-sensitive (K-7) and drought-tolerant (K-9) varieties of groundnut (*Arachis hypogaea* L.) under optimal conditions and PEG-6000 (10%) induced drought stress. C- Plants grown under optimal conditions in Hoagland's nutrient (0.5X) solution (control); CS- Plants grown by adding PEG in Hoagland's nutrient (0.5X) solution for 4 days (drought-stressed); PC- Melatonin primed plants grown under optimal conditions in Hoagland's nutrient (0.5X) solution (primed control); PS - Melatonin primed plants grown by adding 10% PEG-6000 in Hoagland's nutrient (0.5X) solution for 4 days (primed stress). **(A)** Drought-sensitive variety, Kadiri-7 (K-7); **(B)** Drought-tolerant variety, Kadiri-9 (K-9).

3.3 Effect of melatonin on ROS accumulation under drought stress

Reactive oxygen species like superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2) are markers of stress induction. NBT and DAB staining showed higher accumulation of O_2^- (Figure 3A) and H_2O_2 (Figure 3B) respectively, in the unprimed plants during drought stress in the sensitive variety. However, melatonin priming reduced the accumulation of O_2^- and H_2O_2 under drought. In contrast, the tolerant variety did not show any visible differences in the ROS levels in primed as well as unprimed plants under stress. The content of superoxide radicals (Figure 3C) and hydrogen peroxide (Figure 3D) significantly increased in both unprimed K-7 and K-9 under stress. However, the ROS levels were comparatively higher in drought stressed plants of K-7 compared to K-9. Melatonin priming considerably decreased the levels of both the ROS molecules under drought stress compared to unprimed plants in K-7, whereas no significant change was observed in K-9 variety.

3.4 Effect of melatonin on ROS detoxification

The role of melatonin on ROS detoxification was investigated by performing several antioxidant enzyme activity assays *viz*., superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APx) and peroxidase (POX). SOD activity decreased by almost 25% in the unprimed plants during drought stress conditions as compared to control plants in K-7 variety. However, its activity increased by 26% in primed plants in comparison to unprimed ones under stress conditions. On the contrary, in the tolerant variety K-9, SOD activity increased by 34.8% in the unprimed plants during stress while it decreased in primed stressed as compared to unprimed stressed plants (Figure 4A). The enzyme activities of catalase, ascorbate peroxidase and guaiacol peroxidase declined significantly under stress conditions in comparison to control plants of K-7. Nevertheless, priming significantly increased their activities under stress conditions, thereby allowing the plants to combat drought stress more efficiently. However, in the tolerant variety, CAT, APx and POX activities were comparatively higher but surprisingly priming decreased the activities of these enzymes compared to their respective unprimed plants under stress (Figures 4B–D).

3.5 Measurement of malondialdehyde content and electrolytic leakage

A marked increase in MDA content was observed under drought stress in the unprimed plants of K-7. However, the

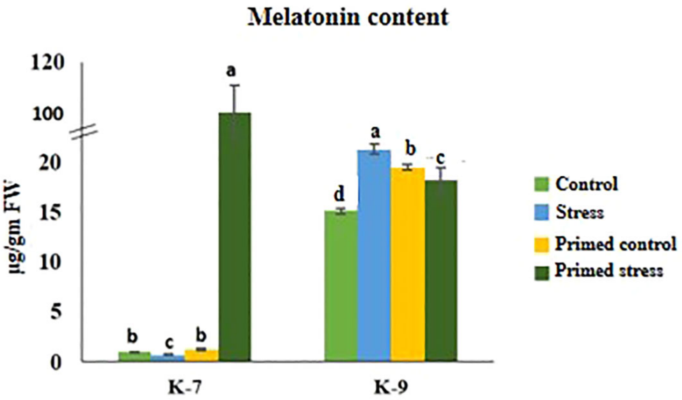


FIGURE 2
Effect of melatonin priming on endogenous melatonin content in drought-sensitive (K-7) and drought-tolerant (K-9) varieties with or without drought stress. Data represents mean values ± SD of minimum 3 independent experiments. Different alphabets within the group represent significant differences among treatments according to Duncan's multiple range test at P < 0.05.

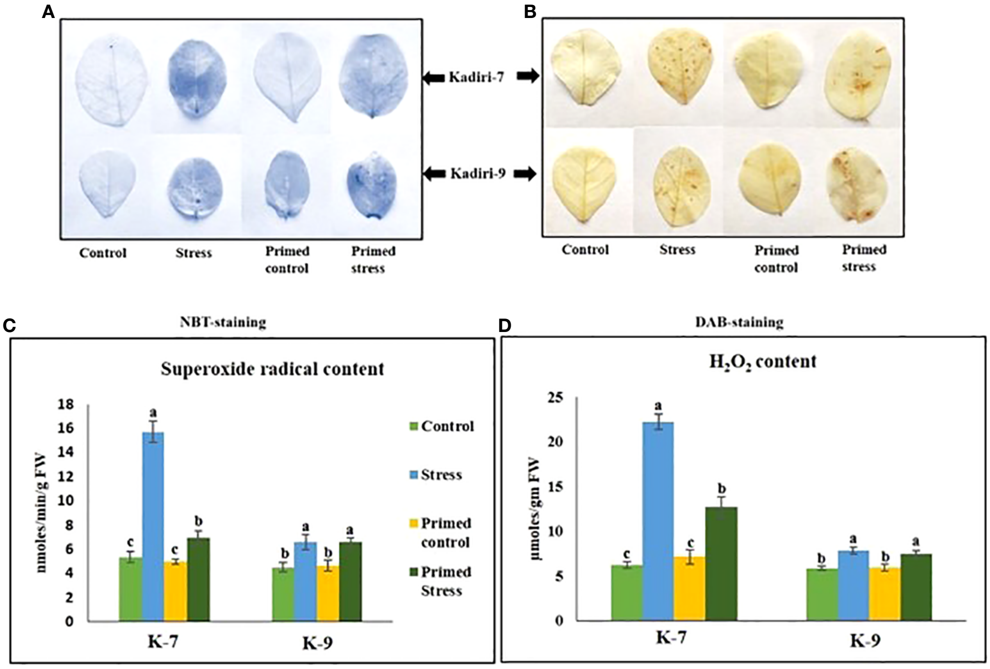


FIGURE 3
Effects of melatonin on the accumulation of superoxide radicals and hydrogen peroxide in the leaves of drought-sensitive (K-7) and drought-tolerant (K-9) varieties after different treatments. **(A)** Visualization of superoxide radicals in the leaves of different treatments by NBT-staining in K-7 and K-9 varieties; **(B)** H_2O_2 accumulation detected by DAB staining in the leaves of K-7 and K-9 varieties after different treatments; **(C)** superoxide radical content and **(D)** H_2O_2 content in the leaves of K-7 and K-9 varieties under stress condition. Data represents mean values ± SD of 3 independent experiments. Different alphabets within the group represent significant differences among treatments according to Duncan's multiple range test at P < 0.05.

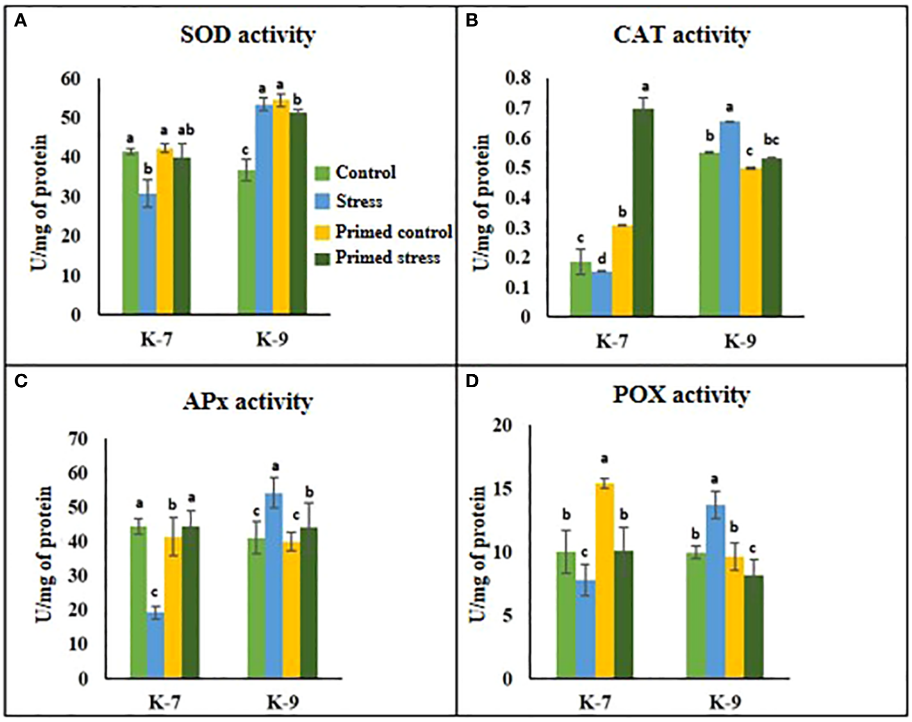


FIGURE 4
Effects of melatonin priming on antioxidant enzyme activities in drought-sensitive (K-7) and drought-tolerant (K-9) varieties with and without drought stress. **(A)** SOD activity; **(B)** CAT activity; **(C)** APx activity and **(D)** POX activity. Data represents mean values ± SD of 3 independent experiments. Different alphabets within the group represent significant differences among treatments according to Duncan's multiple range test at $P < 0.05$.

content decreased significantly in primed plants as compared to unprimed plants under stress. Interestingly, the tolerant variety exhibited no notable change in MDA content in unprimed and primed plants under drought stress (Figure 5A).

Electrolytic leakage, a hallmark of stress response, is an indicator of membrane integrity. The sensitive variety showed a 125.76% increase in the unprimed plants during stress. However, priming was found to decrease the leakage levels by 52.4% as compared to unprimed plants during stress treatment. In the tolerant variety, there was no statistical difference in electrolytic leakage percentage in unprimed and primed stressed plants (Figure 5B).

3.6 Proline content

Exposure to drought stress caused a pronounced increase in proline content in unprimed plants as compared to controls in K-7. However, the proline content decreased considerably in primed plants as compared to unprimed plants under drought stress. On the other hand, the primed plants exhibited higher proline content than unprimed plants under stress in K-9 variety (Figure 6).

3.7 Assessment of photosynthetic parameters (Chlorophyll a fluorescence, chlorophyll content and chlorophyll degrading enzyme activities)

The chlorophyll-a fluorescence characteristic exhibited marked differences between the two varieties under stress conditions. Stress condition dramatically decreased the photosystem II yield Y(II), which reached zero from ≥131 PAR in unprimed plants of K-7 variety. However, the level was found to be similar under all PAR values during stress compared to controls in unprimed plants of K-9 variety. Priming elevated Y(II) values significantly in primed stressed plants compared to unprimed stressed in both varieties although K-7 showed more prominent differences (Figure 7A). Drought stress had a distinct effect on photosystem II electron transport rate (ETR) in K-7 variety where the values decreased significantly from ≥27 PAR and declined to zero from ≥536 PAR onwards. K-9 unprimed stressed plants also showed a decrease in ETR (II) values after ≥58 PAR compared to unprimed control plants. Primed stressed plants exhibited higher ETR (II) compared to unprimed stressed in both varieties (Figure 7B).

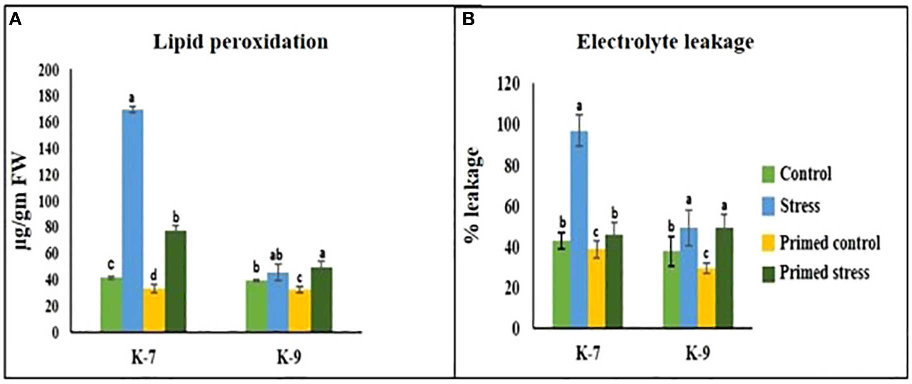


FIGURE 5
Effect of melatonin priming on **(A)** lipid peroxidation and **(B)** electrolytic leakage in drought-sensitive (K-7) and drought-tolerant (K-9) varieties with or without drought stress. Data represents mean values ± SD of minimum 3 independent experiments. Different alphabets within the group represent significant differences among treatments according to Duncan's multiple range test at P < 0.05.

Non-photochemical quenching (NPQ), photochemical quenching (qP) and regulated heat dissipation Y(NPQ) levels were considerably decreased in K-7 plants during stress. However, K-9 unprimed stressed plants showed no marked changes in NPQ and Y(NPQ) levels compared to control plants under stress. Melatonin ameliorated both parameters in primed stressed plants compared to unprimed stressed plants of the sensitive variety. In the case of K-9, priming did not exhibit any beneficial effect (Figures 7C–E). The level of non-regulated heat dissipation Y(NO) increased notably in unprimed stressed plants of K-7 compared to control ones. Priming mitigated Y(NO) level under stress compared to unprimed stressed plants. K-9 variety also showed a significant increase in Y(NO) level in unprimed stressed plants from ≥58 PAR compared to control plants. The Y(NO) of K-9 primed stressed plants exhibited no significant change compared to unprimed stressed plants (Figure 7F). The chlorophyll a and b contents decreased by 31.8% and 45.6% under stress in K-7, respectively. Both the contents increased significantly in primed plants under stress conditions. K-9 variety showed 46.2% and 18.8% higher chlorophyll a and b contents, respectively under stress compared to controls. Surprisingly, there was no significant

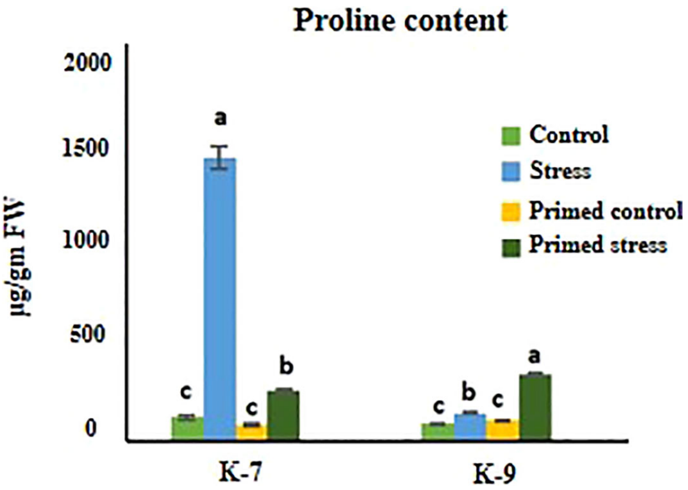


FIGURE 6
Effect of melatonin priming on proline content in drought-sensitive (K-7) and drought-tolerant (K-9) varieties with or without drought stress. Data represents mean values ± SD of minimum 3 independent experiments. Different alphabets within the group represent significant differences among treatments according to Duncan's multiple range test at P < 0.05.

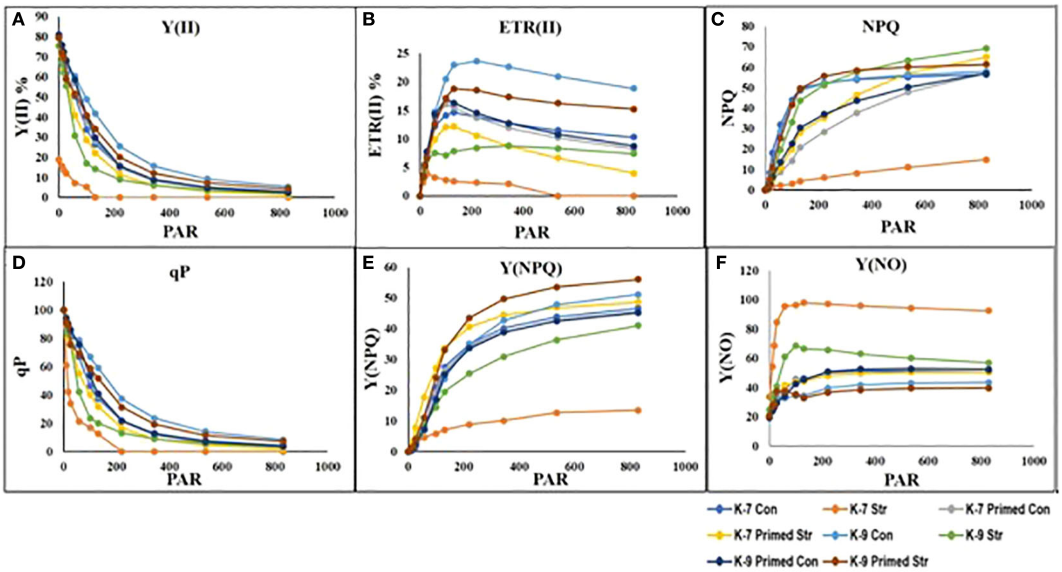


FIGURE 7
Changes in rapid light curves of photosynthetic parameters **(A-F)** in melatonin-primed or unprimed drought-sensitive (K-7) and drought-tolerant (K-9) varieties of groundnut in optimal conditions (control) or after exposure to drought stress. **(A)** Effective photochemical quantum yield [Y (II)]; **(B)** Electron transport rate of PSII [ETR(II)]; **(C)** Non-photochemical quenching (NPQ); **(D)** Photochemical quenching (qP); **(E)** Regulated heat dissipation Y(NPQ); and **(F)** Non-regulated heat dissipation [Y(NO)]. PAR is photosynthetically active radiation.

change in chlorophyll a and b contents in primed stressed plants compared to unprimed stressed plants of K-9 (Figures 8A, B). The total chlorophyll content of unprimed K-7 showed a significant decrease compared to control under stress. But priming caused a notable increase in the total chlorophyll content under stress conditions. The tolerant K-9 variety was found to have significantly higher total chlorophyll content compared to control but surprisingly priming decreased the content compared to unprimed plants during stress (Figure 8C). Drought stress stimulates the chlorophyll degradation process, which is detrimental to plant survival. The chlorophyllase (Figure 9A), pheophytinase (Figure 9B) and chlorophyll degrading peroxidase (Figure 9C) activities increased significantly in unprimed stressed plants, respectively compared to controls in K-7 variety. Priming reduced the chlorophyll degradation as primed stressed plants showed 27.5%, 57.5% and 66.7% decrease in the activities of these enzymes compared to unprimed stressed plants of K-7, respectively. Drought stress decreased the chlorophyllase and chlorophyll degrading peroxidase activities significantly by 16.0% and 33.3% whereas pheophytinase activity did not show any significant change in unprimed plants of K-9. Surprisingly, chlorophyll degrading peroxidase and chlorophyllase activities increased by 65% and 62.7% while pheophytinase activity was similar in primed stressed plants compared to unprimed stressed plants.

3.8 Determination of lipoxygenase activity

Lipoxygenases are a family of enzymes that are reported to have a protective role against abiotic and biotic stresses. Drought stress significantly decreased LOX activity when compared to the control plants in the sensitive variety. Melatonin priming caused a marked increase in its activity in K-7 compared to unprimed stressed plants. On the contrary, drought stressed plants of K-9 variety exhibited higher activity than that of controls. However, primed stressed plants showed significantly decreased LOX activity as compared to unprimed stressed plants in K-9 (Figure 10A). The results of in-gel activity also correlated with the LOX activity assay (Figure 10B).

3.9 JA and ABA quantification

Drought stress significantly reduced the level of JA level as compared to control plants in sensitive variety. However, melatonin priming increased its content by 10.1-fold under stress. Contrarily, the tolerant variety exhibited higher JA levels in unprimed plants under stress but surprisingly, priming led to its decrease under stress conditions (Figure 11A).

The ABA content in sensitive variety was lower as compared to the tolerant variety. Drought stress significantly decreased the

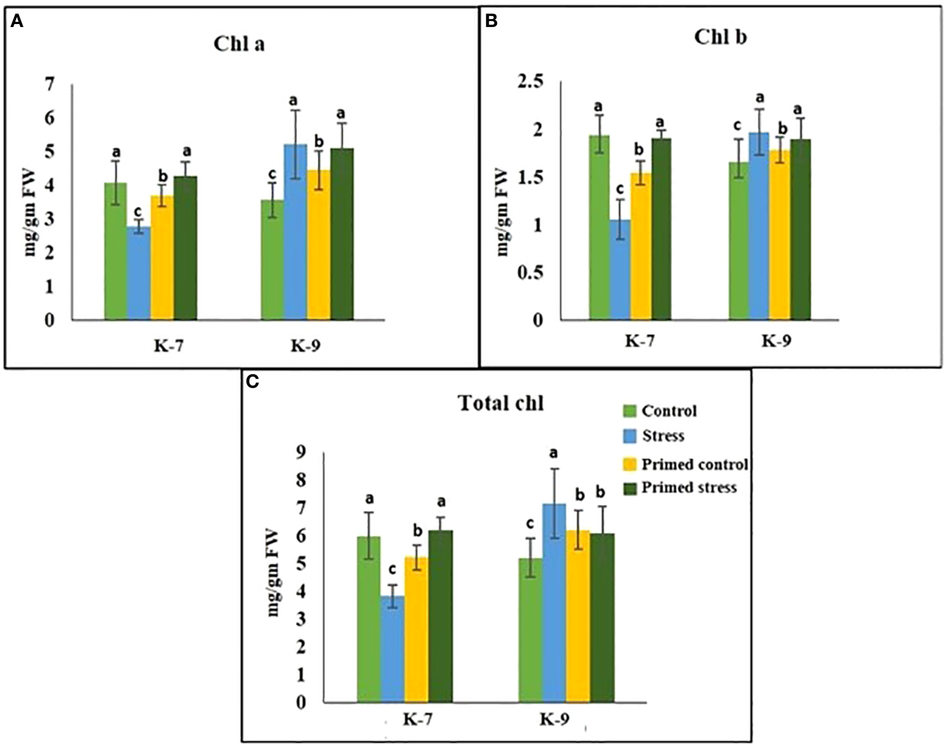


FIGURE 8
Effect of melatonin priming on **(A)** Chl a; **(B)** Chl b; and **(C)** total chlorophyll content in drought-sensitive (K-7) and drought-tolerant varieties (K-9) with or without drought stress. Data represents mean values ± SD of minimum 3 independent experiments. Different alphabets within the group represent significant differences among treatments according to Duncan's multiple range test at P < 0.05.

ABA content whereas priming enhanced the content by 2.1-fold in the sensitive variety. The tolerant variety exhibited higher ABA content under stress whereas priming decreased it by 1.8-fold (Figure 11B).

3.10 Relative expression of genes related to drought stress

The expression level of the genes involved in stress modulation *viz*., ROS regulation (Fe-SOD, Mn-SOD, Zn-SOD, CAT, APx and GR); melatonin biosynthesis (TDC, T-5H, SNAT and ASMT); melatonin receptor (PMTR1); chlorophyll degrading (PAO, SAG13 and SAG39); chlorophyll synthesizing (Chl-Syn); lipoxygenases and related (LOX1, 2, 4, 6, 8, 12, 20, 30, 36, AOC, AOS, OPDAR3, MYC2); ABA related (NCED3, CYP707A2, SnRK2); proline related (P5CS and PDH) was analyzed to confirm biochemical, physiological and molecular data (Figure 12A). Drought stress downregulated the expression of genes of antioxidant enzymes significantly in K-7 compared to controls. Priming was found to have a positive effect as it increased the expression of these genes by 3.0, 4.6, 6.5, 16.2, 7.2 and 4.7-fold compared to unprimed plants under stress, respectively. The tolerant variety, K-9 showed increased expression of these genes under stress in unprimed plants except for the expression of *GR* where there was no significant change in its expression. The expression levels of *Fe-SOD*, *Mn-SOD* and *GR* were elevated, whereas the expression levels of *Zn-SOD*, *CAT* and *APx* decreased significantly in primed stressed plants compared to unprimed stressed plants of K-9 variety (Supplementary Figures 2A–F). Exogenous melatonin modulated the expression of melatonin biosynthesis genes under stress conditions. The expression level of *TDC* (Tryptophan decarboxylase), *T5H* (Tryptophan-5 hydroxylase) and *ASMT* (Acetyl seratonin methyl transferase) decreased significantly whereas *SNAT* (Seratonin N-acetyl transferase) showed no significant change in unprimed stressed plants compared to control stressed plants in K-7 (Supplementary Figures 3A–D). Priming elevated the expression of *TDC*, *T5H*, *SNAT* and *ASMT* notably under stress compared to unprimed stressed plants of K-7. In the tolerant variety K-9, the expression level of *T5H*, *SNAT* and *ASMT* genes showed an increase under stress in unprimed plants, whereas there was no significant change in the expression of *TDC* level in unprimed stressed plants. Interestingly, primed stressed plants either showed no significant change (*TDC* and *SNAT*) or decreased expression

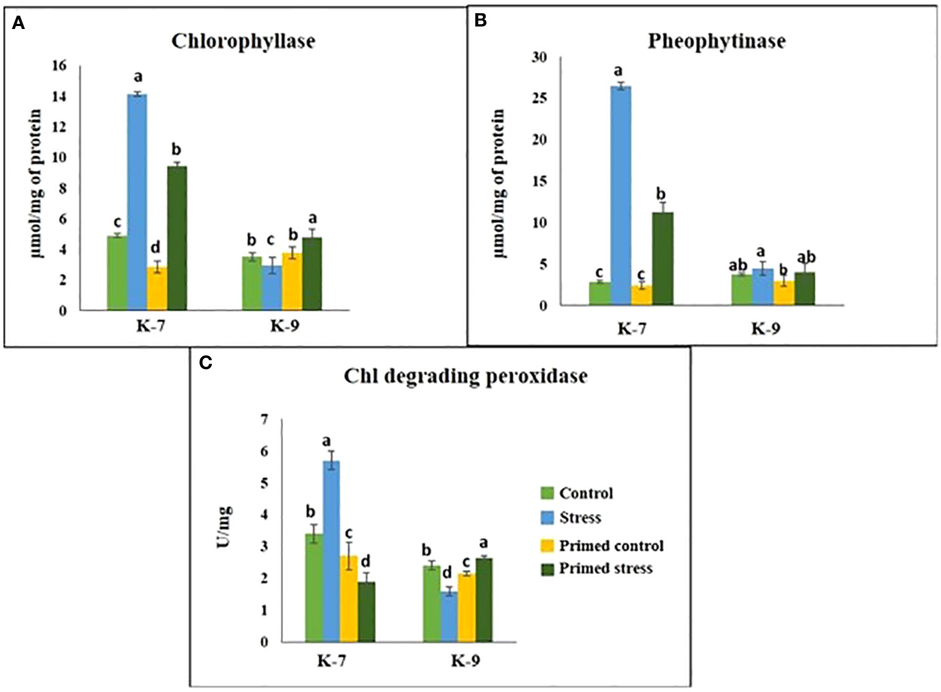


FIGURE 9
Effect of melatonin priming on the activities of chlorophyll degrading enzymes in drought-sensitive (K-7) and drought-tolerant (K-9) varieties under drought stress. **(A)** Chlorophyllase; **(B)** Pheophytinase; and **(C)** Chlorophyll degrading peroxidase. Data represents mean values ± SD of minimum 3 independent experiments. Different alphabets within the group represent significant differences among treatments according to Duncan's multiple range test at $P < 0.05$.

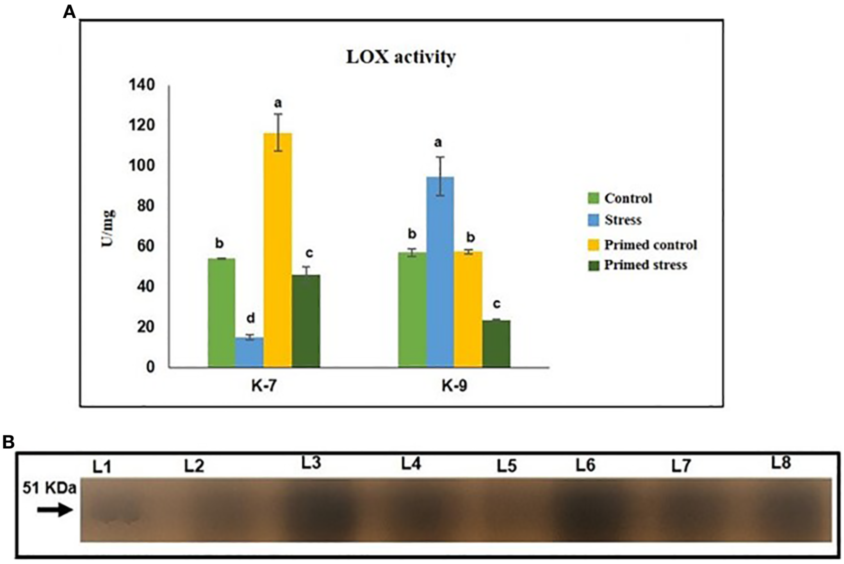


FIGURE 10
Effect of melatonin priming on the activity of lipoxygenase enzyme in drought sensitive (K-7) and drought-tolerant (K-9) varieties under drought stress. **(A)** Lipoxygenase activity (spectrophotometric detection) and; **(B)** in-gel staining activity. Data represents mean values ± SD of minimum 3 independent experiments. Different alphabets within the group represent significant differences among treatments according to Duncan's multiple range test at $P < 0.05$.

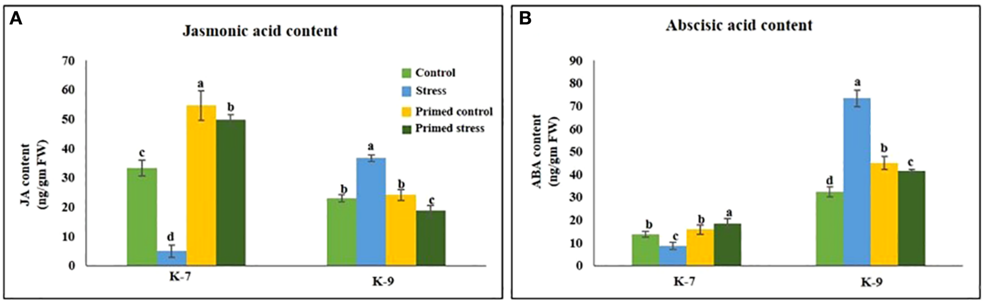


FIGURE 11
Effect of melatonin priming on the contents of **(A)** jasmonic acid and **(B)** abscisic acid in drought-sensitive (K-7) and drought-tolerant (K-9) varieties under drought stress. Data represents mean values ± SD of minimum 3 independent experiments. Different alphabets within the group represent significant differences among treatments according to Duncan's multiple range test at P < 0.05.

(*T5H* and *ASMT*) compared to unprimed stressed plants (Supplementary Figures 3A–D). *PMTR1* (Plant melatonin receptor) exhibited similar expression patterns in both the varieties independent of the treatment (unprimed and primed) and conditions (optimal or drought) (Supplementary Figure 4). The expression of chlorophyll degrading genes *PAO* (Pheophorbide A oxygenase), *SAG13* and *SAG39* (Senescence associated genes) increased significantly by 11.0, 7.2 and 4.3-folds in K-7 in unprimed stressed plants compared to controls (Supplementary Figures 5A–D). Although stress caused an increase in the expression of *PAO*, *SAG13* and *SAG39* genes in K-9 variety, the levels were lower than the unprimed stressed plants of K-7 variety. Priming downregulated their expression by 5.1, 4.0 and 4.8-fold in K-7 compared to unprimed plants under stress. Interestingly, the expression levels of *SAG13* and *SAG39* genes increased while *PAO* expression remained unchanged compared to unprimed stressed plants in K-9 variety. The chlorophyll synthesis gene (*Chl-Syn*) expression decreased 2.7-fold in stressed plants compared to control and increased 7.7-fold in primed stressed plants compared to unprimed stressed

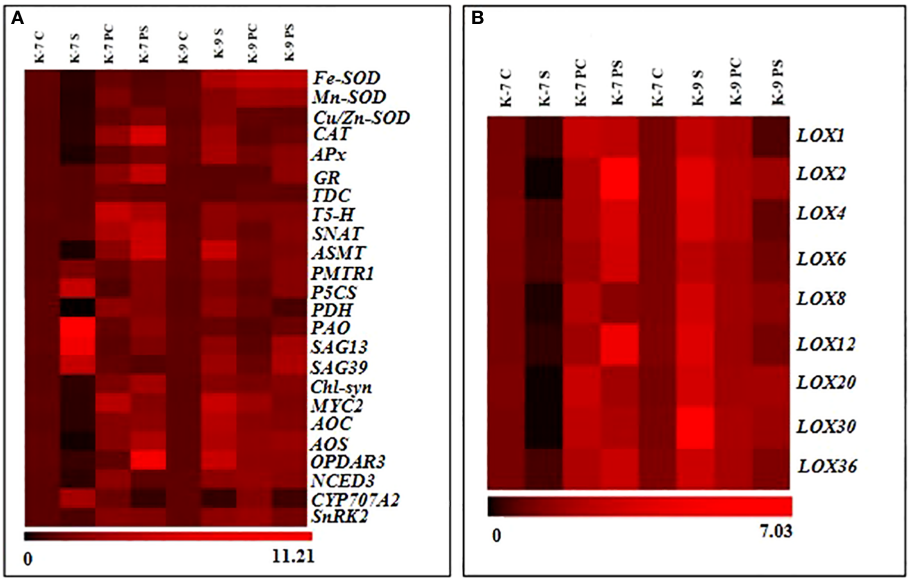


FIGURE 12
Relative abundance of mRNA transcripts of genes (fold-change in comparison to control plants of respective variety) associated with **(A)** antioxidant, melatonin biosynthesis and receptor, chlorophyll degradation and biosynthesis, JA biosynthesis, ABA biosynthesis and degradation, and ABA response element **(B)** lipoxygenase isoforms in primed or unprimed plants K-7 (drought-sensitive) and K-9 (drought-tolerant) varieties, under optimal conditions (control) or drought stress.

plants of K-7. The expression level of the *Chl-Syn* increased in unprimed and primed stressed plants as compared to controls (Supplementary Figure 5A–D). The expression of the proline biosynthesis gene, *P5CS* (Pyrolline-5 carboxylase) increased whereas the expression of the degrading gene, *PDH* (Proline dehydrogenase) decreased significantly in unprimed plants of K-7 exposed to stress. On the other hand, the expression of *P5CS* decreased in primed stressed plants while the expression of *PDH* increased as compared to unprimed stressed plants. *P5CS* and *PDH* gene expressions increased 1.9-fold and 2.2-fold in unprimed stressed plants compared to controls in K-9, respectively. The primed plants showed no change in the expression of *P5CS* whereas *PDH* expression decreased compared to unprimed plants under stress in K-9 (Supplementary Figures 6A, B).

The lipoxygenase isoforms (*LOX1, 2*, *4*, *6*, *8*, *12*, *20*, *30* and *36*) exhibited similar expression patterns (Figure 12B). K-7 showed a decrease in the transcript levels of these isoforms whereas the transcript levels significantly increased in K-9 under stress as compared to their controls. However, priming enhanced the expression in K-7, whereas in K-9, the levels decreased considerably compared to their respective unprimed plants under stress (Supplementary Figures 7A–I). The genes related to jasmonic acid biosynthesis *viz*., *AOC* (Allene oxide cyclase), *AOS* (Allene oxide synthase) and *OPDAR3* (Oxophytodienoic acid reductase 3) decreased significantly by 3.0, 12.6 and 2.3-folds in stressed plants compared to control plants in K-7, respectively (Supplementary Figures 8A–C). Melatonin priming significantly increased their expression compared to unprimed plants under stress. However, the expression of these genes increased in K-9 under stress as compared to controls. Interestingly, priming decreased the expression significantly as compared to unprimed plants under stress. The *MYC2* expression level decreased by 2.71-folds in K-7 and increased by 4.3-folds in K-9 stressed plants compared to their respective control plants. Priming under stress showed increased expression of *MYC2* in K-7 by 6.2-fold, but in K-9 the expression decreased by 2.5-folds as compared to unprimed stressed plants (Supplementary Figure 8D). The expression of ABA biosynthesis gene, *NCED3* (9-cis-epoxycarotenoid dioxygenase 3) was significantly downregulated under stress when compared to its control in the sensitive variety. Priming enhanced its expression under stress compared to unprimed plants under stress. The tolerant variety showed higher transcript levels of *NCED3* under stress. However, melatonin priming caused a decline in its expression under stress (Supplementary Figure 9A). Contrasting expression pattern was observed for the ABA degrading gene *i.e.*, *CYP707A2* (ABA 8'-hydroxylase) where a significant increase in its expression was observed in primed stressed plants of K-7 unlike in K-9 where it decreased under stress. However, its expression in primed stressed plants was lower in K-7 whereas it was found to be higher in K-9 variety in comparison to unprimed stressed plants (Supplementary Figure 9B). *SnRK2* (Snf-related protein kinase 2), an important gene in ABA signaling response, showcased a similar expression pattern as that of *NCED3* (Supplementary Figures 9A–C).

3.11 Immunoblot analysis of LOX

Immunoblot analysis revealed differences in LOX expression in sensitive and tolerant varieties upon exposure to drought stress. The intensity of the protein decreased under stress in the sensitive variety whereas priming increased the intensity of the protein under stress. In contrast, the intensity of protein increased in K-9 stressed plants as compared to unstressed plants whereas it decreased in primed stressed plants than in primed control plants (Figure 13).

4 Discussion

Plant growth is a complex biochemical and physiological phenomenon being modulated by several plant growth regulators such as auxin, cytokinin, jasmonic acid, gibberellins, abscisic acid, ethylene and brassinosteroids (Gray, 2004). Seed priming with many of these growth regulators has been proven to alleviate the adverse effects of abiotic as well as biotic stresses. Melatonin priming also has been found to assist plants to endure drought stress by maintaining ROS homeostasis and also by

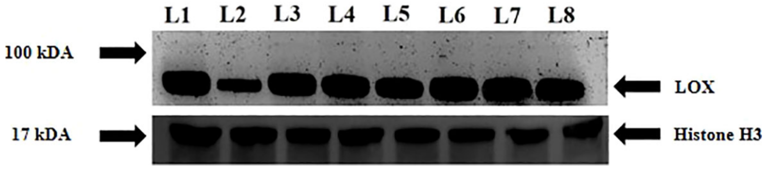


FIGURE 13
Immunoblot analysis of lipoxygenase in melatonin primed and unprimed plants of drought-sensitive (K-7) and drought-tolerant (K-9) varieties of *Arachis hypogaea* L. under optimal and drought stress conditions. Lane 1- K-7 control, Lane 2- K-7 stress, Lane 3- K-7 primed control, Lane 4- K-7 primed stress, Lane 5- K-9 control, Lane 6- K-9 stress, Lane 7- K-9 primed control and Lane 8- K-9 primed stress.

activating stress-related transcription factors and genes. Here, the role of melatonin on redox homeostasis, photosynthesis and lipoxygenase expression to mitigate the negative impacts of drought in *Arachis hypogaea* L. was investigated in K-7 (drought-sensitive) variety in comparison to K-9 (drought-tolerant) of groundnut.

4.1 Melatonin alleviates PEG-induced drought stress in the sensitive variety

Melatonin priming exhibited beneficial effects on the morphology of the sensitive variety as evidenced by fewer wilting symptoms with increased plant height, root length and leaf area after exposure to 4-days of drought stress treatment as compared to unprimed stressed plants. However, melatonin-primed and unprimed plants of tolerant variety (K-9) did not show any differences in plant height, root length and leaf area when subjected to the same duration of drought stress treatment. It can be interpreted that exogenous melatonin has the potential to improve the drought tolerance only when severe stress is prevalent as in the sensitive variety after 4 days of exposure to drought stress, unlike the tolerant variety which was inherently equipped to withstand this duration of stress treatment (Figure 1; Supplementary Figure 1). Similar findings have been reported in cotton where melatonin priming improved the drought tolerance of sensitive variety while no significant phenotypic effect was noticed in the tolerant variety of cotton under drought stress (Supriya et al., 2022).

4.2 Exogenous priming increases endogenous melatonin content

Previous studies have shown that melatonin plays a significant role in drought stress tolerance. In this study, the marked increase in melatonin content with upregulated expression of genes involved in melatonin biosynthesis in primed stressed plants of sensitive variety justifies its implication in stress tolerance. This is further evident by an increase in the melatonin content in unprimed stressed plants of the tolerant variety. However, the decrease in melatonin content in tolerant primed stressed plants compared to unprimed stressed plants (Figure 2) signify the feedback inhibition, which is consistent with findings of Bhowal et al. (2021). Further, down-regulated expression of *T-5H* and *ASMT* in this variety (Supplementary Figures 3B, D) also correlated to the decreased endogenous melatonin content. The unchanged expression of *PMTR1*, which is a receptor of melatonin, indicates that variation in endogenous melatonin was not due to differences in the expression of receptor genes (Figure 4 and 12A). This also edicts the ambiguity about the position and/or function of PMTR1, as suggested by Lee and Back (2020).

4.3 Priming alleviates oxidative damage and maintains ROS homeostasis

Plants produce more ROS under drought stress conditions, which causes oxidative damage to the cells and tissues (Kar, 2011). Melatonin scavenges these ROS molecules and gets converted into metabolites *viz.*, AFMK (N1-acetyl-N2-formyl-5-methoxyknuramine) and AMK (N1-acetyl-5-methoxykynuramine) that are also involved in several cascades of reactions to scavenge other types of ROS, thereby maintaining the redox homeostasis more effectively (Ressmeyer et al., 2003). The role of melatonin in the mitigation of hydrogen peroxide levels and the augmentation of antioxidant systems has been reported in *Malus* (Li et al., 2015). Here, the higher accumulation of superoxide and hydrogen peroxide radicals in leaves of unprimed plants and their decreased levels in primed plants of K-7 under stress justifies the scavenging property of melatonin (Figure 3). On the contrary, melatonin primed stressed plants did not show any notable changes in staining intensity as compared to unprimed stressed plants in the tolerant variety, K-9 indicating that exogenous priming has no further effect on ROS levels.

Plants have developed several enzymatic and non-enzymatic defense systems to combat oxidative damage by minimizing stress-induced ROS accumulation (Qamer et al., 2021). Buttar et al. (2020) reported that exogenous melatonin activates the antioxidant enzymatic activities to reduce stress-induced ROS bursts in wheat seedlings. In our study, decreased antioxidant enzymatic activities in the sensitive variety under stress (Figures 4A–D) correlated with the decrease in the transcript levels of the genes like *Cu-SOD*, *Mn-SOD*, *Zn-SOD*, *CAT*, *APx* and *GR* thereby resulting in increased ROS accumulation. Melatonin aided the activation of the antioxidant defense machinery by upregulating the expression of these stress-related genes and their activities to scavenge intracellular ROS (Figure 12A; Supplementary Figures 2A–F). Thus, melatonin mediated maintenance of cellular redox homeostasis by elevating the antioxidative defense mechanism, thereby improving the drought tolerance of the sensitive variety of groundnut.

4.4 Melatonin ensures membrane integrity and stability by decreasing lipid peroxidation and electrolytic leakage

Unregulated intracellular ROS levels under stress condition lead to membrane lipid peroxidation followed by membrane rancidity. Electrolytic leakage is caused by lipid peroxidation, which leads to membrane damage, a repercussion of drought stress (Jaleel et al., 2007). Our results showed that drought stress intensifies the generation of ROS in groundnut, resulting in higher levels of electrolytic leakage and MDA which are crucial oxidative-damage indicators of cell membrane integrity. Similarly, Khalvandi et al. (2021) reported that drought stress caused damage to the membrane system which increased lipid peroxidation and plasma membrane electrolytic leakage in winter wheat. In this research, lipid peroxidation and electrolytic leakage were alleviated in the sensitive variety upon melatonin priming under stress. The reason might be the amphipathic nature of melatonin that tends to spread across the cytoplasm and lipid membranes. Thus, the interfacially positioned melatonin inhibits lipid peroxidation in biological membranes by directly neutralizing hazardous reactants (de Lima et al., 2010). The reduced lipid peroxidation and electrolyte leakage in the unprimed tolerant variety during stress in comparison to the sensitive variety, explains its inherent ability to tolerate the drought stress (Figures 5A, B).

4.5 Melatonin maintains osmotic potential by regulating proline levels

Plants tend to cope with environmental stresses by regulating the osmotic potential and proline is a crucial osmolyte involved in tolerance to various stresses. Several studies have reported an increase in intracellular proline levels during exposure to stress in different plant species (Liang et al., 2013). Similarly, a significant increase in proline levels was observed under drought stress which is more pronounced in K-7 as compared to K-9 variety in the present study. Besides being an osmoprotectant and cellular stabilizer, it imparted toxic effects if over-accumulated as observed in tomato, where an imbalance in inorganic ions was observed (Heuer, 2003). Similar findings have been reported by Roy et al. (1993) where low concentrations of exogenously applied proline was effective in ameliorating the adverse effects of salinity whereas higher concentrations reduced the seedling growth in rice. Our results showcased higher proline accumulation under stress which might be due to the repression of the proline catabolic gene, *PDH* in mitochondria whose activity might be hampered by the oxidative burst inside the cell in sensitive variety (Figure 6). However, the regulated expression of *P5CS* and *PDH* might have contributed to the balanced proline levels in the tolerant variety under stress. Therefore, our findings suggest a vital role of melatonin in proline metabolism in plants by maintaining an equilibrium between the expression of proline biosynthetic (*P5CS*) and proline catabolic (*PDH*) genes (Figure 12A; Supplementary Figures 6A, B).

4.6 Melatonin inhibits chlorophyll degradation and senescence

Chlorophyll-a fluorescence has been commonly used to assess the plant photosynthetic performance under a variety of stress conditions (Govindjee, 2004). Severe drought stress is reported to cause photoinhibition in the PSII reaction center (Sperdouli and Moustakas, 2012). In line with these observations, we observed that Y(II), ETR(II), qP and NPQ were reduced dramatically in the sensitive variety during drought stress. Melatonin has been reported to boost photosynthetic efficiency in higher plants under drought stress in cucumber (Zhang et al., 2013). In this study, melatonin priming augmented the yield of PSII, electron transport rate (ETR) in K-7, similar to the report of Wang et al. (2013) justifying the role of melatonin in maintaining better photosynthetic activities under drought. As priming did not cause any significant change in endogenous melatonin content in K-9, no change in photosynthetic rate was observed. Y(NO) and Y(NPQ) are two important components of the photosynthetic machinery. Higher Y(NPQ) and lower Y(NO) in primed plants of K-7 justify the proper functioning of its xanthophyll-carotenoid cycle, similar to the report of Khan et al. (2019). As such melatonin priming did not cause any changes in Y(NPQ) and Y(NO) as compared to unprimed plants in the tolerant variety, K-9 under stress showing no additional effect of exogenous melatonin on photosynthesis (Figures 7A–F).

Plants have to be protected from free radicals and associated oxidative stress since chloroplast is the primary location of free radical production. Chlorophyll degradation leads to senescence in plants which is detrimental to plant growth and development (Hörtensteiner, 2006). Previous studies have shown that exogenous melatonin priming induces endogenous melatonin production (Antoniou et al., 2017). Our findings imply that exogenous application can alter endogenous melatonin accumulation in drought-stressed plants of sensitive variety that can help to retain chloroplast integrity and increase the net photosynthetic rate. This is further supported by increased chlorophyll content upon melatonin priming in the sensitive cultivar under stress (Figures 8A–C). An increased expression of the chlorophyll synthesis gene (*Chl-syn*) (Supplementary Figure 5D) and decreased activities of chlorophyll degrading enzymes such as chlorophyllase, pheophytinase and chlorophyll degrading peroxidase upon melatonin priming in the sensitive variety emphasizes the role of melatonin against chlorophyll degradation (Figures 9A–C). Similarly, Ma et al. (2018) reported that drought-induced leaf senescence suppressed by cytokinin and melatonin, was marked by the down-regulation of chlorophyll-degradation genes and enzyme activities in creeping bentgrass (*Agrostis stolonifera*).

Senescence is a critical measure of chlorophyll degradation. The expression levels of *PAO* and senescence-associated genes (*SAG13, 39*) was found to decrease upon priming in K-7 under stress (Figure 12A; Supplementary Figures 5A–C). Thus, the activity of these chlorophyll-degrading enzymes in primed stressed plants was alleviated, thereby inhibiting senescence which could be ascribed to the melatonin treatment. Increased endogenous melatonin concentration could be responsible for these substantial changes, which leads to improved photosynthetic machinery. The enhanced photosynthetic efficiency and lowered expression of chlorophyll degrading genes under stress conditions in unprimed plants of tolerant variety are supportive of its inherent drought tolerance capacity (Figures 9 and 12A; Supplementary Figure 5).

4.7 Melatonin enhances JA and ABA biosynthesis by stimulating lipoxygenases under drought stress

LOXs are non-heme iron-containing dioxygenases enzymes that catalyze the conversion of polyunsaturated fatty acids to hydroperoxy fatty acids, which are involved in the formation of stress-related plant growth regulators such as jasmonic acid (JA), methyl jasmonate (MeJA) and so on (Blée, 2002). Despite greater substrate availability (linoleic and linolenic acid) due to a higher rate of lipid peroxidation, decreased activity and transcripts level of LOX under stress in unprimed K-7 suggested its catalytic inhibition by thiol sensitive feedback regulation, as described by Maynard et al. (2021). The elevated LOX activity and content in primed stressed plants of K-7 (Figures 10 and 13) can be attributed to the regulation of ROS levels by the action of melatonin thereby maintaining lipid peroxidation levels which is enough to provide the substrate for LOX activity. Additionally, our findings suggest that the expression of LOX2 is highest among all other isoforms, which also indicates its exigent role during osmotic stress as reported by Singh et al. (2022). The tolerant variety showcased higher LOX activity and content, which can be correlated to higher melatonin content and its stress tolerance nature. Surprisingly, lower LOX activity in primed stressed plants might be due to the lesser substrate availability. Therefore, endogenous melatonin content shares a direct relationship with LOX. Overall the study has shown the significant role of melatonin in modulating the expression of lipoxygenases under drought stress (Figure 12B).

Abiotic stress responses, including drought, are known to be mediated by jasmonic acid. The negative effects of drought-induced membrane damage in barley were found to be mitigated by JA pre-treatment (Bandurska et al., 2003). Wang et al. (2021) reported that the genes involved in JA biosynthesis and signaling were upregulated by drought priming during drought tolerance in wheat. In consistent with these reports, our studies also indicated that melatonin increased the jasmonic acid content (Figure 11A) and expression of genes (*LOX*, *AOS*, *AOC* and *OPDAR3*) involved in the jasmonic acid biosynthesis pathway under stress condition in K-7 (Figure 12; Supplementary Figures 7 and 8A–C). The JA levels in K-9 also indicate the direct relationship between LOX and JA under stress condition, which could be due to higher endogenous melatonin content.

ABA and JA are the key molecules required for stress tolerance response. Higher levels of JA were found to be essential in ABA accumulation during drought stress in *Arabidopsis* (de Ollas et al., 2015) and rice (Kim et al., 2009). In accordance with these studies, it was observed that melatonin enhanced JA biosynthesis *via* stimulating LOX which further promoted ABA production in K-7 under stress. However, in K-9, enhanced levels of ABA in unprimed stressed plants and a significant decrease in primed plants under stress correlated to JA content suggesting the involvement of JA on ABA accumulation under drought condition (Figure 11B). A correlative expression of Snf-related kinase protein (*SnRK2*) was observed in our study (Supplementary Figure 9C) which is responsible for ABA-mediated dehydration signaling response (Yoshida et al., 2002). These findings indicate that JA might function as an upstream modulator in ABA-mediated signaling, as suggested by Wang et al. (2021) and regulates the tolerance response under drought stress. Moreover, upregulated expression of the *MYC2* transcription factor has been reported to positively regulate the genes involved in the antioxidant defense system (Wang et al., 2021). In consistent with these reports, melatonin was found to enhance the transcript levels of MYC2 in K-7 under stress, thereby enhancing its drought stress tolerance (Supplementary Figure 8D).

The model showing the regulatory differences of melatonin action between drought-sensitive (K-7) and drought-tolerant (K-9) varieties under drought stress is depicted in Figure 14 (this image is made using BioRender).

5 Conclusion

Melatonin is well known for its potential to impart drought stress tolerance in different plant species due to its capability to scavenge ROS, enhance photosynthetic efficiency and modulate the expression of stress-responsive genes. The virtue of exogenous melatonin on drought tolerance might be different in sensitive and tolerant varieties as their redox state differs under drought stress conditions. The present study for the first time reports the differential effects exhibited by exogenous melatonin on PEG-induced water stress attributes in the tolerant and sensitive varieties of groundnut. Melatonin priming improved drought tolerance by regulating redox homeostasis, promoting photosynthetic efficiency and increasing the chlorophyll content in sensitive variety. Additionally, melatonin-primed stressed plants of sensitive variety showed higher endogenous melatonin content with increased LOX expression, accompanied by elevated JA and ABA levels that could have imparted drought stress tolerance. However, melatonin priming led to a decrease in endogenous melatonin content in the tolerant variety under stress conditions possibly due to feedback inhibition to fine-tune melatonin biosynthesis required for maintaining ROS homeostasis towards better plant metabolism. The higher endogenous melatonin content in unprimed stressed plants of tolerant variety was associated with the enhanced antioxidant system, photosynthetic efficiency and LOX expression along with higher JA and ABA, which further

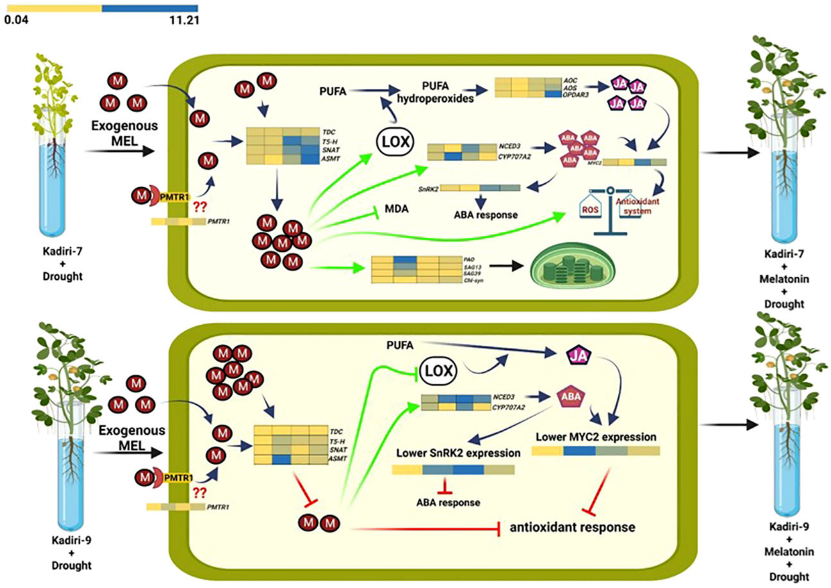


FIGURE 14
Differential effects of melatonin priming on drought-sensitive (Kadiri-7, upper) and drought-tolerant (Kadiri-9, lower) varieties of groundnut under drought stress to alleviate the impact of stress. Melatonin confers the tolerance in Kadiri-7 by improving endogenous melatonin content, antioxidant system and photosynthetic efficiency. Additionally, melatonin-mediated amelioration of LOX expression and activity in turn increases the endogenous JA and ABA contents, which also provides stress tolerance. Furthermore, unprimed Kadiri-9 with higher endogenous melatonin correlates with its inherent tolerance nature. Contrarily, exogenous melatonin priming was found to decrease its endogenous level, possibly as a consequence of feedback inhibition to fine-tune melatonin biosynthesis and maintain redox homeostasis towards better plant metabolism under drought stress condition.

substantiates the role of melatonin in drought stress tolerance. Overall, it can be concluded that exogenous melatonin evokes drought stress responses differently in sensitive and tolerant varieties as reflected by its ability to improve the tolerance of sensitive variety where stress effects are more prevalent in comparison to the tolerant variety that is inherently capable to tolerate the stress treatment given in the study. Thus, the study has advanced our scientific knowledge on the effects of exogenous melatonin with respect to modulation of LOX expression, JA and ABA which provides scope for improving the drought stress tolerance in groundnut.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Author contributions

GP, SS and LS: conceived and designed the study. SS and LS: conducted the experiments, collected the data, prepared the graphs, analyzed and interpreted the results and written the manuscript. GP: supervised, analyzed and interpreted the results and corrected the manuscript. All authors contributed to the article and approved the submitted version.

Funding

GP gratefully acknowledges the research grant of Institute of Eminence (IoE) of UGC (UoH-IoE-RC3-21-041) sanctioned by University of Hyderabad which was used partly to carry out the research work.

Acknowledgments

The infrastructural facilities established in the Department/School with the support of UGC-SAP-DRS-1 (Level-1, Phase-1), DST-FIST-Level-II (Phase-2), DST-PURSE and DBT-BUILDER programmes have been utilized for the research work which is gratefully acknowledged. We are highly grateful to Dr. K. S. S. Naik, Principal Scientist (Groundnut) & Head, Agricultural Research Station, Acharya N. G. Ranga Agricultural University (ANGRAU), Kadiri, Anantapur, Andhra Pradesh for providing seeds of Kadiri-7 and Kadiri-9 varieties, used in this research work. We express our grateful thanks to Prof. S. Rajagopal, Department of Plant Sciences, School of Life Sciences, University of Hyderabad for his help in carrying the experiments of photosynthesis. Our grateful thanks to Dr. M. Muthamilarasan, Department of Plant Sciences, School of Life Sciences, University of Hyderabad for his helpful suggestions during the study. Thanks to Mr. Arpan Chatterjee, Department of Biochemistry, University of Hyderabad for helping out in immunoblot analysis. Mr. Ayon Chatterjee, Department of Biochemistry, University of Hyderabad has helped in HPLC analysis, which is gratefully acknowledged.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2022.1069143/full#supplementary-material

References

Aanangi, R., Kotapati, K. V., Palaka, B. K., Kedam, T., Kanika, N. D., and Ampasala, D. R. (2016). Purification and characterization of lipoxygenase from mung bean (*Vigna radiata* l.) germinating seedlings. *3 Biotech.* 6 (1), 1–8. doi: 10.1007/s13205-016-0427-5

Abeed, A. H., Eissa, M. A., and Abdel-Wahab, D. A. (2021). Effect of exogenously applied jasmonic acid and kinetin on drought tolerance of wheat cultivars based on morpho-physiological evaluation. *J. Soil Sci. Plant Nutr.* 21 (1), 131–144. doi: 10.1007/s42729-020-00348-1

Ahmad, P., Rasool, S., Gul, A., Sheikh, S. A., Akram, N. A., Ashraf, M., et al. (2016). Jasmonates: multifunctional roles in stress tolerance. *Front. Plant Sci.* 7, 813. doi: 10.3389/fpls.2016.00813

Ahmad, S., Wang, G. Y., Muhammad, I., Farooq, S., Kamran, M., Ahmad, I., et al. (2022). Application of melatonin-mediated modulation of drought tolerance by regulating photosynthetic efficiency, chloroplast ultrastructure, and endogenous hormones in maize. *Chem. Biol. Technol. Agric.* 9 (1), 1–14. doi: 10.1186/s40538-021-00272-1

Aiamla-or, S., Kaewsuksaeng, S., Shigyo, M., and Yamauchi, N. (2010). Impact of UV-b irradiation on chlorophyll degradation and chlorophyll-degrading enzyme activities in stored broccoli (*Brassica oleracea* l. italica group) florets. *Food Chem.* 120 (3), 645–651. doi: 10.1016/j.foodchem.2009.10.056

Ajithkumar, I. P., and Panneerselvam, R. (2014). ROS scavenging system, osmotic maintenance, pigment and growth status of *Panicum sumatrense* roth. under drought stress. *Cell Biochem. Biophys.* 68 (3), 587–595. doi: 10.1007/s12013-013-9746-x

Alam, M., Nahar, K., Hasanuzzaman, M., and Fujita, M. (2014). Exogenous jasmonic acid modulates the physiology, antioxidant defense and glyoxalase systems in imparting drought stress tolerance in different brassica species. *Plant Biotechnol. Rep.* 8 (3), 279–293. doi: 10.1007/s11816-014-0321-8

Alexieva, V., Sergiev, I., Mapelli, S., and Karanov, E. (2001). The effect of drought and ultraviolet radiation on growth and stress markers in pea and wheat. *Plant Cell Environ.* 24 (12), 1337–1344. doi: 10.1046/j.1365-3040.2001.00778.x

Alharbi, B. M., Elhakem, A. H., Alnusairi, G. S. H., Soliman, M. H., Hakeem, K. R., Hasan, M. M., et al. (2021). Exogenous application of melatonin alleviates salt stress-induced decline in growth and photosynthesis in *Glycine max* (L.) seedlings by improving mineral uptake, antioxidant and glyoxalase system. *Plant Soil Environ.* 67, 208–220. doi: 10.17221/659/2020-PSE

Antoniou, C., Chatzimichail, G., Xenofontos, R., Pavlou, J. J., Panagiotou, E., Christou, A., et al. (2017). Melatonin systemically ameliorates drought stress-induced damage in *Medicago sativa* plants by modulating nitro-oxidative homeostasis and proline metabolism. *J. Pineal Res.* 62 (4), e12401. doi: 10.1111/jpi.12401

Arnao, M. B., and Hernández-Ruiz, J. (2009). Protective effect of melatonin against chlorophyll degradation during the senescence of barley leaves. *J. Pineal Res.* 46 (1), 58–63. doi: 10.1111/j.1600-079X.2008.00625.x

Bandurska, H., Stroiński, A., and Kubiś, J. (2003). The effect of jasmonic acid on the accumulation of ABA, proline and spermidine and its influence on membrane injury under water deficit in two barley genotypes. *Acta Physiol. Plantarum* 25 (3), 279–285. doi: 10.1007/s11738-003-0009-0

Bates, L. S., Waldren, R. P., and Teare, I. D. (1973). Rapid determination of free proline for water-stress studies. *Plant Soil* 39 (1), 205–207. doi: 10.1007/BF00018060

Beauchamp, C., and Fridovich, I. (1971). Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. *Analytical Biochem.* 44 (1), 276–287. doi: 10.1016/0003-2697(71)90370-8

Bhowal, B., Bhattacharjee, A., Goswami, K., Sanan-Mishra, N., Singla-Pareek, S. L., Kaur, C., et al. (2021). Serotonin and melatonin biosynthesis in plants: genome-wide identification of the genes and their expression reveal a conserved role in stress and development. *Int. J. Mol. Sci.* 22 (20), 11034. doi: 10.3390/ijms222011034

Blée, E. (2002). Impact of phyto-oxylipins in plant defense. *Trends Plant Sci.* 7 (7), 315–322. doi: 10.1016/S1360-1385(02)02290-2

Buttar, Z. A., Wu, S. N., Arnao, M. B., Wang, C., Ullah, I., and Wang, C. (2020). Melatonin suppressed the heat stress-induced damage in wheat seedlings by modulating the antioxidant machinery. *Plants* 9 (7), 809. doi: 10.3390/plants9070809

Carvalho, M. J., Vorasoot, N., Puppala, N., Muitia, A., and Jogloy, S. (2017). Effects of terminal drought on growth, yield and yield components in Valencia peanut genotypes. *SABRAO J. Breed. Genet.* 49 (3), 270–279.

Chen, X., Wang, D., Zhang, C., Wang, X., Yang, K., Wang, Y., et al. (2022). The apple lipoxygenase *MdLOX3* regulates salt tolerance and ABA sensitivity. *Horticulturae* 8 (7), 651. doi: 10.3390/horticulturae8070651

Chung, T., Suttangkakul, A., and Vierstra, R. D. (2009). The ATG autophagic conjugation system in maize: ATG transcripts and abundance of the ATG8-lipid adduct are regulated by development and nutrient availability. *Plant Physiol.* 149 (1), 220–234. doi: 10.1104/pp.108.126714

Debnath, B., Islam, W., Li, M., Sun, Y., Lu, X., Mitra, S., et al. (2019). Melatonin mediates enhancement of stress tolerance in plants. *Int. J. Mol. Sci.* 20 (5), 1040. doi: 10.3390/ijms20051040

de Lima, V. R., Caro, M. S., Munford, M. L., Desbat, B., Dufourc, E., Pasa, A. A., et al. (2010). Influence of melatonin on the order of phosphatidylcholine-based membranes. *J. Pineal Res.* 49 (2), 169–175. doi: 10.1111/j.1600-079X.2010.00782.x

de Ollas, C., Arbona, V., and GóMez-Cadenas, A. (2015). Jasmonoyl isoleucine accumulation is needed for abscisic acid build-up in roots of *Arabidopsis* under water stress conditions. *Plant Cell Environ.* 38 (10), 2157–2170. doi: 10.1111/pce.12536

Dionisio-Sese, M. L., and Tobita, S. (1998). Antioxidant responses of rice seedlings to salinity stress. *Plant Sci.* 135 (1), 1–9. doi: 10.1016/S0168-9452(98)00025-9

Elstner, E. F., and Heupel, A. (1976). Inhibition of nitrite formation from hydroxylammoniumchloride: a simple assay for superoxide dismutase. *Analytical Biochem.* 70 (2), 616–620. doi: 10.1016/0003-2697(76)90488-7

Erbil, E. (2021). The effects of gibberellic acid (GA_3) applications on the physiological features of groundnut (*Arachis hypogaea* l.) under different salt (NaCl) stress conditions. *Legume Research- Int. J.* 44 (10), 1159–1163. doi: 10.18805/LR-620

Fang, Z., Bouwkamp, J. C., and Solomos, T. (1998). Chlorophyllase activities and chlorophyll degradation during leaf senescence in non-yellowing mutant and wild type of *Phaseolus vulgaris* l. *J. Exp. Bot.* 49 (320), 503–510. doi: 10.1093/jxb/49.320.503

Farooq, M., Usman, M., Nadeem, F., ur Rehman, H., Wahid, A., Basra, S. M., et al. (2019). Seed priming in field crops: Potential benefits, adoption and challenges. *Crop Pasture Sci.* 70 (9), 731–771. doi: 10.1071/CP18604

Fu, J., and Huang, B. (2001). Involvement of antioxidants and lipid peroxidation in the adaptation of two cool-season grasses to localized drought stress. *Environ. Exp. Bot.* 45 (2), 105–114. doi: 10.1016/S0098-8472(00)00084-8

Gogorcena, Y., Gordon, A. J., Escuredo, P. R., Minchin, F. R., Witty, J. F., Moran, J. F., et al. (1997). N2 fixation, carbon metabolism, and oxidative damage in nodules of dark-stressed common bean plants. *Plant Physiol.* 113 (4), 1193–1201. doi: 10.1104/pp.113.4.1193

Govindjee, G. (2004). "Chlorophyll a fluorescence: a bit of basics and history," in *Chlorophyll a fluorescence: A signature of photosynthesis* (Dordrecht: Springer), 1–42.

Gray, W. M. (2004). Hormonal regulation of plant growth and development. *PloS Biol.* 2 (9), e311. doi: 10.1371/journal.pbio.0020311

Guo, Y., Zhu, J., Liu, J., Xue, Y., Chang, J., Zhang, Y., et al. (2022). Melatonin delays ABA-induced leaf senescence *via* H_2O_2-dependent calcium signaling. *Plant Cell Environ.* doi: 10.1111/pce.14482

Heath, R. L., and Packer, L. (1968). Photoperoxidation in isolated chloroplasts: I. kinetics and stoichiometry of fatty acid peroxidation. *Arch. Biochem. Biophys.* 125 (1), 189–198. doi: 10.1016/0003-9861(68)90654-1

Heuer, B. (2003). Influence of exogenous application of proline and glycine betaine on growth of salt-stressed tomato plants. *Plant Sci.* 165 (4), 693–699. doi: 10.1016/S0168-9452(03)00222-X

Hörtensteiner, S. (2006). Chlorophyll degradation during senescence. *Annu. Rev. Plant Biol.* 57, 55–77. doi: 10.1146/annurev.arplant.57.032905.105212

Hou, Y., Meng, K., Han, Y., Ban, Q., Wang, B., Suo, J., et al. (2015). The persimmon 9-lipoxygenase gene *DkLOX3* plays positive roles in both promoting senescence and enhancing tolerance to abiotic stress. *Front. Plant Sci.* 6, 1073. doi: 10.3389/fpls.2015.01073

Huangfu, L., Chen, R., Lu, Y., Zhang, E., Miao, J., Zuo, Z., et al. (2022). *OsCOMT*, encoding a caffeic acid O-methyltransferase in melatonin biosynthesis, increases rice grain yield through dual regulation of leaf senescence and vascular development. *Plant Biotechnol. J.* 20 (6), 1122–1139. doi: 10.1111/pbi.13794

Hu, Z., Fu, Q., Zheng, J., Zhang, A., and Wang, H. (2020). Transcriptomic and metabolomic analyses reveal that melatonin promotes melon root development under copper stress by inhibiting jasmonic acid biosynthesis. *Horticult. Res.* 7, 79. doi: 10.1038/s41438-020-0293-5

Ibrahim, E. A. (2016). Seed priming to alleviate salinity stress in germinating seeds. *J. Plant Physiol.* 192, 38–46. doi: 10.1016/j.jplph.2015.12.011

Iriyama, K., Ogura, N., and Takamiya, A. (1974). A simple method for extraction and partial purification of chlorophyll from plant material, using dioxane. *J. Biochem.* 76 (4), 901–904. doi: 10.1093/oxfordjournals.jbchem.a130638

Jaleel, C. A., Gopi, R., and Panneerselvam, R. (2007). Alterations in lipid peroxidation, electrolyte leakage, and proline metabolism in *Catharanthus roseus* under treatment with triadimefon, a systemic fungicide. *Comptes Rendus Biol.* 330 (12), 905–912. doi: 10.1016/j.crvi.2007.10.001

Jisha, K. C., Vijayakumari, K., and Puthur, J. T. (2013). Seed priming for abiotic stress tolerance: an overview. *Acta Physiol. Plantarum* 35 (5), 1381–1396. doi: 10.1007/s11738-012-1186-5

Jung, S. (2004). Variation in antioxidant metabolism of young and mature leaves of *Arabidopsis thaliana* subjected to drought. *Plant Sci.* 166 (2), 459–466. doi: 10.1016/j.plantsci.2003.10.012

Kaewsuksaeng, S., Urano, Y., Aiamla-or, S., Shigyo, M., and Yamauchi, N. (2011). Effect of UV-b irradiation on chlorophyll-degrading enzyme activities and postharvest quality in stored lime (*Citrus latifolia* tan.) fruit. *Postharvest Biol. Technol.* 61 (2-3), 124–130. doi: 10.1016/j.postharvbio.2011.02.014

Kar, R. K. (2011). Plant responses to water stress: role of reactive oxygen species. *Plant Signaling Behav.* 6 (11), 1741–1745. doi: 10.4161/psb.6.11.17729

Khalvandi, M., Siosemardeh, A., Roohi, E., and Keramati, S. (2021). Salicylic acid alleviated the effect of drought stress on photosynthetic characteristics and leaf protein pattern in winter wheat. *Heliyon* 7 (1), e05908. doi: 10.1016/j.heliyon.2021.e05908

Khan, T. A., Ahmed, Z. I., Syed, S., Baloch, A., Malik, M. N., Irfan, N., et al. (2017). Seed priming with iron and zinc improves growth and yield of groundnut (*Arachis hypogaea* l.). *Pure Appl. Biol.* 6 (2), 553–560. doi: 10.19045/bspab.2017.60057

Khan, M. N., Zhang, J., Luo, T., Liu, J., Rizwan, M., Fahad, S., et al. (2019). Seed priming with melatonin coping drought stress in rapeseed by regulating reactive oxygen species detoxification: Antioxidant defense system, osmotic adjustment, stomatal traits and chloroplast ultrastructure perseveration. *Ind. Crops Prod.* 140, 111597. doi: 10.1016/j.indcrop.2019.111597

Kim, E. H., Kim, Y. S., Park, S. H., Koo, Y. J., Choi, Y. D., Chung, Y. Y., et al. (2009). Methyl jasmonate reduces grain yield by mediating stress signals to alter spikelet development in rice. *Plant Physiol.* 149 (4), 1751–1760. doi: 10.1104/pp.108.134684

Lee, H. Y., and Back, K. (2020). The phytomelatonin receptor (PMRT1) *Arabidopsis* Cand2 is not a *bona fide* G protein–coupled melatonin receptor. *Melatonin Res.* 3 (2), 177–186. doi: 10.32794/mr11250055

Lerner, A. B., Case, J. D., Takahashi, Y., Lee, T. H., and Mori, W. (1958). Isolation of melatonin, the pineal gland factor that lightens melanocyte S1. *J. Am. Chem. Soc.* 80 (10), 2587–2587. doi: 10.1021/ja01543a060

Liang, X., Zhang, L., Natarajan, S. K., and Becker, D. F. (2013). Proline mechanisms of stress survival. *Antioxid. Redox Signaling* 19 (9), 998–1011. doi: 10.1089/ars.2012.5074

Lichtenthaler, H. K., and Wellburn, A. R. (1983). Determination of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. *Biochem. Soc. Trans.* 11, 591–592. doi: 10.1042/bst0110591

Li, H., Guo, Y., Lan, Z., Zhang, Z., Ahammed, G. J., Chang, J., et al. (2021). Melatonin antagonizes ABA action to promote seed germination by regulating Ca^{2+} efflux and H_2O_2 accumulation. *Plant Sci.* 303, 110761. doi: 10.1016/j.plantsci.2020.110761

Lim, C. W., Han, S. W., Hwang, I. S., Kim, D. S., Hwang, B. K., and Lee, S. C. (2015). The pepper lipoxygenase *CaLOX1* plays a role in osmotic, drought and high salinity stress response. *Plant Cell Physiol.* 56 (5), 930–942. doi: 10.1093/pcp/pcv020

Lin, C. C., and Kao, C. H. (1999). NaCl Induced changes in ionically bound peroxidase activity in roots of rice seedlings. *Plant Soil* 216 (1), 147–153. doi: 10.1023/A:1004714506156

Li, X., Tan, D. X., Jiang, D., and Liu, F. (2016). Melatonin enhances cold tolerance in drought-primed wild-type and abscisic acid-deficient mutant barley. *J. Pineal Res.* 61 (3), 328–339. doi: 10.1111/jpi.12350

Li, C., Tan, D. X., Liang, D., Chang, C., Jia, D., and Ma, F. (2015). Melatonin mediates the regulation of ABA metabolism, free-radical scavenging, and stomatal behaviour in two *Malus* species under drought stress. *J. Exp. Bot.* 66 (3), 669–680. doi: 10.1093/jxb/eru476

Livak, K. J., and Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods* 25 (4), 402–408. doi: 10.1006/meth.2001.1262

Loggini, B., Scartazza, A., Brugnoli, E., and Navari-Izzo, F. (1999). Antioxidative defense system, pigment composition, and photosynthetic efficiency in two wheat cultivars subjected to drought. *Plant Physiol.* 119 (3), 1091–1100. doi: 10.1104/pp.119.3.1091

Luo, C., Min, W., Akhtar, M., Lu, X., Bai, X., Zhang, Y., et al. (2022). Melatonin enhances drought tolerance in rice seedlings by modulating antioxidant systems, osmoregulation, and corresponding gene expression. *Int. J. Mol. Sci.* 23 (20), 12075. doi: 10.3390/ijms232012075

Maynard, D., Chibani, K., Schmidtpott, S., Seidel, T., Spross, J., Viehhauser, A., et al. (2021). Biochemical characterization of 13-lipoxygenases of *Arabidopsis thaliana*. *Int. J. Mol. Sci.* 22 (19), 10237. doi: 10.3390/ijms221910237

Ma, X., Zhang, J., Burgess, P., Rossi, S., and Huang, B. (2018). Interactive effects of melatonin and cytokinin on alleviating drought-induced leaf senescence in creeping bentgrass (*Agrostis stolonifera*). *Environ. Exp. Bot.* 145, 1–11. doi: 10.1016/j.envexpbot.2017.10.010

Mittler, R. (2002). Oxidative stress, antioxidants and stress tolerance. *Trends Plant Sci.* 7 (9), 405–410. doi: 10.1016/S1360-1385(02)02312-9

Mou, Y., Sun, Q., Yuan, C., Zhao, X., Wang, J., Yan, C., et al. (2022). Identification of the LOX gene family in peanut and functional characterization of *AhLOX29* in drought tolerance. *Front. Plant Sci.* 13. doi: 10.3389/fpls.2022.832785

Nakano, Y., and Asada, K. (1981). Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant Cell Physiol.* 22 (5), 867–880. doi: 10.1093/oxfordjournals.pcp.a076232

Pal, A., Ali, M. A., and Pal, A. K. (2017). Effect of seed priming on germination behavior, oxidative stress and antioxidant enzyme activities in groundnut (*Arachis hypogaea* l.) under salinity stress. *Bull. Environ. Pharmacol. Life Sci.* 6 (1), 479–485.

Pan, X., Welti, R., and Wang, X. (2010). Quantitative analysis of major plant hormones in crude plant extracts by high-performance liquid chromatography–mass spectrometry. *Nat. Protoc.* 5 (6), 986–992. doi: 10.1038/nprot.2010.37

Patterson, B. D., MacRae, E. A., and Ferguson, I. B. (1984). Estimation of hydrogen peroxide in plant extracts using titanium (IV). *Analytical Biochem.* 139 (2), 487–492. doi: 10.1016/0003-2697(84)90039-3

Porta, H., and Rocha-Sosa, M. (2002). Plant lipoxygenases. physiological and molecular features. *Plant Physiol.* 130 (1), 15–21. doi: 10.1104/pp.010787

Production volume of groundnut across India from financial year 2013 to 2021, with estimates until 2022. Available at: https://www.statista.com/statistics/769471/india-groundnut-production-volume/ (Accessed 16 September, 2022).

Qamer, Z., Chaudhary, M. T., Du, X., Hinze, L., and Azhar, M. T. (2021). Review of oxidative stress and antioxidative defense mechanisms in *Gossypium hirsutum* l. @ in response to extreme abiotic conditions. *J. Cotton Res.* 4 (1), 1–9. doi: 10.1186/s42397-021-00086-4

Radogna, F., Sestili, P., Martinelli, C., Paolillo, M., Paternoster, L., Albertini, M. C., et al. (2009). Lipoxygenase-mediated pro-radical effect of melatonin *via* stimulation of arachidonic acid metabolism. *Toxicol. Appl. Pharmacol.* 238 (2), 170–177. doi: 10.1016/j.taap.2009.05.011

Ramel, F., Sulmon, C., Bogard, M., Couée, I., and Gouesbet, G. (2009). Differential patterns of reactive oxygen species and antioxidative mechanisms during atrazine injury and sucrose-induced tolerance in *Arabidopsis thaliana* plantlets. *BMC Plant Biol.* 9 (1), 1–18. doi: 10.1186/1471-2229-9-28

Reddy, T. Y., Reddy, V. R., and Anbumozhi, V. (2003). Physiological responses of groundnut (*Arachis hypogea* l.) to drought stress and its amelioration: a critical review. *Plant Growth Regul.* 41 (1), 75–88. doi: 10.1023/A:1027353430164

Ressmeyer, A. R., Mayo, J. C., Zelosko, V., Sáinz, R. M., Tan, D. X., Poeggeler, B., et al. (2003). Antioxidant properties of the melatonin metabolite N1-acetyl-5-methoxykynuramine (AMK): scavenging of free radicals and prevention of protein destruction. *Redox Rep.* 8 (4), 205–213. doi: 10.1179/135100003225002709

Roy, D., Basu, N., Bhunia, A., and Banerjee, S. K. (1993). Counteraction of exogenous l-proline with NaCl in salt-sensitive cultivar of rice. *Biol. Plantarum* 35 (1), 69–72. doi: 10.1007/BF02921122

Ruan, J., Zhou, Y., Zhou, M., Yan, J., Khurshid, M., Weng, W., et al. (2019). Jasmonic acid signaling pathway in plants. *Int. J. Mol. Sci.* 20 (10), 2479. doi: 10.3390/ijms20102479

Singh, P., Arif, Y., Miszczuk, E., Bajguz, A., and Hayat, S. (2022). Specific roles of lipoxygenases in development and responses to stress in plants. *Plants* 11 (7), 979. doi: 10.3390/plants11070979

Sperdouli, I., and Moustakas, M. (2012). Differential response of photosystem II photochemistry in young and mature leaves of *Arabidopsis thaliana* to the onset of drought stress. *Acta Physiol. Plantarum* 34 (4), 1267–1276. doi: 10.1007/s11738-011-0920-8

Stagnari, F., Galieni, A., and Pisante, M. (2016). "Drought stress effects on crop quality," in *Water stress and crop plants: a sustainable approach*, vol. 2. (New Jersey: Wiley-Blackwell), 375–392.

Stalker, H. T. (1997). Peanut (*Arachis hypogaea* l.). *Field Crops Res.* 53 (1-3), 205–217. doi: 10.1016/S0378-4290(97)00032-4

Supriya, L., Durgeshwar, P., Muthamilarasan, M., and Padmaja, G. (2022). Melatonin mediated differential regulation of drought tolerance in sensitive and tolerant varieties of upland cotton (*Gossypium hirsutum* l.). *Front. Plant Sci.* 13, 821353. doi: 10.3389/fpls.2022.821353

Surrey, K. (1964). Spectrophotometric method for determination of lipoxidase activity. *Plant Physiol.* 39 (1), 65–70. doi: 10.1104/pp.39.1.65

Tan, D. X. (1993). Melatonin: a potent, endogenous hydroxyl radical scavenger. *Endocrinol. J.* 1, 57–60.

Tuteja, N. (2007). Abscisic acid and abiotic stress signaling. *Plant Signaling Behav.* 2 (3), 135–138. doi: 10.4161/psb.2.3.4156

Wang, X., Li, Q., Xie, J., Huang, M., Cai, J., Zhou, Q., et al. (2021). Abscisic acid and jasmonic acid are involved in drought priming-induced tolerance to drought in wheat. *Crop J.* 9 (1), 120–132. doi: 10.1016/j.cj.2020.06.002

Wang, P., Sun, X., Li, C., Wei, Z., Liang, D., and Ma, F. (2013). Long-term exogenous application of melatonin delays drought-induced leaf senescence in apple. *J. Pineal Res.* 54 (3), 292–302. doi: 10.1111/jpi.12017

Warner, K., and Afifi, T. (2014). Where the rain falls: Evidence from 8 countries on how vulnerable households use migration to manage the risk of rainfall variability and food insecurity. *Climate Dev.* 6 (1), 1–17. doi: 10.1080/17565529.2013.835707

Weeda, S., Zhang, N., Zhao, X., Ndip, G., Guo, Y., Buck, G. A., et al. (2014). *Arabidopsis* transcriptome analysis reveals key roles of melatonin in plant defense systems. *PloS One* 9 (3), e93462. doi: 10.1371/journal.pone.0093462

Xing, Q., Liao, J., Cao, S., Li, M., Lv, T., and Qi, H. (2020). *CmLOX10* positively regulates drought tolerance through jasmonic acid-mediated stomatal closure in oriental melon (*Cucumis melo* var. makuwa makino). *Sci. Rep.* 10 (1), 1–14. doi: 10.1038/s41598-020-74550-7

Xing, Q., Zhang, X., Li, Y., Shao, Q., Cao, S., Wang, F., et al. (2019). The lipoxygenase *CmLOX13* from oriental melon enhanced severe drought tolerance *via* regulating ABA accumulation and stomatal closure in *Arabidopsis*. *Environ. Exp. Bot.* 167, 103815. doi: 10.1016/j.envexpbot.2019.103815

Yoshida, R., Hobo, T., Ichimura, K., Mizoguchi, T., Takahashi, F., Aronso, J., et al. (2002). ABA-activated *SnRK2* protein kinase is required for dehydration stress signaling in *Arabidopsis*. *Plant Cell Physiol.* 43 (12), 1473–1483. doi: 10.1093/pcp/pcf188

Zhang, M., He, S., Qin, B., Jin, X., Wang, M., Ren, C., et al. (2020). Exogenous melatonin reduces the inhibitory effect of osmotic stress on antioxidant properties and cell ultrastructure at germination stage of soybean. *PloS One* 15 (12), e0243537. doi: 10.1371/journal.pone.0243537

Zhang, N., Zhao, B., Zhang, H. J., Weeda, S., Yang, C., Yang, Z. C., et al. (2013). Melatonin promotes water-stress tolerance, lateral root formation, and seed germination in cucumber (*Cucumis sativus* l.). *J. Pineal Res.* 54 (1), 15–23. doi: 10.1111/j.1600-079X.2012.01015.x

Zhao, L., Ding, Q., Zeng, J., Wang, F. R., Zhang, J., Fan, S. J., et al. (2012). An improved CTAB–ammonium acetate method for total RNA isolation from cotton. *Phytochem. Anal.* 23 (6), 647–650. doi: 10.1002/pca.2368

frontiers | Frontiers in Plant Science

TYPE Review
PUBLISHED 14 February 2023
DOI 10.3389/fpls.2023.1108507

EDITED BY
Muhammad Ahsan Altaf,
Hainan University, China

REVIEWED BY
Shahbaz Atta Tung,
Pir Mehr Ali Shah Arid Agriculture University, Pakistan
Anis Ali Shah,
University of Education Lahore, Pakistan
Muhammad Faheem Adil,
Zhejiang University, China

*CORRESPONDENCE
Guisheng Zhou
gszhou@yzu.edu.cn

SPECIALTY SECTION
This article was submitted to Plant Abiotic Stress, a section of the journal Frontiers in Plant Science

RECEIVED 26 November 2022
ACCEPTED 09 January 2023
PUBLISHED 14 February 2023

CITATION
Ahmad I, Song X, Hussein Ibrahim ME, Jamal Y, Younas MU, Zhu G, Zhou G and Adam Ali AY (2023) The role of melatonin in plant growth and metabolism, and its interplay with nitric oxide and auxin in plants under different types of abiotic stress.
Front. Plant Sci. 14:1108507.
doi: 10.3389/fpls.2023.1108507

The role of melatonin in plant growth and metabolism, and its interplay with nitric oxide and auxin in plants under different types of abiotic stress

Irshad Ahmad[1], Xudong Song[2], Muhi Eldeen Hussein Ibrahim[1,3], Yousaf Jamal[4], Muhammad Usama Younas[5], Guanglong Zhu[1], Guisheng Zhou[1,6]* and Adam Yousif Adam Ali[7]

[1]Joint International Research Laboratory of Agriculture and Agri-Product Safety of the Ministry of Education of China, College of Agriculture, Yangzhou University, Yangzhou, China, [2]Department of Agronomy, Institute of Agricultural, Sudan University of Science and Technology, Khartoum, Sudan, [3]Jiangsu Yanjiang Area, Institute of Agricultural Sciences, Nantong, China, [4]Department of Agronomy, Faculty of Agriculture, University of Swabi, Swabi, Pakistan, [5]Department of Crop Genetics and Breeding, College of Agriculture, Yangzhou University, Yangzhou, China, [6]Key Lab of Crop Genetics & Physiology of Jiangsu Province, Yangzhou University, Yangzhou, China, [7]Department of Agronomy, Faculty of Agricultural and Environmental Science, University of Gadarif, Al Gadarif, Sudan

Melatonin is a pleiotropic signaling molecule that reduces the adverse effects of abiotic stresses, and enhances the growth and physiological function of many plant species. Several recent studies have demonstrated the pivotal role of melatonin in plant functions, specifically its regulation of crop growth and yield. However, a comprehensive understanding of melatonin, which regulates crop growth and yield under abiotic stress conditions, is not yet available. This review focuses on the progress of research on the biosynthesis, distribution, and metabolism of melatonin, and its multiple complex functions in plants and its role in the mechanisms of metabolism regulation in plants grown under abiotic stresses. In this review, we focused on the pivotal role of melatonin in the enhancement of plant growth and regulation of crop yield, and elucidated its interactions with nitric oxide (NO) and auxin (IAA, indole-3-acetic acid) when plants are grown under various abiotic stresses. The present review revealed that the endogenousapplication of melatonin to plants, and its interactions with NO and IAA, enhanced plant growth and yield under various abiotic stresses. The interaction of melatonin with NO regulated plant morphophysiological and biochemical activities, mediated by the G protein-coupled receptor and synthesis genes. The interaction of melatonin with IAA enhanced plant growth and physiological function by increasing the levels of IAA, synthesis, and polar transport. Our aim was to provide a comprehensive review of the performance of melatonin under various abiotic stresses, and, therefore, further explicate the mechanisms that plant hormones use to regulate plant growth and yield under abiotic stresses.

KEYWORDS

abiotic stresses, auxin, nitric oxide, phytomelatonin, plant growth and metabolism

Introduction

Abiotic stresses continuously reduce the growth and yield of different crops (Zhang et al., 2021a; Ahmad et al., 2022a). The growing of plants in altered environments often creates abiotic stresses such as salinity, drought, heat, cold, and heavy metals. The imposition of abiotic stresses can certainly affect plants' morphophysiological, biochemical, and molecular activity, from seed germination to maturity, and, eventually, cause higher losses in plant yields (Rahman et al., 2022). It has been demonstrated that about 70% of staple food crop yields are adversely affected by abiotic stresses (Khan et al., 2015). These stresses induce numerous changes in the metabolism of plants by producing reactive oxygen species (ROS), which in turn disturb homeostasis and ion distribution in plants (Raza et al., 2022). Improving the response of plants to these stresses is particularly important for sustainable plant production (Gonzalez Guzman et al., 2022). Over the last few decades, tremendous efforts have been made by research scientists to enhance plant growth and yields *via* the extensive application of chemicals.

Melatonin (*N*-acetyl-5-methoxytryptamine) is an important bioactive compound in vascular plants, discovered in 1995 (Dubbels et al., 1995). Initially, it was regarded as a powerful antioxidant that had different beneficial roles in various stages of plant growth and development (Sheshadri et al., 2018), such as germination (Zhang et al., 2017), root elongation (Arnao and Hernández-Ruiz, 2019), photosynthesis (Li et al., 2017), and leaf senescence (Wang et al., 2022). It has also been a plant hormonewith an important role in enhancing the growth and regulation of plants (Arnao and Hernández-Ruiz, 2019). It is found in various plants tissues, such as the seeds, roots, leaves, and fruits (Zhang et al., 2017). Thepotential role melatonin could play in the enhancement of plant growth andregulation has been widely investigated by scientific researchers (Sun et al., 2020).

Recently, it has been reported that melatonin increases the fatty acid content and enhances the profile of alkaloids in coffee and soybean plants (Ramakrishna et al., 2012). However, the mechanism of enhanced fatty acid production *via* melatonin is far from clear and needs to be further investigated in different crops under various abiotic stresses. As a multiregulatory molecule, melatonin regulates the expression of genes involved in plant growth and development (Byeon and Back, 2014), redox reactions (Tomas and Montes, 2005), abiotic stress resistance (Boccalandro et al., 2011), sucrose metabolism [cell wall invertase (CWIN) and sucrose synthase (SUSY)] (Solfanelli et al., 2006; Dutta et al., 2013; Payyavula et al., 2013), and specialized metabolism [phenylpropanoid metabolism: phenylalanine ammonia lyase (PAL), chalcone synthase (CHS), chalcone isomerase (CHI), flavanone 3-hydroxylase (F3H), dihydroflavonol reductase (DFR), and anthocyanidin synthase (ANS)] (Weeda et al., 2014). The phytomelatonin receptor PMTR1 mediates the signaling of ROS, regulates homeostasis or, and delivers a dark indication to promote night stomatal closure (thus avoiding water loss during the night), thereby facilitating plant adaptation to dry land environments (Li et al., 2020). However, what genes participate in the signaling pathway to promote night stomatal closure, and how these genes evolved to facilitate plant adaptation to dry land environments, is still far from clear. In addition, because of the limitation of experimental methods, there is still no definitive evidence showing that melatonin function in plant organs is significantly enhanced at night as compared with the daytime (Van Tassel et al., 2001; Xie et al., 2022). The findings of various studies related to the role of phytomelatonin are of huge significance (Zhang et al., 2021b). Melatonin gives plants resistance to drought (Wang et al., 2014), salt (Hernández et al., 2015), osmotic stress (Zhang et al., 2013), high temperature (Byeon and Back, 2014), cold (Bajwa et al., 2014), and copper stress (Posmyk et al., 2009a).

It has been confirmed that the application of exogenous melatonin can mitigate the effects of abiotic stresses in various crops (Cao et al., 2019). Lower doses of melatonin (i.e., <10 μM) have been shown to promote seed germination and lateral root formation in cucumber plants under cold and drought stresses (Zhang et al., 2013; Simlat et al., 2018). In corn seedlings, melatonin increased drought resistance by alleviating oxidative damage and drought-induced photosynthetic inhibition (Ye et al., 2016). The pretreatment of melatonin also increased endogenous melatonin and inhibited the up-regulation of *NCED1* genes, but selectively up-regulated catabolic genes, such as *ABA80x1* and *ABA80x3*, and abscisic acid (ABA)-related synthesis genes, and decreased the accumulation of ABA and induced stomatal reopening in corn under drought stress (Li et al., 2021). In apple trees, melatonin maintained drought tolerance by regulating the concentrations of ABA metabolism and stomatal behavior (Li et al., 2015). In barley, the exogenous supply of melatonin increased photosynthetic carbon assimilation by improving the antioxidant defense of organelles under low temperature or drought stresses (Li et al., 2016). To date, most of the components in melatonin-related signaling pathways remain unclear and need to be further investigated, especially in plants under abiotic stresses (Zhou et al., 2020). In previous studies, melatonin has been shown to be present at high concentrations in several crops (e.g., wheat, rice, barley, corn, grape, oats, and tobacco), and in popular beverages (e.g., tea, coffee, and wine) (Arnao and Hernández-Ruiz, 2009; Ramakrishna et al., 2012; Arnao and Hernández-Ruiz, 2013; Shi et al., 2015a). However, it is still unknown if the response of melatonin in plants under various stresses is the same across different crops.

Therefore, in this manuscript we have aimed to provide a comprehensive review of advances in our knowledge of the roles, biosynthesis, distribution, metabolism, functions, and mechanisms of melatonin in regulating the growth and development of various crops under abiotic stresses. In addition, the interactions of melatonin with other phytohormones, such as nitric oxide (NO) and auxin (IAA, indole-3-acetic acid), are analyzed.

Melatonin biosynthesis

The biosynthetic pathway of melatonin in plants is well documented (Park et al., 2012; Kang et al., 2013). The concept of plant-synthesized melatonin was first introduced in an isotope tracer study (Murch et al., 2000). The biosynthetic pathway of phytomelatonin in vascular plants is thought to be similar to that in animals, although there is much debate surrounding this (Murch et al., 2000; Tan et al., 2013; Zhao et al., 2019). Based on a number of findings, tryptophan is considered as the initial substrate of melatonin synthesis and is involved in four enzymatic steps catalyzed by at least six enzymes, including tryptophan decarboxylase (TDC), tryptophan hydroxylase (TPH), tryptamine 5-hydroxylase (T5H), serotonin *N*-acetyltransferase (SNAT), *N*-acetylserotonin methyltransferase (ASMT), and caffeic acid *O*-methyl transferase (COMT) (Back et al., 2016; Sun et al., 2021) (Figure 1). The two required processes that contribute to tryptophan are hydroxylation and decarboxylation for melatonin biosynthesis. They have been identified in herbivorous plants (Park et al., 2012). Auxin [indole-3-acetic acid (IAA)], which occurs naturally in plants, is biosynthesized from tryptophan *via* four proposed routes, that is, indole-3-acetaldoxime (IAOx), indole-3-pyruvic acid (IPyA), indole-3-acetamide (IAM), and tryptamine (TAM). The biosynthesis pathway of auxin from tryptophan is still unknown and needs to be further investigated in different crops under abiotic stresses. Serotonin is catalyzed *via* SNATs to form *N*-acetylserotonin, which is then methoxylated by ASMTs to form melatonin (Wang et al., 2017). Serotonin performs various important functions in plants, such as growth regulation and stress defense (Figure 1). Currently, the presence and function of serotonin in plants is an increasingly popular research area, but to date, there are only minor studies available about the functions of serotonin under different abiotic stresses. It has been shown that in rice TDC-catalyzed decarboxylation of tryptophan is the first step in melatonin biosynthesis, followed by T5H-catalyzed hydroxylation (Park et al., 2012). The *T5H* gene is considered an essential gene for serotonin biosynthesis. It has been found that suppression of the *T5H* gene in transgenic rice increases the melatonin concentration, suggesting that melatonin concentration in plants is not proportional to serotonin concentration (Sun et al., 2021). The increase in melatonin concentration and the up-regulation of the *T5H* gene for serotonin biosynthesis under abiotic stresses in other crops are still far from being clearly understood.

Role of melatonin in plants under abiotic stresses

The distribution, metabolism, and complex functions of melatonin in plants under abiotic stresses

The immunohistochemical localization of melatonin has demonstrated that the compound is present in the primary roots and seeds of sunflower and *Arabidopsis* seedlings (Pelagio-Flores et al., 2012; Mukherjee et al., 2014; Figure 2). The accumulation of melatonin was observed in the oily body of plants, including in the cotyledon cells of both control and salt-treated seedlings, thus showing the effect of long-distance signaling, induced by sodium chloride (NaCl) stress, from roots to cotyledons (Mukherjee et al., 2014). A study found that NaCl stress induced slower mobilization in the cotyledons of sunflower seedlings (David et al., 2010). NaCl stress caused melatonin accumulation in seedling cotyledons, and, as a result, reduced degradation of the oily body. The mobilization of the oily body and the activity of fatty acid-metabolizing enzymes are considered to mitigate the effects of salt stress (David et al., 2010). The accumulation of melatonin in cotyledons played a positive antioxidative role, in that it maintained the activity of the enzymes required for lipid mobilization during seedling growth (David et al., 2010). The accumulation mechanism of melatonin in the oily body of plants, including in the cotyledon cells of control and salt-treated seedlings, is less well documented, and further studies are required so that, ultimately, antioxidant defense systems of various crops can be improved.

Moreover, melatonin can trigger the accumulation of nitric oxide *via* its up-regulation of nitrate reductase expression and down-regulation of *S*-nitrosoglutathione reductase (GSNOR) expression.

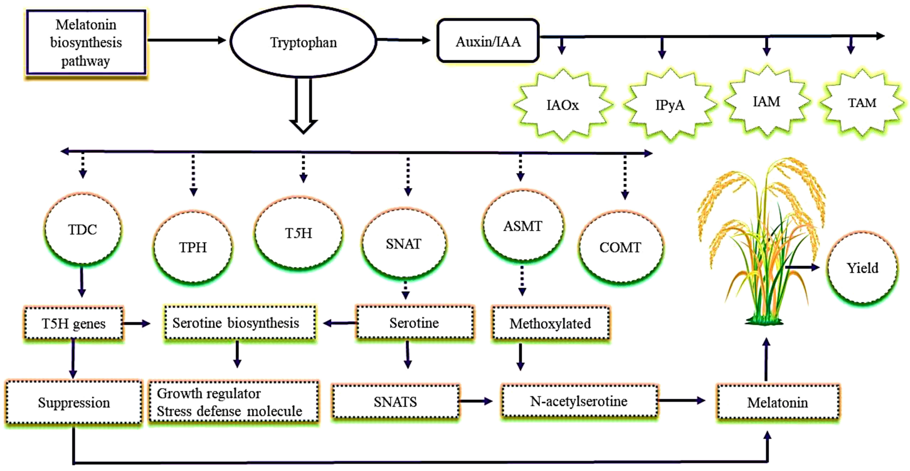


FIGURE 1
The regulatory role of biosynthetic melatonin under stress conditions. Tryptophan is the initial substrate of melatonin synthesis and is divided into four enzymatic steps catalyzed by six enzymes: tryptophan decarboxylase (TDC), tryptophan hydroxylase (TPH), tryptamine 5-hydroxylase (T5H), serotonin *N*-acetyltransferase (SNAT), *N*-acetylserotonin methyltransferase (ASMT), and caffeic acid *O*-methyl transferase (COMT). Serotonin is catalyzed *via* SNAT to form *N*-acetylserotonin, which is further methoxylated by ASMTs to form melatonin and acts as a growth regulator stress defense molecule. The *T5H* gene improves serotonin biosynthesis. TDC catalyzes decarboxylation of tryptophan, and it is considered the first step of melatonin biosynthesis. Suppression of the *T5H* gene increases the concentration of melatonin in rice plants and increases yield. Auxin produced naturally in plants is biosynthesized from tryptophan in four ways: indole-3-acetaldoxime (IAOx), indole-3-pyruvic acid (IPyA), indole-3-acetamide (IAM), and tryptamine (TAM).

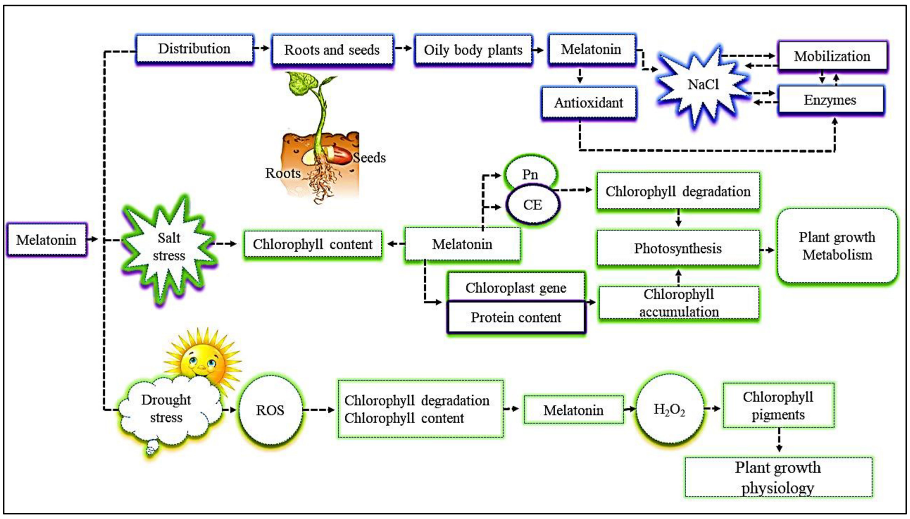


FIGURE 2
The distribution and regulatory roles of an exogenous supply of melatonin in mitigating abiotic stresses are divided into three parts. (i) Melatonin distributed in the roots and seeds. Sodium chloride (NaCl) induces slow mobilization of enzymes and reduces the enzymes' activity in the oily body of plants in seedlings and roots and, as a result, alters salt stress. The supply of exogenous melatonin enhances the level of plant antioxidants and enzyme activities. (ii) Salt stress reduces chlorophyll content, but exogenous melatonin improves chlorophyll content, chloroplast gene expression, and protein content, and, as a result, enhances photosynthesis activity and chlorophyll accumulation. In addition, it enhances plant *Pn* and *CE* and, as a result, inhibits chlorophyll degradation. The improvement of all these traits enhances plant growth and metabolism. (iii) Drought stress causes ROS in plants, which induces *Ch* degradation and *Ch* reduction. The application of endogenous melatonin during drought reduces ROS and O_2^- content and, as a result, increases chlorophyll content and plant growth physiological function. *CE*, carboxylation efficiency; *Ch*, chlorophyll; *Pn*, net photosynthetic rate; ROS, reactive oxygen species.

The application of melatonin can alter the levels of NO in plants, and, as a result, affect the level of endogenous melatonin. The molecular interaction mechanisms of melatonin and NO are indispensable to different physiological activitiesin plants. However, the molecular interaction mechanisms of melatonin with NO in plants is still far from clear (He and He, 2020). Melatonin can mediate the crosstalk between NO and ethylene and regulate the ripening of fruits *via N*-nitrosomelatonin (NOMela) signaling (Mukherjee, 2019). In pear fruits, for example, melatonin reduced ethylene production and delayed post-harvest senescence by regulating NO synthesis (Liu et al., 2019). A recent discovery identified that the interaction between melatonin and NO resulted in the formation of NOMela (i.e., *N*-nitrosomelatonin), and the promised roles in plant morphophysiological activity (Martínez-Lorente et al., 2022). However, owing to the limited available knowledge on melatonin, the interaction of these two compounds and fruit ripening occurring *via* NOMela in various plants are poorly understood when these plants are under abiotic stresses. In addition, melatonin induces the accumulation of IAA *via* NO and, as a result, affects the formation of adventitious roots in tomato seedlings (Xie et al., 2022). Melatonin also regulates the transport and distribution of auxin, in turn promoting the formation of adventitious roots in tomato plants (Wen et al., 2016).

Melatonin has been widely shown to promote plant growth and photosynthetic activity under salt stress (Wang et al., 2016; Figure 2). Melatonin greatly reduced the decrease in chlorophyll *a* (*Chl a*), chlorophyll *b* (*Chl b*), and total chlorophyll (*Chls*) contents caused by salt stress, and promoted the net photosynthetic rate (*Pn*) and carboxylation efficiency (*CE*), showing that it can alleviate chlorophyll degradation caused by salt stress in plants (Kudoh and Sonoike, 2002; Yin et al., 2019). Previous studies found that the contents of *Chl a*, *Chl b*, and *Chls* were much higher in plants treated with melatonin than in untreated plants, indicating that melatonin facilitated both chloroplast gene expression and protein content turnover to promote the accumulation of chlorophyll content (Suo et al., 2015; Siddiqui et al., 2019). Melatonin can act as an antioxidant agent, reducing ROS activity and, as a result, inhibits chlorophyll degradation (Ma et al., 2018; Figure 2). There are studies that indicate that melatonin reduces the degradation of chlorophyll by down-regulating the expression of chlorophyll degradation-related genes during methyl jasmonate-induced senescence (Wang et al., 2019). However, more studies are needed to identify the various genes and measure gene expression involved in reducing chlorophyll degradation in various plants under abiotic stresses.

Interaction of melatonin with nitric oxide

It is essential to study the physiological responses of crops regulated by the interactions between melatonin and NO to ensure higher yields of these crops. Melatonin and NO affect several physiological processes, such as root growth, mitigation of iron deficiency, and aging (Kaya et al., 2020; Figure 3). The interactions between the two compounds regulate many genes involved in hormone synthesis and, as a result, change the levels of phytohormones (Zhu et al., 2019; Singhal et al., 2021). Interactions

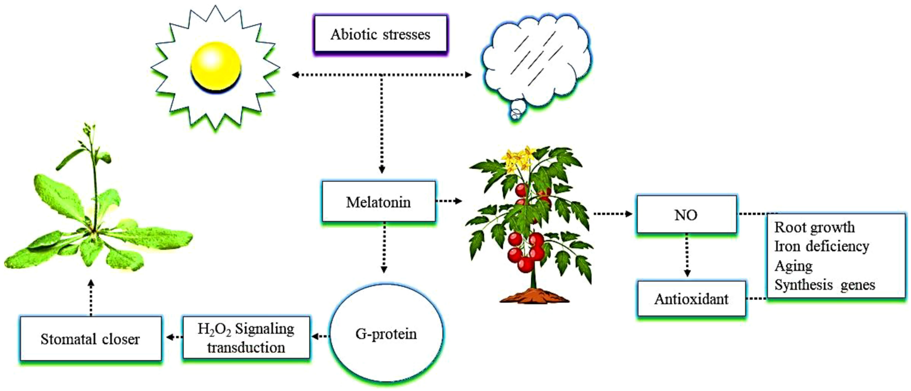


FIGURE 3
The interactive role of melatonin with nitric oxide (NO) in mitigating abiotic stress. The application of melatonin in tomato plants enhances NO content. NO further triggers antioxidant enzymes activity in plants, which shows resistance to abiotic stress and enhances root growth, iron deficiency, aging, and the expression of synthesis genes. In addition, G protein as a melatonin receptor enhances root growth, iron deficiency, aging, and the expression of synthesis genes mediates hydrogen peroxide (H_2O_2) signaling transduction that is involved in a melatonin-induced stomatal closure in *Arabidopsis*.

between melatonin and NO have recently been identified under conditions of plant stress (Arnao and Hernández-Ruiz, 2018). A previous study demonstrated that melatonin triggers the endogenous accumulation and synthesis of NO, which acts as an antioxidant and regulates other plant defense mechanisms (Okant and Kaya, 2019). The G protein-coupled receptor, as a melatonin receptor, mediates hydrogen peroxide (H_2O_2) signaling transduction, which is in turn involved in melatonin-induced stomatal closure in *Arabidopsis* plants (Wei et al., 2018; He and He, 2020). An example of this is melatonin promoting the production of NO in tomato plants when they were exposed to alkaline stress. In this situation, NO could be a downstream signal that plays an crucial role in the tolerance enhanced by melatonin in tomato plants grown under alkaline stress (Liu et al., 2015). Melatonin, together with NO, promotes plant growth and physiological function. The current review suggests that the mechanism of melatonin's interaction with NO in plants under abiotic stress is still not clearly understood, and the various genes activated as a result of that interaction have not yet been identified.

Melatonin regulated the transport and distribution of auxin

Previous studies of the relationship between melatonin and auxin have focused on their chemical similarity (Arnao and Hernández-Ruiz, 2021). Melatonin promotes growth by increasing the concentration of IAA, synthesis of IAA, and polar IAA transport (Wang et al., 2016; Figure 4). Various studies have also identified the ability of melatonin and auxin to regulate root and shoot growth and to promote photosynthesis in a similar way (Tan et al., 2019; Mao et al., 2020). A study of plants under drought stress showed that melatonin encouraged the plants to produce more IAA, which helped to increase plant growth and yield. During the maturity stage, the concentration of melatonin decreased, and the increase in IAA concentration was negligible (Ahmad et al., 2022). This decrease in IAA concentration seen in plants in the later growth stages is due to the decreased demand for IAA (Jia et al., 2020). Another similar study showed that the content of IAA decreased from the early growth to the maturity stages in plants under drought stress. Thus, it is

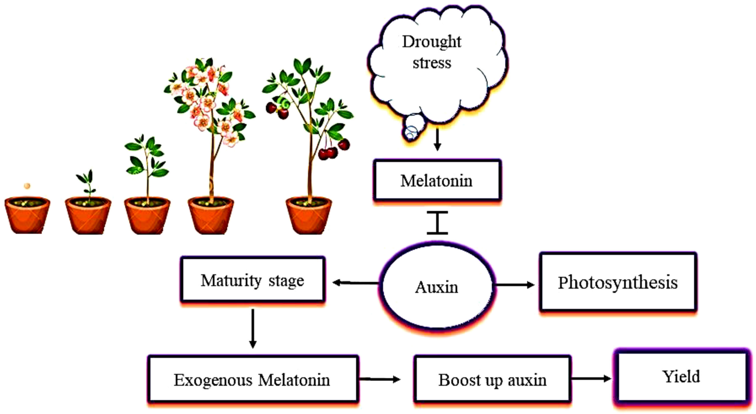


FIGURE 4
The interactive role of melatonin with auxin (IAA, indole-3-acetic acid) in mitigating the effects of drought stress. The application of melatonin increases IAA content in plants. Melatonin and IAA promote plant photosynthetic activity in the same way. When plants are under drought stress, melatonin interacts with IAA at the maturity stage, and increases plant growth and yield.

conceivable that plants need higher levels of IAA during seedling growth. Plants require certain hormones during their growth and development. Melatonin boosts the IAA levels in plants, and IAA plays an indispensable role in the growth of plants and their development from germination to maturity.

TABLE 1 Published examples of melatonin in mitigating abiotic stress in various crops.

Abiotic stress	Crop	Response of plants treated with melatonin under abiotic stress	References
Salt stress	Rice	Increased salt tolerance	Liu et al., 2020
	Tobacco	Increased salt tolerance	Cao et al., 2006
	Melon	Increased salt tolerance	Castañares and Bouzo, 2019
	Limonium bicolor	Increased salt tolerance	Li et al., 2019
	Grapevine	Increased salt tolerance	Xu et al., 2019
Drought stress	Corn	Increased drought tolerance	Li et al., 2021
	Apple	Increased drought tolerance	Li et al., 2015
Heat stress	*Arabidopsis*	Increased heat tolerance	Hernández et al., 2015
	Tomato	Increased heat tolerance	Wang et al., 2018
Cold stress	Corn	Increased cold stress tolerance	Posmyk et al., 2009b
	Cucumber	Increased cold stress tolerance	Kolodziejczyk et al., 2016
	Arabidopsis	Increased cold stress tolerance	Shi et al., 2015b
	Bermuda grass	Increased cold stress tolerance	Khalid et al., 2022
Heavy metal stress	Wheat	Increased heavy metal tolerance	Zeng et al., 2022
	Tomato	Increased heavy metal tolerance	Hasan et al., 2019
	Arabidopsis	Increased heavy metal tolerance	Yin et al., 2022
	Rice	Increased heavy metal tolerance	Maharajan et al., 2022
Other stresses	*Malus hupehensis*	Increased UV stress tolerance	Wei et al., 2019
	Mediterranean	Increased UV stress tolerance	Nawaz et al., 2022
	Alpine species	Increased UV stress tolerance	Nawaz et al., 2022

UV, ultraviolet light.

Role of melatonin in plants under abiotic stress

Salinity

Publishedexamples of melatonin mitigating abiotic stresses in various species of cropplants are shown in Table 1. Salt stress decreased chlorophyll content and photosynthetic activity and enhanced ROS activity and photoperiod regulation (Yin et al., 2019). Melatonin improved thegrowth of green bean seedlings under salt stress, increased photosynthetic activity, and mitigated the oxidative damage caused by ROS by improving antioxidant defense systems in plants (Hasanuzzaman et al., 2020; Elsayed et al., 2021). Multiple studies indicate that melatonin plays a vital role in adaptive responses to salt stress in various plant species (Chen et al., 2018; Liang et al., 2018). However, most of these studies are observational and the findings have not been supported by physiological and molecular research (Liu et al., 2020). In rice, melatonin enhanced salt stress by enabling K^+ retention (a vital component of plant tissue tolerance mechanisms) in the roots of plants, and by enabling the process that required Oryza sativa (OS) respiratory burstoxidase homolog F (OsRBOHF)-dependent ROS signaling to trigger stress-responsive genes, which in turn increased the expression of K^+ uptake transporters (particularly *OsHAK5*) in the tips of roots *(*Liu et al., 2020*). Potassium is an essential element for plant growth and development, and its reduction has been observed under salt stress* (Chen et al., 2018; Liu et al., 2019). These results correspond with the findings of Huang et al. (2019), who reported that NaCl-induced respiratory burst oxidase homolog (RBOH)-mediated production of H_2O_2 may be essential for stress signaling and plant adaptation to saline stress. However, studies on the role of OsRBOHF-dependent ROS signaling in the activation of stress-responsive genes and increased expression of K^+ uptake transporters *in the root tip of plants are lacking, as they have not been conducted on a large variety of plants under abiotic stresses. In addition, further research should focus on identifying responsive genes from OsRBOHF-dependent ROS signaling to increase the uptake of K^+ transporter ions in the root tips of different crops under various stresses* (Yu et al., 2018).

Melatonin also promotes ethylene biosynthesis, and the application of melatonin was found to strongly induce *MYB108A* and *ACS1* genes during grape berry ripening (Xu et al., 2017). The *MYB108A* and *ACS1* genes, which perform their function as transcription and essential genes that participate in the production of ethylene, were induced by the application of melatonin (Dong et al., 2011). *ACS* genes are considered a significant target under abiotic stresses to regulate ethylene production in plants. The salt-responsive gene *VviACS1* has been identified as being responsible for ethylene production in plants (Xu et al., 2019). In addition, the *ACSa* and *ACS1* genes are significant in that they are considered a primary target for salt tolerance in corn and tobacco (Cao et al., 2006; Lee and Back, 2016). *Melatonin, combined with 1-aminocyclopropane-1-carboxylic acid (ACC, an ethylene precursor), improved salt tolerance in grapevine plants. In addition, ethylene production was involved in* *melatonin-induced salt tolerance (*Xu et al., 2019*). The mechanism and function of MYB108A, ACS1, ACSa*, and *VviACS1* genes in ethylene production due to the melatonin induction under abiotic stresses in different plants is largely unknown.

Drought

Drought stress negatively affects plants' morphophysiological and biochemical activity, leading to a decrease in crop yields (Singh et al., 2015; Chen et al., 2019). Drought stress is the cause of oxidative stress and damages plant cells, and, *via* the higher accumulation of ROS, decreases stomatal closure and photosynthetic activity, and results in a deterioration of antioxidant defense systems. The accumulation of ROS is considered a threat to the survival of plant cells as it leads to electron leakage, lipid peroxidation, and subsequent membrane injury, as well as damaged protein and nucleic acid contents (Maksup et al., 2014). To prevent this damage, plants have developed various strategies to regulate their growth under different environmental stresses (Kim and Kim, 2020). As a new plant growth regulator, melatonin is thought to be involved in drought stress responses (Zhang et al., 2015; Li et al., 2021). Drought stress reduced morphological activity in plants, including that pertaining to leaf size and the relative water conductivity of corn seedlings. Meanwhile, both leaf size and relative water conductivity were significantly enhanced by the application of melatonin (Li et al., 2021). A similar result was revealed by Ye et al. (2016), who reported that melatonin improved the shoot dry weight and leaf size of corn seedlings. In plants, physiological processes in leaves, such as photosynthesis, respiration, and transpiration, are maintained by stomata, the opening and closing of which are controlled by complex signal transduction pathways and water balance. In the presence of drought stress, plants regulate their cellular moisture content by regulating stomatal closure and reducing their transpiration rate. However, the density of stomata significantly increases with the contraction of guard cells, and deteriorates under drought stress (Xue et al., 2021). In general, the application of melatonin has shown resistance against the deterioration of stomata cells and increased its length and width under drought stress in corn (Li et al., 2021). The contrasting results in the study by Li et al. (2015), however, demonstrated that drought stress did not reduce stomatal cell density in apples. Nevertheless, the exogenous supply of melatonin maintained high turgor pressure and kept the stomata open. The difference in the findings might be because of the differences in the regulatory mechanism of melatonin in different plant species (Li et al., 2021). The present review demonstrates that melatonin's quantity, performance, and mechanisms of action differ from plant species to plant species, but fewer morphophysiological responses have been documented under drought stress in different plants.

Heat stress

High levels of heat stress increase endogenous melatonin concentrations and, thereby, enhance thermotolerance, because of the potent antioxidant capacity of melatonin in plants (Liang et al., 2018; Ahammed et al., 2019). A previous study on *Arabidopsis* plants demonstrated that melatonin increased the seed germination rate from approximately 30% to 39% under heat stress (Hernández et al., 2015). It has been confirmed by the correlation between the synthesis of phytomelatonin and seed germination that phytomelatonin is synthesized during the germination of cucumber seeds, and that its synthesis peaks 14 hours after germination (Zhang et al., 2014). Nevertheless, further research on various crops is still needed. Melatonin improved germination capability by promoting soluble sugar utilization and synthesis of new proteins, and increased amylase and α-amylase activities in melon and *Limonium bicolor* seeds (Castañares and Bouzo, 2019; Li et al., 2019). Recent research has revealed the mechanisms by which melatonin significantly mitigates the effects of heat stress on plant seeds. First, because of the high potency of melatonin, it maintained high viability and germination capacity (Hernández et al., 2015). When plants are exposed to high levels of heat stress, the activities of antioxidant enzymes, such as superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT), are increased (Wang et al., 2022), and melatonin inhibits the accumulation of H_2O_2 (Marta et al., 2016). Melatonin treatment up-regulates genes, such as GA20ox and GA3ox, which are involved in gibberellin (GA) biosynthesis. The content of GA, particularly GA_4, is also increased by melatonin. However, unfortunately, melatonin down-regulates the expression of the essential gene *NECD2*, which is mainly involved in ABA biosynthesis (Zhang et al., 2014; Li et al., 2019). The mechanisms of the up-regulation and down-regulation of gene expression should be studied further in plant cells under heat stress.

Furthermore, heat stress can deteriorate the balance between antioxidants, resulting in ROS accumulation and causing peroxidative damage to cell membranes (Sun et al., 2021). The exogenous application of melatonin in tomatoes and rice reversed the adverse effects of heat stress on plant shoot and root growth (Wang et al., 2018). Melatonin also reduced the damage caused by heat stress by regulating redox homeostasis, and modulating NO and polyamine biosynthesis in tomato seedlings (Jahan et al., 2019). In *Arabidopsis* plants, the heat shock protein HSP_{90} and heat shock factors (i.e., $HSFA_2$ and $HSFA_{32}$) contributed to the alleviation of melatonin-mediated heat stress (Shi et al., 2015a). A study demonstrated that HSPs prevented the cellular proteins of tomatoes, grown under heat stress, from refolding or degrading denatured proteins (Xu et al., 2016). Heat shock proteins (HSPs) can isolate and store unfolded proteins. In addition, HSPs can act as chaperones by protecting cells against stresses that can induce protein denaturation and block protein aggregation, and by enhancing the survival of cells and, in turn, of the cellular activity during high levels of heat stress. However, our current understanding of how heat shock proteins and heat shock factors relate to melatonin-mediated heat stress is limited, and in need of further investigation.

Cold stress

Cold stress is one of the major abiotic stresses that reduces crop growth and yield, especially in temperate zones and highly elevated areas (Bhat et al., 2022). Plants exposed to cold stress experience changes in various physiological, molecular, metabolic, and biochemical activities. Examples include variations in membrane fluidity, metabolism homeostasis, and enzyme activity (Wu et al., 2022). Photosynthesis is a pivotal plant metabolism process, and one that is highly sensitive to cold stress. This is because low temperature hinders many major components of photosynthesis (Dahal et al., 2012). Chlorophyll content decreases under cold stress, leading to chlorosis in leaves (Kaura et al., 2022). The chlorophyll content of leaves provides important information about the effectiveness of physiological processes in plants in plants (Gitelson et al., 2003). Plants treated with melatonin had a higher concentration of chlorophyll than non-treated plants under cold stress (Yang et al., 2022). Plant growth at low temperatures induces the excessive production or inefficient deactivation of ROS, such as H_2O_2, superoxide anions (i.e., O_2^-), and hydroxyl radicals (i.e., OH), which in turn can cause injury to plants (Ghaderian et al., 2015). In addition, ROS accumulation causes the oxidation of proteins and peroxidation of lipids within plant cells, resulting in reduced plant growth (Nahar et al., 2015). For self-defense against oxidative injury caused by ROS, plants have evolved effective antioxidant systems to scavenge ROS, such as SOD, POD, and CAT, as well as non-enzymatic antioxidants, including proline and glutathione (Erdal et al., 2015; Ghaderian et al., 2015). Several studies have demonstrated that exogenous melatonin can stimulate plant growth in various plants, such as corn, and can promote the germination of cucumber seeds under cold stress (Posmyk et al., 2009b; Kolodziejczyk et al., 2016). In *Arabidopsis* plants, melatonin modulates leaf senescence against cold stress (Shi et al., 2015b). Melatonin applications enhance the resistance of Bermuda grass to cold stress by improving cell membrane stability, and by regulating photosynthesis and metabolic activity (Khalid et al., 2022). Melatonin played a role as both a first-line defense and internal sensor of oxidative stress in a study of different species of plants (Iqbal and Khan, 2022). For example, in barley, exogenous melatonin can enhance photosynthetic carbon assimilation by improving the plant antioxidant defense systems of organelles under cold stress (Li et al., 2016). Therefore, the improved performance of primed seeds in terms of seedling growth and germination might be the result of improved antioxidant defense systems under cold stress (Cao et al., 2022). However, an understanding of the growth of waxy corn and other crop seeds primed with melatonin in response to cold stress is still limited (Cao et al., 2022).

Heavy metal stress

Certain heavy metals, such as zinc (Zn), cadmium (Cd), iron (Fe), and copper (Cu), are essential for plant growth and metabolism, but their accumulation to higher levels can negatively affect plant growth and yield. Heavy metal stress inhibits plant photosynthetic activity, the activity of enzymes involved in the Calvin cycle, and carbohydrate metabolism (Hasan et al., 2019). In addition, higher levels of ROS accumulation during heavy metal stress inhibit root growth and promote leaf senescence in turn, chloroplasts (Zeng et al., 2022). Previous studies have demonstrated that plants treated with melatonin can improve their growth and yield by improving their morphophysiological activities under heavy metal stress conditions. The production of endogenous melatonin in plants can be triggered by the application of exogenous melatonin which builds up heavy metal tolerance (Menhas et al., 2022). Melatonin enhanced plant metabolism and antioxidant enzymes activity, and triggered the ascorbate–glutathione cycle to counteract the effects of heavy metal stress (Moustafa-Farag et al., 2020). In wheat seedlings, exogenous melatonin increased endogenous melatonin and, as a result, enhanced root and shoot growth under cadmium (Cd) toxicity (Zeng et al., 2022). The increase of endogenous melatonin mitigates cadmium toxicity by balancing H_2O_2 homeostasis and activating antioxidant defense systems in wheat (Ni et al., 2018). Melatonin effectively mitigated Cd toxicity by improving H^+-ATPase activity and phytochelatin and glutathione content, and by facilitating Cd sequestration in tomato plant cells (Hasan et al., 2015). Melatonin impacted sulfur metabolism, which plays an important role in plant tolerance against Cd stress (Menhas et al., 2022). In tomatoes, melatonin deficiency reduced the sulfur concentration and increased the accumulation of cadmium (Hasan et al., 2019). The overexpression of melatonin biosynthetic enzymes genes improved heavy metal stress in *Arabidopsis* plants (Yin et al., 2022). Similarly, in rice, various gene families, such as *NRAMP*, *HMA*, *MTP*, *YSL*, and *ZIP*, are involved in heavy metal stress (Maharajan et al., 2022). These genes reduced the uptake of heavy metals and accumulation in rice grains (Peris-Peris et al., 2017). Several studies investigating the role of melatonin in plant morphophysiological activity, antioxidant capacity, and biosynthetic genes in various crops have recently been undertaken. However, the role of melatonin in improving these activities, as well as the transduction pathways of different genes in cotton, rice, and other crops under heavy metal stress, is still unknown.

Other stresses

Ultraviolet (UV) radiations negatively effects plant growth and development, andtheir intensity continuously increasing caused by rapid ozone layer depletion. The higher levels of UV radiation can substantially reduce crop productivity by hindering plant PSII, photosynthetic activity, nucleic acids, and biomass accumulation and portioning (Bera et al., 2022). Plants exposed to higher levels of UV radiation have reduced expression and synthesis of key photosynthetic proteins, such as chlorophyll *a/b* binding proteins (Khudyakova et al., 2019). Melatonin plays a vital role in mitigating the negative effects of UV radiation on crop productivity. It has been demonstrated that exogenous melatonin in *Malus hupehensis* and *Nicotiana sylvestris* plants facilitates the UV-induced damage to DNA and UV radiation induced by ROS (Wei et al., 2019). Melatonin is considered a potent antioxidant that protects plants against UV radiation; it regulates the expression of various UV signaling pathways, such as transcription factors RUP1/2, HY5, and HYH, and the ubiquitin-degrading enzyme COP1 (Yao et al., 2021). Exogenous melatonin improved the expression of RUP1/1, HY5, HYH, and COP1, which perform a key role in the protection against UV radiation (Hassan et al., 2022). Hence, melatonin regulates antioxidant defense systems to prevent plants from the negative impacts of UV stress (Yao et al., 2021). Endogenous melatonin is substantially increased in *Glycyrrhiza uralensis* plants when their roots are exposed to UV radiation, and as a result plant DNA damage is reduced (Wei et al., 2019). Similarly, the accumulation of endogenous melatonin induces a tolerance response to UV stress in Mediterranean and alpine species of plants (Nawaz et al., 2022). Although limited research has demonstrated a role for melatonin in UV stress tolerance (Hassan et al., 2022), more studies are required to investigate the role of melatonin in regulating various UV signaling pathways that are involved in mitigating the negative effects of UV radiation in various crops.

Conclusions and future directions

The impact of abiotic stresses on plant development is considered a significant threat to agricultural productivity. Plants adopt different physiological, biochemical, and molecular responses to overcome the negative effects of abiotic stresses (Ahmad et al., 2022b). Phytomelatonin has potential to be used as a tool for reducing or alleviating the adverse effects of abiotic stresses in various crops. The exogenous application of melatonin is essential for plant growth and development under abiotic stresses. Phytomelatonin plays a key role in plant metabolism and the complex mechanism of plant function; however, the role that melatonin plays in the underlying mechanisms in plants grown under abiotic stress is still poorly understood. Moreover, the interaction of melatonin with NO and with IAA/auxin, and their responses to abiotic stresses, make for attractive targets in molecular research. The relationship between melatonin and NO regulates morphophysiological and biochemical activities by way of the G protein-coupled receptor and synthesis genes. Furthermore, the mechanism by which G protein regulates the morphophysiological activity and the different genes involved in the regulation by melatonin are still unclear. In addition, the interaction of melatonin with auxin enhanced growth and physiological function by increasing the levels of auxin, synthesis, and polar transport. In the later growth stage, the content of auxin is decreased because of the decreased melatonin concentrations in plants. To grow and achieve a higher yield, plants need a continuous supply of IAA from sowing to maturity. In the lateral growth stage, the effects of an exogenous supply of melatonin, and the mechanism by which melatonin boosts IAA levels in various crops, are still unknown.

Melatonin has an important role in regulating plant metabolism and increasing yield under various abiotic stresses. In addition, the OsRBOHF-dependent ROS signaling that activated stress-responsive genes in plants grown under abiotic stress enhanced the uptake of potassium (K^+) transporter (*OsHAK5*) in the roots. The potassium transporter *OsHAK5* plays a vital role in potassium acquisition and transport from root tissue to the shoots, especially in plants exposed to low potassium concentrations, enhancing plant metabolism and physiological function under salt stress. The mechanism of the K^+ transporter *OsHAK5* and the activation of gene identification, which are due to the OsRBOHF-dependent ROS signaling in various crops under abiotic stresses, however, is poorly understood.

Ethylene in plants is considered a multifunctional phytohormone that significantly improves plant growth and senescence. However, the role of genes such as *MYB108A*, *ACS1*, *ACSa*, and *VviACS1* in ethylene production in different plants under various stresses is still poorly understood.

In addition, serotonin plays a vital function in plants, acting as a growth regulator and as a stress defense molecule. The relationship of melatonin with *T5H* genes in serotonin biosynthesis under abiotic stress is still unknown in many plants.

Stomata density is closely associated with plant growth properties, and photosynthetic activity is improved by melatonin application. Stomatal cells undergo deterioration when plants are exposed to drought stress (Salehi-Lisar and Bakhshayeshan-Agdam, 2016). In contrast, no adverse effects on stomatal density were observed in apple plants grown under drought stress (Li et al., 2015). The reason for these different results might be that there are different signaling pathways in different crops. The mechanisms of this phenomenon in various crops are still poorly understood. These mechanisms need to be further investigated under various stresses because the amount performance, and mechanism of action of melatonin vary among plant species.

It has been confirmed that, melatonin up-regulates *GA20ox* and *GA3ox*, genes that are involved in GA biosynthesis and result in increased GA_4 while down-regulating the *NECD2*, which is involved in ABA biosynthesis. The role of melatonin in the up- and down-regulation of genes involved in the biosynthesis of GA and ABA under various abiotic stresses remains unclear.

The heat shock proteins of tomatoes protect the plants' cellular protein against heat stress due to refolding or degradation of denatured proteins. However, the response and activity of heat shock proteins and heat factors in plants with a melatonin supply under abiotic stresses in different crops is still poorly understood.

Author contributions

IA was involved in the conceptualization, writing/reviewing, and editing the original draft. GuiZ was involved in supervision. MI and GuaZ were involved in collection of the literature. YJ and XS contributed to the writing of the manuscript. AA and MY eliminated grammatical errors. All authors contributed to the article and approved the submitted version.

Funding

The research was financially supported by National Key Research and Development Project of China (2022YFE0113400).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

Ahammed, G. J., Xu, W., Liu, A., and Chen, S. (2019). Endogenous melatonin deficiency aggravates high temperature-induced oxidative stress in solanum lycopersicum l. *Environ. Exp. Bot.* 161, 303–311. doi: 10.1016/j.envexpbot.2018.06.006

Ahmad, S., Wang, G. Y., Muhammad, I., Farooq, S., Kamran, M., Ahmad, I., et al. (2022). Application of melatonin-mediated modulation of drought tolerance by regulating photosynthetic efficiency, chloroplast ultrastructure, and endogenous hormones in maize. *Chem. Biol. Technol. Agric.* 9, 1–14. doi: 10.1186/s40538-021-00272-1

Ahmad, I., Zhu, G., Zhou, G., Song, X., Hussein Ibrahim, M. E., and Ibrahim Salih, E. G. (2022a). Effect of n on growth, antioxidant capacity, and chlorophyll content of sorghum. *Agronomy* 12, 501. doi: 10.3390/agronomy12020501

Ahmad, I., Zhu, G., Zhou, G., Song, X., Hussein Ibrahim, M. E., Ibrahim Salih, E. G., et al. (2022b). Pivotal role of phytohormones and their responsive genes in plant growth and their signaling and transduction pathway under salt stress in cotton. *Int. J. Mol. Sci.* 23, 7339. doi: 10.3390/ijms23137339

Arnao, M. B., and Hernández-Ruiz, J. (2009). Chemical stress by different agents affects the melatonin content of barley roots. *J. Pineal Res.* 46, 295–299. doi: 10.1111/j.1600-079X.2008.00660.x

Arnao, M. B., and Hernández-Ruiz, J. (2013). Growth conditions determine different melatonin levels in l upinus albus l. *J. Pineal Res.* 55, 149–155. doi: 10.1111/jpi.12055

Arnao, M. B., and Hernández-Ruiz, J. (2018). Melatonin and its relationship to plant hormones. *Ann. Bot.* 121, 195–207. doi: 10.1093/aob/mcx114

Arnao, M. B., and Hernández-Ruiz, J. (2019). Melatonin: A new plant hormone and/or a plant master regulator? *Trends Plant Sci.* 24, 38–48. doi: 10.1016/j.tplants.2018.10.010

Arnao, M., and Hernández-Ruiz, J. (2021). Melatonin as a regulatory hub of plant hormone levels and action in stress situations. *Plant Biol.* 23, 7–19. doi: 10.1111/plb.13202

Back, K., Tan, D. X., and Reiter, R. J. (2016). Melatonin biosynthesis in plants: multiple pathways catalyze tryptophan to melatonin in the cytoplasm or chloroplasts. *J. Pineal Res.* 61, 426–437. doi: 10.1111/jpi.12364

Bajwa, V. S., Shukla, M. R., Sherif, S. M., Murch, S. J., and Saxena, P. K. (2014). Role of melatonin in alleviating cold stress in a rabidopsis thaliana. *J. Pineal Res.* 56, 238–245. doi: 10.1111/jpi.12115

Bera, K., Ball, K., Ghosh, S., Sadhukhan, S., and Dutta, P. (2022). UV Radiation: plant responses and an in-depth mechanism of sustainability under climatic extremities. doi: 10.21203/rs.3.rs-2219238/v1

Bhat, K. A., Mahajan, R., Pakhtoon, M. M., Urwat, U., Bashir, Z., Shah, A. A., et al. (2022). Low temperature stress tolerance: An insight into the omics approaches for legume crops. *Front. Plant Sci.* 13. doi: 10.3389/fpls.2022.888710

Boccalandro, H. E., González, C. V., Wunderlin, D. A., and Silva, M. F. (2011). Melatonin levels, determined by LC-ESI-MS/MS, fluctuate during the day/night cycle in vitis vinifera cv Malbec: evidence of its antioxidant role in fruits. *J. Pineal Res.* 51, 226–232. doi: 10.1111/j.1600-079X.2011.00884.x

Byeon, Y., and Back, K. (2014). Melatonin synthesis in rice seedlings *in vivo* is enhanced at high temperatures and under dark conditions due to increased serotonin n-acetyltransferase and n-acetylserotonin methyltransferase activities. *J. Pineal Res.* 56, 189–195. doi: 10.1111/jpi.12111

Cao, Q., Li, G., Cui, Z., Yang, F., Jiang, X., Diallo, L., et al. (2019). Seed priming with melatonin improves the seed germination of waxy maize under chilling stress *via* promoting the antioxidant system and starch metabolism. *Sci. Rep.* 9, 1–12. doi: 10.1038/s41598-019-51122-y

Cao, W. H., Liu, J., Zhou, Q. Y., Cao, Y. R., Zheng, S. F., Du, B. X., et al. (2006). Expression of tobacco ethylene receptor NTHK1 alters plant responses to salt stress. *Plant Cell Environ.* 29, 1210–1219. doi: 10.1111/j.1365-3040.2006.01501.x

Cao, Q., Wang, W., Akhtar, S. S., Yang, F., Kong, F., Cui, Z., et al. (2022). Exogenous foliar spray of selenium (Se) alleviates cold stress by promoting photosynthesis and antioxidant defense in waxy maize. doi: 10.21203/rs.3.rs-1719103/v1

Castañares, J. L., and Bouzo, C. A. (2019). Effect of exogenous melatonin on seed germination and seedling growth in melon (Cucumis melo l.) under salt stress. *Hortic. Plant J.* 5, 79–87. doi: 10.1016/j.hpj.2019.01.002

Chen, J., Li, Y., Luo, Y., Tu, W., and Wan, T. (2019). Drought differently affects growth properties, leaf ultrastructure, nitrogen absorption and metabolism of two dominant species of hippophae in Tibet plateau. *Acta Physiol. Plant* 41, 1–12. doi: 10.1007/s11738-018-2785-6

Chen, G., Liu, C., Gao, Z., Zhang, Y., Zhang, A., Zhu, L., et al. (2018). Variation in the abundance of OsHAK1 transcript underlies the differential salinity tolerance of an indica and a japonica rice cultivar. *Front. Plant Sci.* 8, 2216. doi: 10.3389/fpls.2017.02216

Dahal, K., Kane, K., Gadapati, W., Webb, E., Savitch, L. V., Singh, J., et al. (2012). The effects of phenotypic plasticity on photosynthetic performance in winter rye, winter wheat and brassica napus. *Physiol. Plant* 144, 169–188. doi: 10.1111/j.1399-3054.2011.01513.x

David, A., Yadav, S., and Bhatla, S. C. (2010). Sodium chloride stress induces nitric oxide accumulation in root tips and oil body surface accompanying slower oleosin degradation in sunflower seedlings. *Physiol. Plant* 140, 342–354. doi: 10.1111/j.1399-3054.2010.01408.x

Dong, H., Zhen, Z., Peng, J., Chang, L., Gong, Q., and Wang, N. N. (2011). Loss of ACS7 confers abiotic stress tolerance by modulating ABA sensitivity and accumulation in arabidopsis. *J. Exp. Bot.* 62, 4875–4887. doi: 10.1093/jxb/err143

Dubbels, R., Reiter, R., Klenke, E., Goebel, A., Schnakenberg, E., Ehlers, C., et al. (1995). Melatonin in edible plants identified by radioimmunoassay and by high performance liquid chromatography-mass spectrometry. *J. Pineal Res.* 18, 28–31. doi: 10.1111/j.1600-079X.1995.tb00136.x

Dutta, A., Sen, J., and Deswal, R. (2013). New evidences about strictosidine synthase (Str) regulation by salinity, cold stress and nitric oxide in catharanthus roseus. *J. Plant Biochem.* 22, 124–131. doi: 10.1007/s13562-012-0118-1

Elsayed, A. I., Rafudeen, M. S., Gomaa, A. M., and Hasanuzzaman, M. (2021). Exogenous melatonin enhances the reactive oxygen species metabolism, antioxidant defense-related gene expression, and photosynthetic capacity of phaseolus vulgaris l. @ to confer salt stress tolerance. *Physiol. Plant* 173, 1369–1381. doi: 10.1111/ppl.13372

Erdal, S., Genisel, M., Turk, H., Dumlupinar, R., and Demir, Y. (2015). Modulation of alternative oxidase to enhance tolerance against cold stress of chickpea by chemical treatments. *J. Plant Physiol.* 175, 95–101. doi: 10.1016/j.jplph.2014.10.014

Ghaderian, S. M., Ghasemi, R., Heidari, H., and Vazirifar, S. (2015). Effects of Ni on superoxide dismutase and glutathione reductase activities and thiol groups: a comparative study between alyssum hyperaccumulator and non-accumulator species. *Aust. J. Bot.* 63, 65–71. doi: 10.1071/BT14282

Gitelson, A. A., Gritz, Y., and Merzlyak, M. N. (2003). Relationships between leaf chlorophyll content and spectral reflectance and algorithms for non-destructive chlorophyll assessment in higher plant leaves. *J. Plant Physiol.* 160, 271–282. doi: 10.1078/0176-1617-00887

Gonzalez Guzman, M., Cellini, F., Fotopoulos, V., Balestrini, R., and Arbona, V. (2022). New approaches to improve crop tolerance to biotic and abiotic stresses. *Physiol. Plant* 174, e13547. doi: 10.1111/ppl.13547

Hasan, M. K., Ahammed, G. J., Sun, S., Li, M., Yin, H., and Zhou, J. (2019). Melatonin inhibits cadmium translocation and enhances plant tolerance by regulating sulfur uptake and assimilation in *Solanum lycopersicum* l. *J. Agric. Food Chem.* 67, 10563–10576. doi: 10.1021/acs.jafc.9b02404

Hasan, M. K., Ahammed, G. J., Yin, L., Shi, K., Xia, X., Zhou, Y., et al. (2015). Melatonin mitigates cadmium phytotoxicity through modulation of phytochelatins biosynthesis, vacuolar sequestration, and antioxidant potential in solanum lycopersicum l. *Front. Plant Sci.* 6. doi: 10.3389/fpls.2015.00601

Hasanuzzaman, M., Bhuyan, M., Zulfiqar, F., Raza, A., Mohsin, S. M., Mahmud, J. A., et al. (2020). Reactive oxygen species and antioxidant defense in plants under abiotic stress: Revisiting the crucial role of a universal defense regulator. *Antioxidants* 9, 681. doi: 10.3390/antiox9080681

Hassan, M. U., Mahmood, A., Awan, M. I., Maqbool, R., Aamer, M., Alhaithloul, H. A., et al. (2022). Melatonin-induced protection against plant abiotic stress: mechanisms and prospects. *Front. Plant Sci.* 13. doi: 10.3389/fpls.2022.902694

He, H., and He, L. F. (2020). Crosstalk between melatonin and nitric oxide in plant development and stress responses. *Physiol. Plant* 170, 218–226. doi: 10.1111/ppl.13143

Hernández, I. G., Gomez, F. J. V., Cerutti, S., Arana, M. V., and Silva, M. F. (2015). Melatonin in arabidopsis thaliana acts as plant growth regulator at low concentrations and preserves seed viability at high concentrations. *Plant Physiol. Biochem.* 94, 191–196. doi: 10.1016/j.plaphy.2015.06.011

Huang, Y., Cao, H., Yang, L., Chen, C., Shabala, L., Xiong, M., et al. (2019). Tissue-specific respiratory burst oxidase homolog-dependent H2O2 signaling to the plasma membrane h+-ATPase confers potassium uptake and salinity tolerance in cucurbitaceae. *J. Exp. Bot.* 70, 5879–5893. doi: 10.1093/jxb/erz328

Iqbal, R., and Khan, T. (2022). Application of exogenous melatonin *in vitro* and in planta: a review of its effects and mechanisms of action. *Biotechnol. Lett.* 1-18. doi: 10.1007/s10529-022-03270-x

Jahan, M. S., Shu, S., Wang, Y., Chen, Z., He, M., Tao, M., et al. (2019). Melatonin alleviates heat-induced damage of tomato seedlings by balancing redox homeostasis and modulating polyamine and nitric oxide biosynthesis. *BMC Plant Biol.* 19, 1–16. doi: 10.1186/s12870-019-1992-7

Jia, C., Yu, X., Zhang, M., Liu, Z., Zou, P., Ma, J., et al. (2020). Application of melatonin-enhanced tolerance to high-temperature stress in cherry radish (Raphanus sativus l. var. radculus pers). *J. Plant Growth Regul.* 39, 631–640. doi: 10.1007/s00344-019-10006-1

Kang, K., Lee, K., Park, S., Byeon, Y., and Back, K. (2013). Molecular cloning of rice serotonin n-acetyltransferase, the penultimate gene in plant melatonin biosynthesis. *J. Pineal Res.* 55, 7–13. doi: 10.1111/jpi.12011

Kaura, V., Malhotra, P., Mittal, A., Sanghera, G., Kaur, N., Bhardwaj, R., et al. (2022). Physiological, biochemical, and gene expression responses of sugarcane under cold, drought and salt stresses. *J. Plant Growth Regul.*, 1–10. doi: 10.1007/s00344-022-10850-8

Kaya, C., Ashraf, M., Alyemeni, M. N., and Ahmad, P. (2020). Responses of nitric oxide and hydrogen sulfide in regulating oxidative defence system in wheat plants grown under cadmium stress. *Physiol. Plant* 168, 345–360. doi: 10.1111/ppl.13012

Khalid, M., Rehman, H. M., Ahmed, N., Nawaz, S., Saleem, F., Ahmad, S., et al. (2022). Using exogenous melatonin, glutathione, proline, and glycine betaine treatments to combat abiotic stresses in crops. *Int. J. Mol. Sci.* 23, 12913. doi: 10.3390/ijms232112913

Khan, M. I. R., Fatma, M., Per, T. S., Anjum, N. A., and Khan, N. A. (2015). Salicylic acid-induced abiotic stress tolerance and underlying mechanisms in plants. *Front. Plant Sci.* 6. doi: 10.3389/fpls.2015.00462

Khudyakova, A. Y., Kreslavski, V. D., Shmarev, A. N., Lyubimov, V. Y., Shirshikova, G. N., Pashkovskiy, P. P., et al. (2019). Impact of UV-b radiation on the photosystem II activity, pro-/antioxidant balance and expression of light-activated genes in arabidopsis thaliana hy4 mutants grown under light of different spectral composition. *J. Photochem. Photobiol.* 194, 14–20. doi: 10.1016/j.jphotobiol.2019.02.003

Kim, S., and Kim, T.-H. (2020). Alternative splicing for improving abiotic stress tolerance and agronomic traits in crop plants. *J. Plant Biol.* 63, 409–420. doi: 10.1007/s12374-020-09282-2

Kołodziejczyk, I., Dzitko, K., Szewczyk, R., and Posmyk, M. M. (2016). Exogenous melatonin improves corn (*Zea mays* l.) embryo proteome in seeds subjected to chilling stress. *J. Plant Physiol.* 193, 47–56. doi: 10.1016/j.jplph.2016.01.012

Kudoh, H., and Sonoike, K. (2002). Irreversible damage to photosystem I by chilling in the light: Cause of the degradation of chlorophyll after returning to normal growth temperature. *Planta* 215, 541–548. doi: 10.1007/s00425-002-0790-9

Lee, H. J., and Back, K. (2016). 2-hydroxymelatonin promotes the resistance of rice plant to multiple simultaneous abiotic stresses (combined cold and drought). *J. Pineal Res.* 61, 303–316. doi: 10.1111/jpi.12347

Liang, D., Gao, F., Ni, Z., Lin, L., Deng, Q., Tang, Y., et al. (2018). Melatonin improves heat tolerance in kiwifruit seedlings through promoting antioxidant enzymatic activity and glutathione s-transferase transcription. *Molecules* 23, 584. doi: 10.3390/molecules23030584

Li, H., Chang, J., Chen, H., Wang, Z., Gu, X., Wei, C., et al. (2017). Exogenous melatonin confers salt stress tolerance to watermelon by improving photosynthesis and redox homeostasis. *Front. Plant Sci.* 8. doi: 10.3389/fpls.2017.00295

Li, Z., Su, X., Chen, Y., Fan, X., He, L., Guo, J., et al. (2021). Melatonin improves drought resistance in maize seedlings by enhancing the antioxidant system and regulating abscisic acid metabolism to maintain stomatal opening under PEG-induced drought. *J. Plant Biol.* 64, 299–312. doi: 10.1007/s12374-021-09297-3

Li, X., Tan, D. X., Jiang, D., and Liu, F. (2016a). Melatonin enhances cold tolerance in drought-primed wild-type and abscisic acid-deficient mutant barley. *J. Pineal Res.* 61, 328–339. doi: 10.1111/jpi.12350

Li, X., Tan, D. X., Jiang, D., and Liu, F. (2016b). Melatonin enhances cold tolerance in drought-primed wild-type and abscisic acid-deficient mutant barley. *J. Pineal Res.* 61, 328–339. doi: 10.1111/jpi.12350

Li, C., Tan, D.-X., Liang, D., Chang, C., Jia, D., and Ma, F. (2015). Melatonin mediates the regulation of ABA metabolism, free-radical scavenging, and stomatal behaviour in two malus species under drought stress. *J. Exp. Bot.* 66, 669–680. doi: 10.1093/jxb/eru476

Liu, N., Gong, B., Jin, Z., Wang, X., Wei, M., Yang, F., et al. (2015). Sodic alkaline stress mitigation by exogenous melatonin in tomato needs nitric oxide as a downstream signal. *J. Plant Physiol.* 186, 68–77. doi: 10.1016/j.jplph.2015.07.012

Liu, J., Shabala, S., Shabala, L., Zhou, M., Meinke, H., Venkataraman, G., et al. (2019). Tissue-specific regulation of na+ and k+ transporters explains genotypic differences in salinity stress tolerance in rice. *Front. Plant Sci.* 10. doi: 10.3389/fpls.2019.01361

Liu, J., Shabala, S., Zhang, J., Ma, G., Chen, D., Shabala, L., et al. (2020). Melatonin improves rice salinity stress tolerance by NADPH oxidase-dependent control of the plasma membrane k+ transporters and k+ homeostasis. *Plant Cell Environ.* 43, 2591–2605. doi: 10.1111/pce.13759

Li, D., Wei, J., Peng, Z., Ma, W., Yang, Q., Song, Z., et al. (2020). Daily rhythms of phytomelatonin signaling modulate diurnal stomatal closure *via* regulating reactive oxygen species dynamics in arabidopsis. *J. pineal Res.* 68, e12640. doi: 10.1111/jpi.12640

Li, J., Zhao, C., Zhang, M., Yuan, F., and Chen, M. (2019). Exogenous melatonin improves seed germination in limonium bicolor under salt stress. *Plant Signal.* 14, 1659705. doi: 10.1080/15592324.2019.1659705

Maharajan, T., Chellasamy, G., Tp, A. K., Ceasar, S. A., and Yun, K. (2022). The role of metal transporters in phytoremediation: A closer look at *Arabidopsis*. *Chemosphere* 136881. doi: 10.1016/j.chemosphere.2022.136881

Maksup, S., Roytrakul, S., and Supaibulwatana, K. (2014). Physiological and comparative proteomic analyses of Thai jasmine rice and two check cultivars in response to drought stress. *J. Plant Interact.* 9, 43–55. doi: 10.1080/17429145.2012.752042

Mao, J., Niu, C., Li, K., Chen, S., Tahir, M. M., Han, M., et al. (2020). Melatonin promotes adventitious root formation in apple by promoting the function of MdWOX11. *BMC Plant Biol.* 20, 1–11. doi: 10.1186/s12870-020-02747-z

Marta, B., Szafrańska, K., and Posmyk, M. M. (2016). Exogenous melatonin improves antioxidant defense in cucumber seeds (Cucumis sativus l.) germinated under chilling stress. *Front. Plant Sci.* 7. doi: 10.3389/fpls.2016.00575

Martínez-Lorente, S. E., Pardo-Hernández, M., Martí-Guillén, J. M., López-Delacalle, M., and Rivero, R. M. (2022). Interaction between melatonin and no: action mechanisms, main targets, and putative roles of the emerging molecule NOMela. *Int. J. Mol. Sci.* 23, 6646. doi: 10.3390/ijms23126646

Ma, X., Zhang, J., Burgess, P., Rossi, S., and Huang, B. (2018). Interactive effects of melatonin and cytokinin on alleviating drought-induced leaf senescence in creeping bentgrass (Agrostis stolonifera). *Environ. Exp. Bot.* 145, 1–11. doi: 10.1016/j.envexpbot.2017.10.010

Menhas, S., Yang, X., Hayat, K., Aftab, T., Bundschuh, J., Arnao, M. B., et al. (2022). Exogenous melatonin enhances cd tolerance and phytoremediation efficiency by ameliorating cd-induced stress in oilseed crops: A review. *J. Plant Growth Regul.* 41, 922–935. doi: 10.1007/s00344-021-10349-8

Moustafa-Farag, M., Mahmoud, A., Arnao, M. B., Sheteiwy, M. S., Dafea, M., Soltan, M., et al. (2020). Melatonin-induced water stress tolerance in plants: Recent advances. *Antioxidants* 9, 809. doi: 10.3390/antiox9090809

Mukherjee, S. (2019). Insights into nitric oxide–melatonin crosstalk and n-nitrosomelatonin functioning in plants. *J. Exp. Bot.* 70, 6035–6047. doi: 10.1093/jxb/erz375

Mukherjee, S., David, A., Yadav, S., Baluška, F., and Bhatla, S. C. (2014). Salt stress-induced seedling growth inhibition coincides with differential distribution of serotonin and melatonin in sunflower seedling roots and cotyledons. *Physiol. Plant* 152, 714–728. doi: 10.1111/ppl.12218

Murch, S., Krishnaraj, S., and Saxena, P. (2000). Tryptophan is a precursor for melatonin and serotonin biosynthesis in *in vitro* regenerated st. john's wort (Hypericum perforatum l. cv. anthos) plants. *Plant Cell Rep.* 19, 698–704. doi: 10.1007/s002990000206

Nahar, K., Hasanuzzaman, M., Alam, M., and Fujita, M. (2015). Roles of exogenous glutathione in antioxidant defense system and methylglyoxal detoxification during salt stress in mung bean. *Biol. Plant* 59, 745–756. doi: 10.1007/s10535-015-0542-x

Nawaz, K., Chaudhary, R., Sarwar, A., Ahmad, B., Gul, A., Hano, C., et al. (2020). Melatonin as master regulator in plant growth, development and stress alleviator for sustainable agricultural production: Current status and future perspectives. *Sustainability* 13, 294. doi: 10.3390/su13010294

Ni, J., Wang, Q., Shah, F. A., Liu, W., Wang, D., Huang, S., et al. (2018). Exogenous melatonin confers cadmium tolerance by counterbalancing the hydrogen peroxide homeostasis in wheat seedlings. *Molecules* 23, 799. doi: 10.3390/molecules23040799

Okant, M., and Kaya, C. (2019). The role of endogenous nitric oxide in melatonin-improved tolerance to lead toxicity in maize plants. *Environ. Sci. pollut. Res.* 26, 11864–11874. doi: 10.1007/s11356-019-04517-3

Park, S., Lee, K., Kim, Y. S., and Back, K. (2012). Tryptamine 5-hydroxylase-deficient sekiguchi rice induces synthesis of 5-hydroxytryptophan and n-acetyltryptamine but decreases melatonin biosynthesis during senescence process of detached leaves. *J. Pineal Res.* 52, 211–216. doi: 10.1111/j.1600-079X.2011.00930.x

Payyavula, R. S., Singh, R. K., and Navarre, D. A. (2013). Transcription factors, sucrose, and sucrose metabolic genes interact to regulate potato phenylpropanoid metabolism. *J. Exp. Bot.* 64, 5115–5131. doi: 10.1093/jxb/ert303

Pelagio-Flores, R., Muñoz-Parra, E., Ortiz-Castro, R., and López-Bucio, J. (2012). Melatonin regulates arabidopsis root system architecture likely acting independently of auxin signaling. *J. Pineal Res.* 53, 279–288. doi: 10.1111/j.1600-079X.2012.00996.x

Peris-Peris, C., Serra-Cardona, A., Sánchez-Sanuy, F., Campo, S., Ariño, J., and San Segundo, B. (2017). Two NRAMP6 isoforms function as iron and manganese transporters and contribute to disease resistance in rice. *Mol. Plant Microbe Interact.* 30, 385–398. doi: 10.1094/MPMI-01-17-0005-R

Posmyk, M. M., Bałabusta, M., Wieczorek, M., Sliwinska, E., and Janas, K. (2009a). Melatonin applied to cucumber (Cucumis sativus l.) seeds improves germination during chilling stress. *J. Pineal Res.* 46, 214–223. doi: 10.1111/j.1600-079X.2008.00652.x

Posmyk, M. M., Janas, K. M., and Kontek, R. (2009). Red cabbage anthocyanin extract alleviates copper-induced cytological disturbances in plant meristematic tissue and human lymphocytes. *Biometals* 22, 479–490. doi: 10.1007/s10534-009-9205-8

Rahman, M. M., Ibrahim, M., Muktadir, M. A., Sadeque, A., Abdel Latef, A., and Ashrafuzzaman, M. (2022). "Rice: Role and responses under abiotic stress," in *Sustainable remedies for abiotic stress in cereals* (Springer), 125–147. doi: 10.1007/978-981-19-5121-3_6

Ramakrishna, A., Giridhar, P., Sankar, K. U., and Ravishankar, G. A. (2012). Melatonin and serotonin profiles in beans of coffea species. *J. Pineal Res.* 52, 470–476. doi: 10.1111/j.1600-079X.2011.00964.x

Raza, A., Salehi, H., Rahman, M. A., Zahid, Z., Madadkar Haghjou, M., Najafi-Kakavand, S., et al. (2022). Plant hormones and neurotransmitter interactions mediate antioxidant defenses under induced oxidative stress in plants. *Front. Plant Sci.* 13. doi: 10.3389/fpls.2022.961872

Salehi-Lisar, S. Y., and Bakhshayeshan-Agdam, H. (2016). "Drought stress in plants: Causes, consequences, and tolerance," in *Drought stress tolerance in plants*, vol. 1. (Springer), 1–16. doi: 10.1007/978-3-319-28899-4_1

Sheshadri, S., Nishanth, M., Yamine, V., and Simon, B. (2018). Effect of melatonin on the stability and expression of reference genes in catharanthus roseus. *Sci. Rep.* 8, 1–11. doi: 10.1038/s41598-018-20474-2

Shi, H., Jiang, C., Ye, T., Tan, D.-X., Reiter, R. J., Zhang, H., et al. (2015b). Comparative physiological, metabolomic, and transcriptomic analyses reveal mechanisms of improved abiotic stress resistance in bermudagrass [Cynodon dactylon (L). pers.] by exogenous melatonin. *J. Exp. Bot.* 66, 681–694. doi: 10.1093/jxb/eru373

Shi, H., Reiter, R. J., Tan, D. X., and Chan, Z. (2015a). Indole-3-acetic acid inducible 17 positively modulates natural leaf senescence through melatonin-mediated pathway in *Arabidopsis*. *J. Pineal Res.* 58, 26–33. doi: 10.1111/jpi.12188

Siddiqui, M. H., Alamri, S., Al-Khaishany, M. Y., Khan, M. N., Al-Amri, A., Ali, H. M., et al. (2019). Exogenous melatonin counteracts NaCl-induced damage by regulating the antioxidant system, proline and carbohydrates metabolism in tomato seedlings. *Int. J. Mol. Sci.* 20, 353. doi: 10.3390/ijms20020353

Simlat, M., Ptak, A., Skrzypek, E., Warchoł, M., Morańska, E., and Piórkowska, E. (2018). Melatonin significantly influences seed germination and seedling growth of stevia rebaudiana bertoni. *PeerJ* 6, e5009. doi: 10.7717/peerj.5009

Singhal, R. K., Jatav, H. S., Aftab, T., Pandey, S., Mishra, U. N., Chauhan, J., et al. (2021). Roles of nitric oxide in conferring multiple abiotic stress tolerance in plants and crosstalk with other plant growth regulators. *J. Plant Growth Regul.* 40, 2303–2328. doi: 10.1007/s00344-021-10446-8

Singh, R., Mishra, A., Dhawan, S. S., Shirke, P. A., Gupta, M. M., and Sharma, A. (2015). Physiological performance, secondary metabolite and expression profiling of genes associated with drought tolerance in withania somnifera. *Protoplasma* 252, 1439–1450. doi: 10.1007/s00709-015-0771-z

Solfanelli, C., Poggi, A., Loreti, E., Alpi, A., and Perata, P. (2006). Sucrose-specific induction of the anthocyanin biosynthetic pathway in arabidopsis. *Plant Physiol.* 140, 637–646. doi: 10.1104/pp.105.072579

Sun, Y.-D., Guo, D.-L., Yang, S.-D., Zhang, H.-C., Wang, L.-L., and Yu, Y.-H. (2020). Melatonin treatment improves the shelf-life and postharvest quality of table grape (Vitis labrusca l. cv.'Fengzao'). *J. Berry Res.* 10, 665–676. doi: 10.3233/JBR-200569

Sun, C., Liu, L., Wang, L., Li, B., Jin, C., and Lin, X. (2021). Melatonin: A master regulator of plant development and stress responses. *J. Integr. Plant Biol.* 63, 126–145. doi: 10.1111/jipb.12993

Suo, J., Zhao, Q., Zhang, Z., Chen, S., Liu, G., Wei, X., et al. (2015). Cytological and proteomic analyses of osmunda cinnamomea germinating spores reveal characteristics of fern spore germination and rhizoid tip growth. *Mol. Cell. Proteomics* 14, 2510–2534. doi: 10.1074/mcp.M114.047225

Tan, X. L., Fan, Z. Q., Kuang, J. F., Lu, W. J., Reiter, R. J., Lakshmanan, P., et al. (2019). Melatonin delays leaf senescence of Chinese flowering cabbage by suppressing ABFs-mediated abscisic acid biosynthesis and chlorophyll degradation. *J. Pineal Res.* 67, e12570. doi: 10.1111/jpi.12570

Tan, D. X., Manchester, L. C., Liu, X., Rosales-Corral, S. A., Acuna-Castroviejo, D., and Reiter, R. J. (2013). Mitochondria and chloroplasts as the original sites of melatonin synthesis: a hypothesis related to melatonin's primary function and evolution in eukaryotes. *J. Pineal Res.* 54, 127–138. doi: 10.1111/jpi.12026

Tomas, C., and Montes, A. (2005). A proposed mechanism to explain the stimulatory effect of melatonin on antioxidantive en-zymes. *J. Pineal Res.* 39, 99–104. doi: 10.1111/j.1600-079X.2005.00248.x

Van Tassel, D. L., Roberts, N., Lewy, A., and O'neill, S. D. (2001). Melatonin in plant organs. *J. Pineal Res.* 31, 8–15. doi: 10.1034/j.1600-079X.2001.310102.x

Wang, Q., An, B., Wei, Y., Reiter, R. J., Shi, H., Luo, H., et al. (2016). Melatonin regulates root meristem by repressing auxin synthesis and polar auxin transport in arabidopsis. *Front. Plant Sci.* 7. doi: 10.3389/fpls.2016.01882

Wang, L., Feng, C., Zheng, X., Guo, Y., Zhou, F., Shan, D., et al. (2017). Plant mitochondria synthesize melatonin and enhance the tolerance of plants to drought stress. *J. Pineal Res.* 63, e12429. doi: 10.1111/jpi.12429

Wang, Y., Reiter, R. J., and Chan, Z. (2018). Phytomelatonin: A universal abiotic stress regulator. *J. Exp. Bot.* 69, 963–974. doi: 10.1093/jxb/erx473

Wang, K., Xing, Q., Ahammed, G. J., and Zhou, J. (2022). Functions and prospects of melatonin in plant growth, yield and quality. *J. Exp. Bot.* doi: 10.1093/jxb/erac233

Wang, M., Zhang, T., and Ding, F. (2019). Exogenous melatonin delays methyl jasmonate-triggered senescence in tomato leaves. *Agronomy* 9, 795. doi: 10.3390/agronomy9120795

Wang, L., Zhao, Y., Reiter, R. J., He, C., Liu, G., Lei, Q., et al. (2014). Changes in melatonin levels in transgenic 'Micro-tom'tomato overexpressing ovine AANAT and ovine HIOMT genes. *J. Pineal Res.* 56, 134–142. doi: 10.1111/jpi.12105

Weeda, S., Zhang, N., Zhao, X., Ndip, G., Guo, Y., Buck, G. A., et al. (2014). Arabidopsis transcriptome analysis reveals key roles of melatonin in plant defense systems. *PLoS One* 9, e93462. doi: 10.1371/journal.pone.0093462

Wei, Z., Li, C., Gao, T., Zhang, Z., Liang, B., Lv, Z., et al. (2019). Melatonin increases the performance of malus hupehensis after UV-b exposure. *Plant Physiol. Biochem.* 139, 630–641. doi: 10.1016/j.plaphy.2019.04.026

Wei, J., Li, D. X., Zhang, J. R., Shan, C., Rengel, Z., Song, Z. B., et al. (2018). Phytomelatonin receptor PMTR 1-mediated signaling regulates stomatal closure in arabidopsis thaliana. *J. Pineal Res.* 65, e12500. doi: 10.1111/jpi.12500

Wen, D., Gong, B., Sun, S., Liu, S., Wang, X., Wei, M., et al. (2016). Promoting roles of melatonin in adventitious root development of solanum lycopersicum l. by regulating auxin and nitric oxide signaling. *Front. Plant Sci.* 7. doi: 10.3389/fpls.2016.00718

Wu, J., Nadeem, M., Galagedara, L., Thomas, R., and Cheema, M. (2022). Recent insights into cell responses to cold stress in plants: Signaling, defence, and potential functions of phosphatidic acid. *Environ. Exp. Bot.*, 105068. doi: 10.1016/j.envexpbot.2022.105068

Xie, Q., Zhang, Y., Cheng, Y., Tian, Y., Luo, J., Hu, Z., et al. (2022). The role of melatonin in tomato stress response, growth and development. *Plant Cell Rep.*, 1–20. doi: 10.1007/s00299-022-02876-9

Xu, W., Cai, S. Y., Zhang, Y., Wang, Y., Ahammed, G. J., Xia, X. J., et al. (2016). Melatonin enhances thermotolerance by promoting cellular protein protection in tomato plants. *J. Pineal Res.* 61, 457–469. doi: 10.1111/jpi.12359

Xue, F., Liu, W., Cao, H., Song, L., Ji, S., Tong, L., et al. (2021). Stomatal conductance of tomato leaves is regulated by both abscisic acid and leaf water potential under combined water and salt stress. *Physiol. Plant* 172, 2070–2078. doi: 10.1111/ppl.13441

Xu, L., Xiang, G., Sun, Q., Ni, Y., Jin, Z., Gao, S., et al. (2019). Melatonin enhances salt tolerance by promoting MYB108A-mediated ethylene biosynthesis in grapevines. *Hortic. Res.* 6. doi: 10.1038/s41438-019-0197-4

Xu, L., Yue, Q., Bian, F. E., Sun, H., Zhai, H., and Yao, Y. (2017). Melatonin enhances phenolics accumulation partially *via* ethylene signaling and resulted in high antioxidant capacity in grape berries. *Front. Plant Sci.* 8. doi: 10.3389/fpls.2017.01426

Yang, X., Ren, J., Li, J., Lin, X., Xia, X., Yan, W., et al. (2022). Meta-analysis of the effect of melatonin application on abiotic stress tolerance in plants. *Plant Biotechnol. Rep.*, 1–14. doi: 10.1007/s11816-022-00770-0

Yao, J. W., Ma, Z., Ma, Y. Q., Zhu, Y., Lei, M. Q., Hao, C. Y., et al. (2021). Role of melatonin in UV-b signaling pathway and UV-b stress resistance in *Arabidopsis thaliana*. *Plant Cell Environ.* 44, 114–129. doi: 10.1111/pce.13879

Ye, J., Wang, S., Deng, X., Yin, L., Xiong, B., and Wang, X. (2016). Melatonin increased maize (Zea mays l.) seedling drought tolerance by alleviating drought-induced photosynthetic inhibition and oxidative damage. *Acta Physiol. Plant* 38, 1–13. doi: 10.1007/s11738-015-2045-y

Yin, X., Bai, Y. L., Gong, C., Song, W., Wu, Y., Ye, T., et al. (2022). The phytomelatonin receptor PMTR1 regulates seed development and germination by modulating abscisic acid homeostasis in *Arabidopsis thaliana*. *J. Pineal Res.* 72, e12797. doi: 10.1111/jpi.12797

Yin, Z., Lu, J., Meng, S., Liu, Y., Mostafa, I., Qi, M., et al. (2019). Exogenous melatonin improves salt tolerance in tomato by regulating photosynthetic electron flux and the ascorbate–glutathione cycle. *J. Plant Interact.* 14, 453–463. doi: 10.1080/17429145.2019.1645895

Yu, Y., Lv, Y., Shi, Y., Li, T., Chen, Y., Zhao, D., et al. (2018). The role of phyto-melatonin and related metabolites in response to stress. *Molecules* 23, 1887. doi: 10.3390/molecules23081887

Zeng, W., Mostafa, S., Lu, Z., and Jin, B. (2022). Melatonin-mediated abiotic stress tolerance in plants. *Front. Plant Sci.* 13. doi: 10.3389/fpls.2022.847175

Zhang, N., Sun, Q., Zhang, H., Cao, Y., Weeda, S., Ren, S., et al. (2015). Roles of melatonin in abiotic stress resistance in plants. *J. Exp. Bot.* 66, 647–656. doi: 10.1093/jxb/eru336

Zhang, Y., Wang, J., Chen, X., Lu, X., Wang, D., Wang, J., et al. (2021b). Genome-wide identification and characteristic analysis of the downstream melatonin metabolism gene GhM2H in *Gossypium hirsutum* l. *Biol. Res.* 54, 1–17. doi: 10.1186/s40659-021-00358-y

Zhang, T., Wang, J., Sun, Y., Zhang, L., and Zheng, S. (2021a). Versatile roles of melatonin in growth and stress tolerance in plants. *J. Plant Growth Regul.*, 1–17. doi: 10.1007/s00344-021-10317-2

Zhang, N., Zhang, H.-J., Sun, Q.-Q., Cao, Y.-Y., Li, X., Zhao, B., et al. (2017). Proteomic analysis reveals a role of melatonin in promoting cucumber seed germination under high salinity by regulating energy production. *Sci. Rep.* 7, 1–15. doi: 10.1038/s41598-017-00566-1

Zhang, H. J., Zhang, N., Yang, R. C., Wang, L., Sun, Q. Q., Li, D. B., et al. (2014). Melatonin promotes seed germination under high salinity by regulating antioxidant systems, ABA and GA 4 interaction in cucumber (C ucumis sativus l.). *J. Pineal Res.* 57, 269–279. doi: 10.1111/jpi.12167

Zhang, N., Zhao, B., Zhang, H. J., Weeda, S., Yang, C., Yang, Z. C., et al. (2013). Melatonin promotes water-stress tolerance, lateral root formation, and seed germination in cucumber (Cucumis sativus l.). *J. Pineal Res.* 54, 15–23. doi: 10.1111/j.1600-079X.2012.01015.x

Zhao, D., Yu, Y., Shen, Y., Liu, Q., Zhao, Z., Sharma, R., et al. (2019). Melatonin synthesis and function: evolutionary history in animals and plants. *Front. Endocrinol.* 10. doi: 10.3389/fendo.2019.00249

Zhou, Y., Chen, M., Guo, J., Wang, Y., Min, D., Jiang, Q., et al. (2020). Overexpression of soybean DREB1 enhances drought stress tolerance of transgenic wheat in the field. *J. Exp. Bot.* 71, 1842–1857. doi: 10.1093/jxb/erz569

Zhu, Y., Gao, H., Lu, M., Hao, C., Pu, Z., Guo, M., et al. (2019). Melatonin-nitric oxide crosstalk and their roles in the redox network in plants. *Int. J. Mol. Sci.* 20, 6200. doi: 10.3390/ijms20246200

frontiers | Frontiers in Plant Science

TYPE Original Research
PUBLISHED 06 March 2023
DOI 10.3389/fpls.2023.1149873

Pre-treatment of melatonin enhances the seed germination responses and physiological mechanisms of soybean (*Glycine max* L.) under abiotic stresses

Samrah Afzal Awan[1,2†], Imran Khan[3†], Qi Wang[1,2], Jing Gao[1,2], Xianming Tan[1,2] and Feng Yang[1,2*]

[1]College of Agronomy, Sichuan Agricultural University, Chengdu, China, [2]Sichuan Engineering Research Center for Crop Strip Intercropping System, Chengdu, China, [3]State Key Laboratory of Grassland Agro-Ecosystems, Ministry of Agriculture, College of Pastoral Agriculture Science and Technology, Lanzhou University, Lanzhou, China

EDITED BY
Safina Naz,
Bahauddin Zakariya University, Pakistan

REVIEWED BY
Wajid Zaman,
Yeungnam University, Republic of Korea
Fazal Ullah,
Key Laboratory of Mountain Ecological Rehabilitation and Biological Resource Utilization (CAS), China
Mohammad Shah Jahan,
Sher-e-Bangla Agricultural University, Bangladesh
Mohamed Omar Kaseb,
Agricultural Research Center, Egypt

*CORRESPONDENCE
Feng Yang
✉ f.yang@sicau.edu.cn

†These authors have contributed equally to this work

SPECIALTY SECTION
This article was submitted to Plant Abiotic Stress, a section of the journal Frontiers in Plant Science

RECEIVED 23 January 2023
ACCEPTED 21 February 2023
PUBLISHED 06 March 2023

CITATION
Awan SA, Khan I, Wang Q, Gao J, Tan X and Yang F (2023) Pre-treatment of melatonin enhances the seed germination responses and physiological mechanisms of soybean (*Glycine max* L.) under abiotic stresses.
Front. Plant Sci. 14:1149873.
doi: 10.3389/fpls.2023.1149873

The germination of soybean (*Glycine max* L.) seeds is critically affected by abiotic stresses which resulting in decreasing crop growth and yield. However; little is known about the physiological mechanisms of germination and the potential role of melatonin on soybean seed germination under drought, salt, cold, and heat stresses. Therefore, the current study investigated the possible effects of melatonin to enhance germination indices and other physiological attributes by alleviating the harmful impacts of these stresses during germination. Seeds of soybean were pre-treated (seed priming) with melatonin at MT1 (20 µmol L^{-1}), MT2 (50 µmol L^{-1}), MT3 (100 µmol L^{-1}), MT4 (200 µmol L^{-1}), and MT5 (300 µmol L^{-1}) and exposed to the four stresses (drought at PEG 15%, salt at 150mM, cold at 10 °C, and heat at 30 °C) . It was noted that MT1 (20 µmol L^{-1}), MT2 (50 µmol L^{-1}), and MT3 (100 µmol L^{-1}) remarkably improved the germination potential, germination rate, radical length, and biomass under given stresses. Furthermore, MT1, MT2, and MT3 progressively increased the proline to minimize the impact of drought, salt, cold, and heat stresses. In addition, all stresses significantly induced oxidative damage however, salt (150 mM NaCl) and heat (30 °C) stresses highly increased the malondialdehyde content (MDA) and hydrogen peroxide (H2O2) as compared to drought (PEG 15%) and cold (10 °C) stresses. Moreover, MT2 and MT3 significantly enhanced the activities of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and ascorbate peroxidase (APX) to reduce the oxidative damage in soybean seeds during the germination. Overall, melatonin at 50 µmol L^{-1} and 100 µmol L^{-1} considerably mitigated the harmful impacts of drought, salt, cold, and heat stress by enhancing germination and other physiological mechanisms of soybean. This study could provide bases to enhance the melatonin-mediated tolerance of soybean and other related crops at early growth stages when exposed to abiotic stresses.

KEYWORDS

soybean, melatonin, germination, oxidative damage, antioxidant enzymes

Introduction

Seed germination starts as dry seed absorbs water and ends as radical protrudes from the seed coat, and it is a crucial stage of a plant's life cycle (Xiao et al., 2019). A series of metabolic, cellular, and molecular events are usually involved in this complex process for the successful establishment of crops at early growth stages (Zhang et al., 2020). The seed germination stage is significantly influenced by external environmental factors and is very sensitive to abiotic stresses such as drought, salt, and low and high temperatures. Therefore, this stage makes a strong interaction between growth and the final yield of crops which refers to economic and ecological importance (Weitbrecht et al., 2011). Abiotic stresses cause irregular seedling emergence and lead to decrease in number of plants and final crop production (Okçu et al., 2005). Changes in the germination indicators including germination index (GI), germination potential (GP), and germination rate (GR) could be considered for the evaluation of abiotic stress tolerance during seed germination (Zhang et al., 2020). It has been reported that mechanical strength provided by seed coat increases under stressful condition which directly inhibits seed germination (Debeaujon et al., 2000). Abiotic stress condition induces oxidative damage by increasing the excessive production of reactive oxygen species (ROS) in the plant cell, resulting in cell death. However, a complex antioxidant defense system efficiently regulates the oxidative profile by enhancing the activities of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and ascorbate peroxidase (APX), and reduces the impacts of stresses (Xiao et al., 2019). Therefore, it is crucial to reduce the impacts of abiotic stresses on seed germination for better seedling growth and crop yield.

Different conventional methods have been applied to lessen the deleterious effects of abiotic stresses on seed germination and radical emergence (Seleiman et al., 2021). Among them, seed pre-treatment with biological substances, biomolecules, or phytohormones could be important to enhance seed germination and tolerance to abiotic stresses (Awan et al., 2021). Melatonin is a biological molecule produced by L-tryptophan and performs a variety of physiological functions in plants (Imran et al., 2021). Recently, melatonin is being widely used by different researchers to improve growth, productivity, and tolerance of plants under various types of stresses and found beneficial for plants under normal and stressful conditions (Wang et al., 2018). The production of endogenous melatonin is not enough and cannot significantly reduce the impact of abiotic stresses due to high degree of oxidative damage caused by over production of ROS (Fu et al., 2017). Melatonin effectively regulates seed germination, growth and development, and antioxidant profile in plants. Furthermore, the impact of melatonin on seed germination is dose-dependent, however; its pre-treatment usually promotes seed germination and seedling emergence even under stressful conditions (Xiao et al., 2019). Pieces of evidence reported that exogenous melatonin reduced the malondialdehyde (MDA) content, H_2O_2, oxygen radical, and electrolyte leakage in the seedlings under stressful conditions (Bai et al., 2020; Wei et al., 2022). Therefore, the use of melatonin as a seed germination-promoting agent under stressful conditions could be crucial for crop growth and development.

Soybean (*Glycine max* [L.] Merr.) is one of the most important economical grain crops cultivated for oil and protein worldwide (Papadaki et al., 2019; Zhao et al., 2021). Soybean roots have good nitrogen fixation ability, which can reduce the excessive use of fertilizer and is beneficial to the sustainable development of the environment (Meena et al., 2018). Soybeans are used as a major source of food and for feeding and have high economic value, high content of protein and oil, minerals, nutrients, and vitamins (Yang et al., 2018b). Besides, soybean is also being used in intercropping with maize and other crops to achieve the goal of safe and enough production within the limited space (Yang et al., 2014; Yang et al., 2018a) due to the increasing world population. The worldwide production of soybean was reported 311.1 million tonnes in 2020 and estimated to reach 371.3 million tonnes in 2030, which indicated 1.8% more growth rate as compared to 2010-2020 (Siamabele, 2021). According to a conducted survey, soybean farming increased the agriculture business income by 37.77% and household income by 18.87% in Indonesia (Roessali et al., 2019). In 2017, the total production of soybean was 14.3 million tonnes in China and due to less land available for soybean cultivation, China imported soybean about 95.5 million tonnes to fulfill the need of soybean (Guo et al., 2021). This suggested that soybean farming (increasing growth rate) could improve its production that can increase the agriculture business income and household's income in China and could be important to economy of China. In addition, the soybean intercropping with other crops usually creates a low-light environment that could be a challenge to successful growth and sufficient production of soybean because it also refers to a stressful environment (Yang et al., 2017; Yang et al., 2020). However, soybean production hardly meets the needs of the increasing global population. At the same time, unfavorable environmental settings and a decrease in the germination potential in terms of low germination rate due to abiotic stresses negatively affect its early growth which leads to fatal disorders (Zhang et al., 2020). Soybean is highly sensitive to drought, salt, and heat stresses that inhibited seed germination, vegetative growth, and physiological, and biochemical attributes (Zhang et al., 2020; Imran et al., 2021). Abiotic stresses generally lead to reduce water supply to seed or germinating seeds which results in slow metabolic processes and inhibits or prolongs seed germination that negatively affects its later growth stages (Bai et al., 2020). The protective role of melatonin pre-treatment on soybean seed germination and seedling growth under combination of abiotic stresses (drought, salt, cold, and heat) is still unclear that needs to be investigated for clear understandings.

Therefore, the present study was designed to investigate the potential role of melatonin to mitigate the impact of abiotic stress and promote seed germination of soybean. Moreover, how melatonin can regulate the different physiological mechanisms during seed germination of soybean under different types of stresses is also crucial to be explored for future research work. This is because, little is known about the pre-treatment (seed priming) of melatonin on soybean seed germination, its physiological profile, and antioxidant enzyme activities under drought, salt, cold, and heat stresses. Thus, the main objectives of the current study were to investigate the role of pre-treatment of melatonin on soybean seed germination indices, seedling growth, degree of oxidative damage, activities of antioxidant enzyme activities, and range of tolerance under drought, salt, cold, and heat stresses

Materials and methods

Plat materials, treatments, and growth conditions

The seeds of soybean (*Glycine max* [L.] Merr.) cultivar Nandou-12 (ND-12) were used in this experiment and obtained from Nanchong Academy of Agricultural Sciences, Sichuan Province, China. Healthy and uniform size seeds of the soybean cultivar "Nandou 12" were surface sterilized by following the method given by (Awan et al., 2022). After that seeds were air-dried and subjected to different melatonin (MT) treatments. Moreover, the melatonin (molecular weight: 232.28) was purchased from Sigma Aldrich with >99% purity. To make a stock solution of melatonin, the amount of melatonin 0.232g powder was weighed and dissolved in an appropriate amount of anhydrous ethanol. Later, deionized water was added and made the final volume of the solution up to 100 mL which gave 10,000 µmol L^{-1}. Further dilutions were carried out to prepare different concentrations of melatonin as CK, MT1 (20 µmol L^{-1}), MT2 (50 µmol L^{-1}), MT3 (100 µmol L^{-1}), MT4 (200 µmol L^{-1}), and MT5 (300 µmol L^{-1}), and seeds were dipped in the prepared concentrations for 12 hours (pre-treatment). After that seeds were air-dried at room temperature for 10 min and cultivated in petri plates having moistened blotting paper. A total of 100 seeds were cultivated for each treatment and placed petri plates in the growth chambers under growing conditions of 8 hours at night and 12 hours a day.

Drought (PEG), salt, cold, and heat stress treatments

All the seed pre-treated with melatonin was separated into four groups and exposed to these four stresses in petri plates. After immediate cultivation of soybean seeds into petri plates, the 15% PEG-6000 by dissolving in distilled water, and 150 mM NaCl by dissolving in distilled water up to final concentration (150mM), cold (10°C), and heat (30°C) were given to seeds. For PEG and NaCl, 10 mL of solution was applied after every 24 hours to each petri plate except for the control (CK). The germination of seeds was observed and noted daily and after 7 days, the related parameters were measured.

Germination indices

Germination indices including germination potential and germination rate of soybean seeds were measured according to the method given by (Cao et al., 2019; Chen et al., 2020) under all given treatments by using the following formulae;

$$\text{Germination potential (\%)} = \text{number of seeds germinated at 3 days/ total no. of seeds} \times 100$$

$$\text{Germination rate (\%)} = \text{number of germinated seeds at 7 days/ total no. of seeds} \times 100$$

Proline content and Electrolyte Leakage

Proline content was measured according to the method given by (Bates et al., 1973) with slight modifications. The fresh radical sample (0.5 g) from each treatment was homogenized with 3 mL of 3% (w/v) sulfosalicylic acid and centrifuged. The supernatant was collected and 2mL of each glacial acetic acid and ninhydrin was added to the supernatant and heated for 30 min at 100˚C in a water bath. Later, the mixture was placed in an ice bath to stop the reaction. After cooling, the mixture was again centrifuged at 10,000 rpm for 5 min and absorbance of the mixture was measured at 520nm using a spectrophotometer. The proline content was expressed as µmol g^{-1} FW. Moreover, the electrolyte leakage (EL) of fresh sample material was determined according to the method described by (Chen et al., 2020).

Determination of malondialdehyde and hydrogen peroxide

The MDA and H_2O_2 were determined according to the instructions of the kits (Solarbio, Beijing) as reported by (Gu et al., 2018; Zhao et al., 2018). In brief, 0.5 g of sample material was ground with liquid nitrogen and extraction buffer was added to it. Later, the reaction mixture was centrifuged at 12,000 g for 10 min and the supernatant was collected. Finally, the MDA and H_2O_2 were determined as per manufacturer instructions.

Measurements of antioxidant enzymes activities

The activities of antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and ascorbate peroxidase (APX) were measured by following the instructions of antioxidant enzyme assay kits (Solarbio) as reported by (Zhao et al., 2018). In short, 0.5g of the sample was ground in liquid nitrogen and extracted through an extraction buffer. Later, the extraction mixture was centrifuged at 12, 000 g for 10 min, and the supernatant was collected. The activities of

SOD, CAT, POD, and APX were measured by using a spectrophotometer.

Statistical analyses

All the data were analyzed by one-way analyses of variance (ANOVA) using statistics 8.1. Data are the mean of triplicate per treatment with standard deviation (mean ± SD). The statistical differences among different treatments were achieved by the least significance difference (LSD) test. The level of significance was considered at $p < 0.05$. The graphical figures of the present study were generated by using Microsoft excel. Principal component analysis (PCA) among various measured variables of soybean under different types of stresses was achieved using "past" software.

Results

The results of the present study depicted that PEG, NaCl, Cold, and Heat stresses remarkably declined the germination indices including germination potential, germination rate, radical lengths, and fresh and dry weights of germinated soybean seeds (Table 1). However, different concentrations of melatonin differentially improved the morphology and germination indices by reducing the harmful effects of these four stresses (Figure 1). Furthermore, PEG, NaCl, Cold, and Heat stress reduced the germination potential by 33%, 36%, 36%, and 41% and germination rate by 30%, 27%, 29%, and 36% over the respective controls. The PEG stress differently influenced the radical length and fresh and dry weight of soybean seeds during germination on exposure to different concentrations of melatonin. In addition, NaCl, Cold, and Heat

TABLE 1 Effect of different concentrations of melatonin on germination indices, radical length, and biomass of germinated soybean seeds under PEG, NaCl, Cold, and Heat stress.

Treatments		Germination Potential (%)	Germination Rate (%)	Radical Length (cm)	Fresh Weight (g)	Dry Weight (g)
PEG-stress	CK	86.0 ± 3.4a	86.0 ± 3.4a	13.5 ± 0.20a	0.71 ± 0.008c	0.042 ± 0.0022b
	stress	57.3 ± 2.3d	60.0 ± 2.6c	20.6 ± 0.31f	0.73 ± 0.012c	0.035 ± 0.0013c
	MT1 (20µM)	71.7 ± 2.6bc	75.0 ± 1.7b	18.3 ± 0.16e	0.72 ± 0.005c	0.041 ± 0.0011b
	MT2 (50µM)	78.3 ± 2.0ab	83.3 ± 2.3a	15.4 ± 0.17d	0.77 ± 0.012b	0.043 ± 0.0015b
	MT3 (100µM)	80.6 ± 2.9a	85.3 ± 2.4a	21.9 ± 0.28b	0.82 ± 0.008a	0.049 ± 0.0011a
	MT4 (200µM)	70.1 ± 2.1bc	70.6 ± 2.6b	15.3 ± 0.24c	0.78 ± 0.006b	0.034 ± 0.0015c
	MT5 (300µM)	65.0 ± 3.0cd	74.6 ± 2.1b	11.4 ± 0.14c	0.72 ± 0.006c	0.032 ± 0.0018c
NaCl-stress	CK	86.0 ± 3.4a	86.0 ± 3.4a	13.5 ± 0.20a	0.71 ± 0.008c	0.042 ± 0.0022ab
	stress	55.0 ± 3.4f	62.0 ± 1.5d	10.9 ± 0.20f	0.64 ± 0.008b	0.032 ± 0.0017c
	MT1 (20µM)	71.3 ± 2.0cd	76.3 ± 2.1b	14.5 ± 0.20de	0.64 ± 0.005c	0.036 ± 0.0018bc
	MT2 (50µM)	82.2 ± 2.0ab	87.0 ± 1.0a	13.8 ± 0.03cd	0.68 ± 0.003c	0.033 ± 0.0022c
	MT3 (100µM)	76.0 ± 2.6bc	77.6 ± 2.4b	14.8 ± 0.20b	0.74 ± 0.005b	0.044 ± 0.0019a
	MT4 (200µM)	65.6 ± 1.4de	71.6 ± 2.0bc	12.6 ± 0.12c	0.59 ± 0.011a	0.035 ± 0.0016c
	MT5 (300µM)	63 ± 2.0e	68.0 ± 1.7cd	10.7 ± 0.21ef	0.49 ± 0.012d	0.033 ± 0.0018c
Cold-stress	CK	86.0 ± 3.4a	86.0 ± 3.4a	13.5 ± 0.20b	0.71 ± 0.008e	0.042 ± 0.0022a
	stress	54.6 ± 2.4e	60.3 ± 2.0e	3.9 ± 0.08d	0.38 ± 0.014a	0.021 ± 0.0016e
	MT1 (20µM)	65.0 ± 1.7cd	71.6 ± 2.0c	4.4 ± 0.06a	0.42 ± 0.005f	0.025 ± 0.0011cd
	MT2 (50µM)	70.3 ± 2.9bc	72.6 ± 2.3bc	4.7 ± 0.05b	0.47 ± 0.011e	0.021 ± 0.0014de
	MT3 (100µM)	77.0 ± 2.0b	79.3 ± 2.6ab	5.1 ± 0.10a	0.52 ± 0.008cd	0.032 ± 0.0015b
	MT4 (200µM)	63.0 ± 2.8cd	64.6 ± 1.7de	4.7 ± 0.03c	0.47 ± 0.006b	0.027 ± 0.0010c

(Continued)

TABLE 1 Continued

Treatments		Germination Potential (%)	Germination Rate (%)	Radical Length (cm)	Fresh Weight (g)	Dry Weight (g)
	MT5 (300μM)	60.7 ± 2.1de	69.6 ± 1.2cd	4.2 ± 0.06d	0.44 ± 0.008c	0.023 ± 0.0010cde
Heat-stress	CK	86.0 ± 3.4a	86.0 ± 3.4a	13.5 ± 0.20e	0.71 ± 0.008a	0.042 ± 0.0022a
	stress	50.3 ± 1.8d	54.6 ± 1.4d	3.9 ± 0.11b	0.29 ± 0.017e	0.014 ± 0.0017e
	MT1 (20μM)	59.3 ± 1.8c	64.0 ± 1.0c	5.1 ± 0.12c	0.32 ± 0.006e	0.018 ± 0.0011de
	MT2 (50μM)	72.3 ± 2.1b	75.0 ± 1.5b	6.2 ± 0.21d	0.39 ± 0.008cd	0.021 ± 0.0029cd
	MT3 (100μM)	74.0 ± 3.0b	81.6 ± 1.4a	8.1 ± 0.15a	0.48 ± 0.005b	0.030 ± 0.0012b
	MT4 (200μM)	63.0 ± 1.5c	66.0 ± 1.5c	7.3 ± 0.08d	0.40 ± 0.008c	0.023 ± 0.0012cd
	MT5 (300μM)	70.3 ± 2.3b	73.6 ± 2.3b	7.0 ± 0.12f	0.37 ± 0.008d	0.024 ± 0.0020c

Values are the means ± SD (n=3). Different letters show a statistical significance level at $p<0.05$. Here, μM is indicating (μmole L^{-1}).

stress reduced the radical length by 19%, 71%, and 71% and dry weight by 22%, 49%, and 65% as compared to the control, respectively.

In addition, the combined application of melatonin with PEG at MT1, MT2, MT3, MT4, and MT5 increased the germination potential by 25%, 36%, 40%, 23%, and 13%, germination rate by

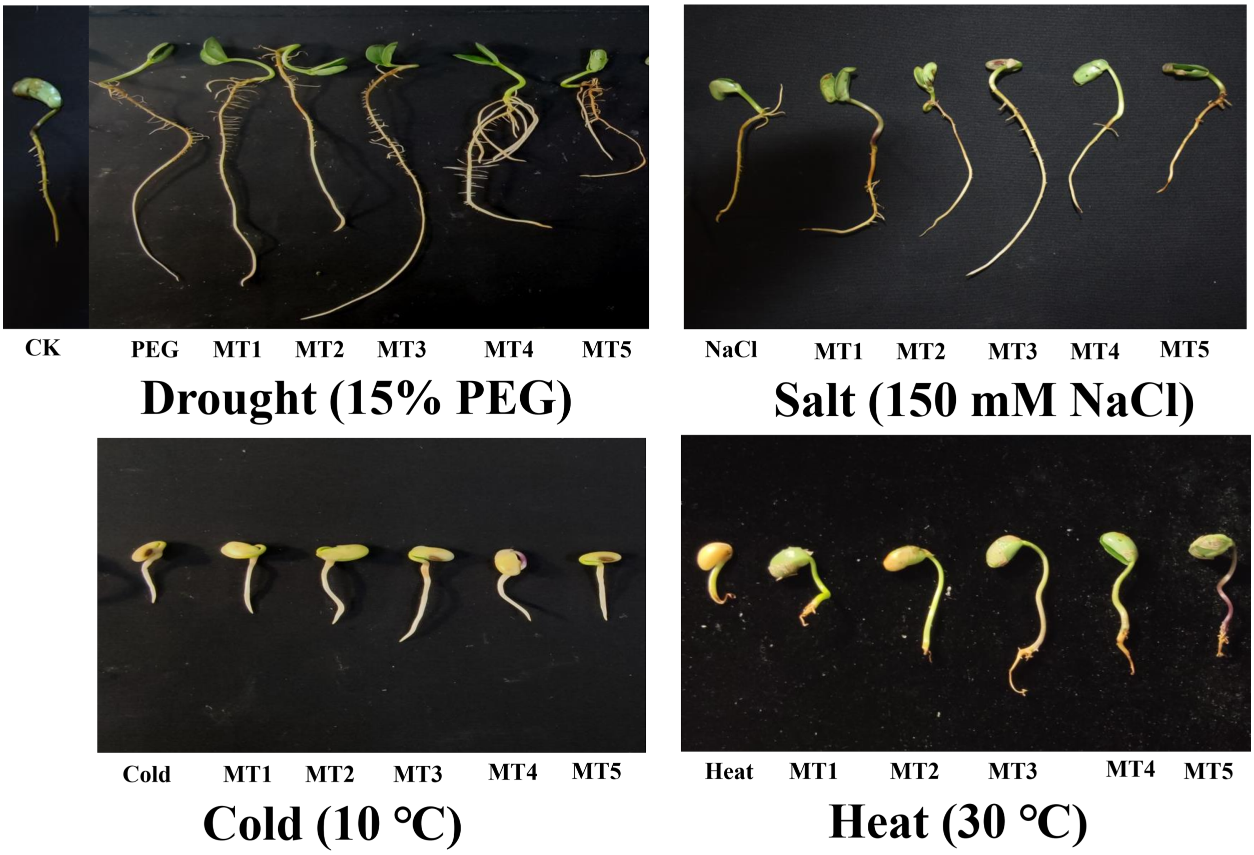


FIGURE 1
Effect of different concentrations of melatonin on the morphological representation of soybean germination PEG (15%), NaCl (150mM), Cold (10 °C), and Heat (30°C).

25%, 38%, 42%, 17%, and 24%, over the PEG, respectively. The applied concentrations of melatonin showed a progressive role under NaCl, Cold, and Heat stresses. Moreover, the MT1, MT2, MT3, MT4, and MT5 improved the germination potential by 29%, 49%, 38%, 19%, and 14%, germination rate by 23%, 40%, 25%, 15%, and 9%, over the NaCl, respectively. Similarly, MT1, MT2, MT3, MT4, and MT5 enhanced the germination potential by 19%, 28%, 40%, 15%, and 10%, germination rate by 19%, 20%, 31%, 7%, and 15%, over the Cold, respectively. In the same context, MT1, MT2, MT3, MT4, and MT5 increased the germination potential by 17%, 43%, 47%, 25%, and 39%, germination rate by 17%, 37%, 49%, 20%, and 34%, over the Heat, respectively (Table 1). In addition, the seed priming at different concentrations of melatonin differentially impacted the fresh and dry biomass of germinated soybean seeds under drought, salt, cold, and heat stresses. However, the optimized concentrations of melatonin significantly improved the fresh and dry biomass of germinated soybean seeds as shown in Table 1.

Among all treated concentrations of melatonin, the 20µmol L^{-1} (MT1), 50µmol L^{-1} (MT2), and 100µmol L^{-1} (MT3) showed significant impacts on morphology and germination indices of soybean seeds as compared to other concentrations under PEG, NaCl, Cold, and Heat stresses. Therefore, we selected these three concentrations of melatonin to carry out further analyses.

Proline is an important osmoprotectant that facilitates plants to withstand stressful conditions (Bai et al., 2020). In the present study, PEG, NaCl, Cold, and Heat stress increased the proline content by 27%, 39%, 18%, and 46% as compared to the respective control (Figure 2). However, melatonin at 20µmol L^{-1}, 50µmol L^{-1}, and 100µmol L^{-1} progressively improved the proline content under all these stresses and maximum enhancement was noticed at 50 µmol L^{-1} and 100 µmol L^{-1}. Melatonin at 20 µmol L^{-1}, 50 µmol L^{-1} and 100 µmol L^{-1} improved the proline by 8%, 19%, and 31% over the PEG (Figure 2A), 6%, 16%, and 27% over the NaCl (Figure 2B), 11%, 25% and 26% over the Cold (Figure 2C), 9%, 26% and 27% over the Heat (Figure 2D), respectively. On the other hand, electrolyte leakage (EL) was found to be increased due to PEG, NaCl, Cold, and Heat stress whereas, the application of melatonin reduced EL under these stresses. The EL was found to increase by PEG (151%), NaCl (178%), Cold (182%), and Heat (222%) over the respective controls (Figure 3). Furthermore, 20 µmol L^{-1}, 50 µmol L^{-1}, and 100 µmol L^{-1} decreased EL by 17%, 31%, and 33% over PEG, 8%, 25%, and 23% over NaCl, 9%, 26%, and 22% over Cold, and 16%, 29%, and 30% over Heat stress, respectively. Overall, melatonin at 50 µmol L^{-1}, and 100 µmol L^{-1} remarkably improved the proline and reduced the EL under different types of abiotic stresses.

Moreover, oxidative damage in terms of MDA and H_2O_2 was found increased due to PEG, NaCl, Cold, and Heat however, melatonin at different concentrations significantly reduced MDA and H_2O_2 under these stresses (Figures 4, 5). In the present study, PEG, NaCl, Cold, and Heat stress increased the MDA content by 105%, 135%, 103%, and 152% as compared to the respective control (Figure 4). However, melatonin at 20 µmol L^{-1}, 50 µmol L^{-1}, and 100 µmol L^{-1} linearly decreased the MDA content under all these stresses. Melatonin at 20 µmol L^{-1}, 50 µmol L^{-1} and 100 µmol L^{-1} reduced MDA by 13%, 25%, and 28% over the PEG (Figure 4A), 11%, 20%, and 25% over the NaCl (Figure 4B), 12% 29% and 23% over the Cold (Figure 4C), 14%, 23% and 24% over the Heat (Figure 4D), respectively. On the other hand, H_2O_2 was also increased due to PEG, NaCl, Cold, and Heat stresses by 121%, 154%, 105%, and 162% over their respective controls. Furthermore, 20 µmol L^{-1}, 50 µmol L^{-1}, and 100 µmol L^{-1} decreased H_2O_2 by 9%, 27%, and 37% over PEG, 8%, 27%, and 29% over NaCl, 10%, 31%, and 29% over Cold, and 12%, 27%, and 36% over Heat stress respectively (Figure 5). Overall, melatonin at 50µM, and 100µM remarkably reduced the MDA and H_2O_2 (oxidative damage) under different types of abiotic stresses.

In addition, the activities of antioxidant enzymes including SOD, CAT, POD, and APX were found negatively regulated by PEG, NaCl, Cold, and Heat stresses whereas melatonin positively impacted these activities to reduce the effects of oxidative damage. PEG and Cold increased the SOD by 72% and 6% as compared to control; however, melatonin at different levels progressively improved the SOD activity under PEG and Cold (Figure 6). Moreover, NaCl and Heat decreased the SOD activity by 9% and 5% over the control. In contrast, melatonin at 20 µmol L^{-1}, 50 µmol L^{-1}, and 100 µmol L^{-1} increased the SOD by 14%, 30%, and 47% over NaCl (Figure 6B), and 27%, 32% and 60% over Heat stress (Figure 6D) respectively. Furthermore, CAT activity was found to improve due to melatonin under all these stresses (Figure 7).

The melatonin at 20 µmol L^{-1}, 50 µmol L^{-1}, and 100 µmol L^{-1} enhanced the CAT activity by 0.3%, 50%, and 36% over PEG, 17%, 40%, and 67% over NaCl, 14%, 30%, and 54% over Cold, and 19%, 54%, and 58% over Heat. In the same line, the POD activity was differentially regulated by PEG, NaCl, Cold, and Heat stress however, progressively increased due to different concentrations of melatonin under all the stresses (Figure 8). PEG and cold decreased the POD activity whereas; NaCl and Heat increased the POD activity as compared to the control. Besides, melatonin at 20 µmol L^{-1}, 50 µmol L^{-1}, and 100 µmol L^{-1} increased the POD activity by 5%, 15%, and 19% over PEG (Figure 8A), 8%, 30%, and 27% over NaCl (Figure 8B), 2%, 30%, and 34% over Cold (Figure 8C), and 42%, 67%, and 78% over Heat (Figure 8D). In addition, the APX activity was also found to increase due to PEG, NaCl, Cold, and Heat stresses, and different levels of melatonin further boosted the APX activity under these stresses (Figure 9). Melatonin at 20 µmol L^{-1}, increased the APX activity by 22%, 14%, and 24% over the NaCl, cold, and heat stresses respectively. Furthermore, 50 µmol L^{-1} increased the APX by 22%, 69%, 36%, and 73%, and 100 µmol L^{-1} by 49%, 70%, 51%, and 64% over the PEG, NaCl, Cold, and Heat, respectively. Overall, the melatonin at all levels improved the activities of SOD, CAT, POD, and APX but 50 µmol L^{-1} and 100 µmol L^{-1} significantly impacted antioxidant enzymes activities under these stresses.

The loading plots of principal component analysis (PCA) evaluated the impact of drought, salt, cold, and heat stresses and exogenous melatonin on different parameters of soybean seeds during germination (Figure 10). Among all the components, the two components i.e. component 1 and component 2 covered almost 90-95% of whole dataset that made the largest portion of all components. The component 1 distributed about 50.67% and component 2 distributed about 42.12% (Figure 10A), component 1 distributed about 50.71% and component 2 distributed about 37.64% (Figure 10B), component 1 distributed about 59.20% and component 2 distributed about 37.06% (Figure 10C), and component 1 distributed about 62.93% and component 2

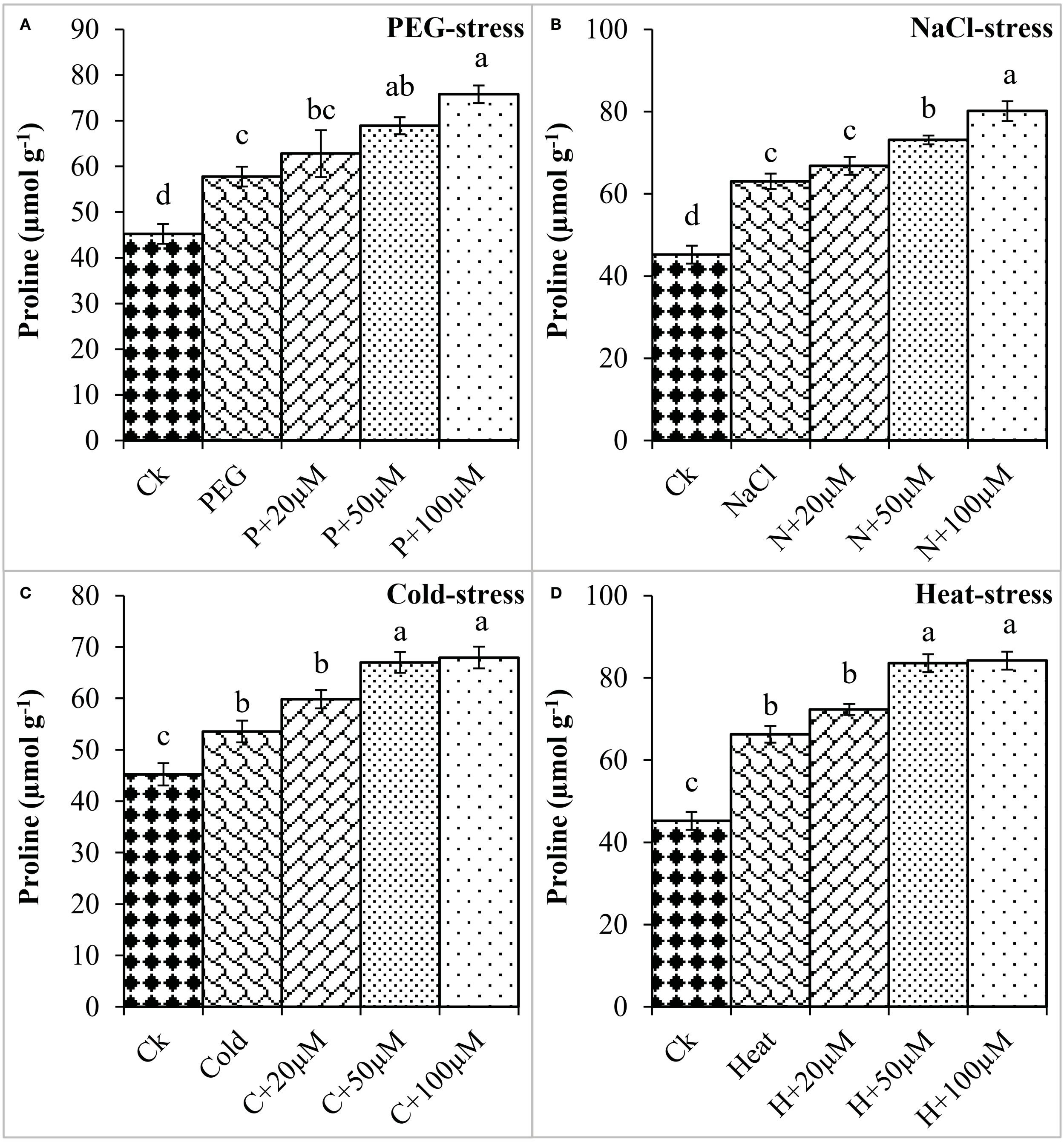


FIGURE 2
Effect of different concentrations of melatonin on the activity of Proline content under PEG **(A)**, NaCl **(B)**, Cold **(C)**, and Heat **(D)**. Values are the means ± SD (n=3). Different letters on the bars show a statistical significance level at $p<0.05$. Here, µM is indicating (µmole L^{-1}).

distributed about 35.14% (Figure 10D), of whole dataset. All the measured parameters were dispersed in the dataset of these two components.

Discussion

Soybean is an important leguminous crop and a major source of plant protein for humans, and animals intake it as an essential grain, oil, and feed (Yang et al., 2018b). Global production of soybean is facing major challenges to meet the increasing demand of the world's population. Climatic changes are critical and devastating factors to decrease the growth and production of plants (Chaudhry and Sidhu, 2022). The most sensitive and crucial stage of plant growth is seed germination (Patanè et al., 2016). However, abiotic stresses including drought, salt, cold, heat, and heavy metals negatively affect the germination of seeds by influencing the internal metabolic and physiological processes (Sethy and Ghosh,

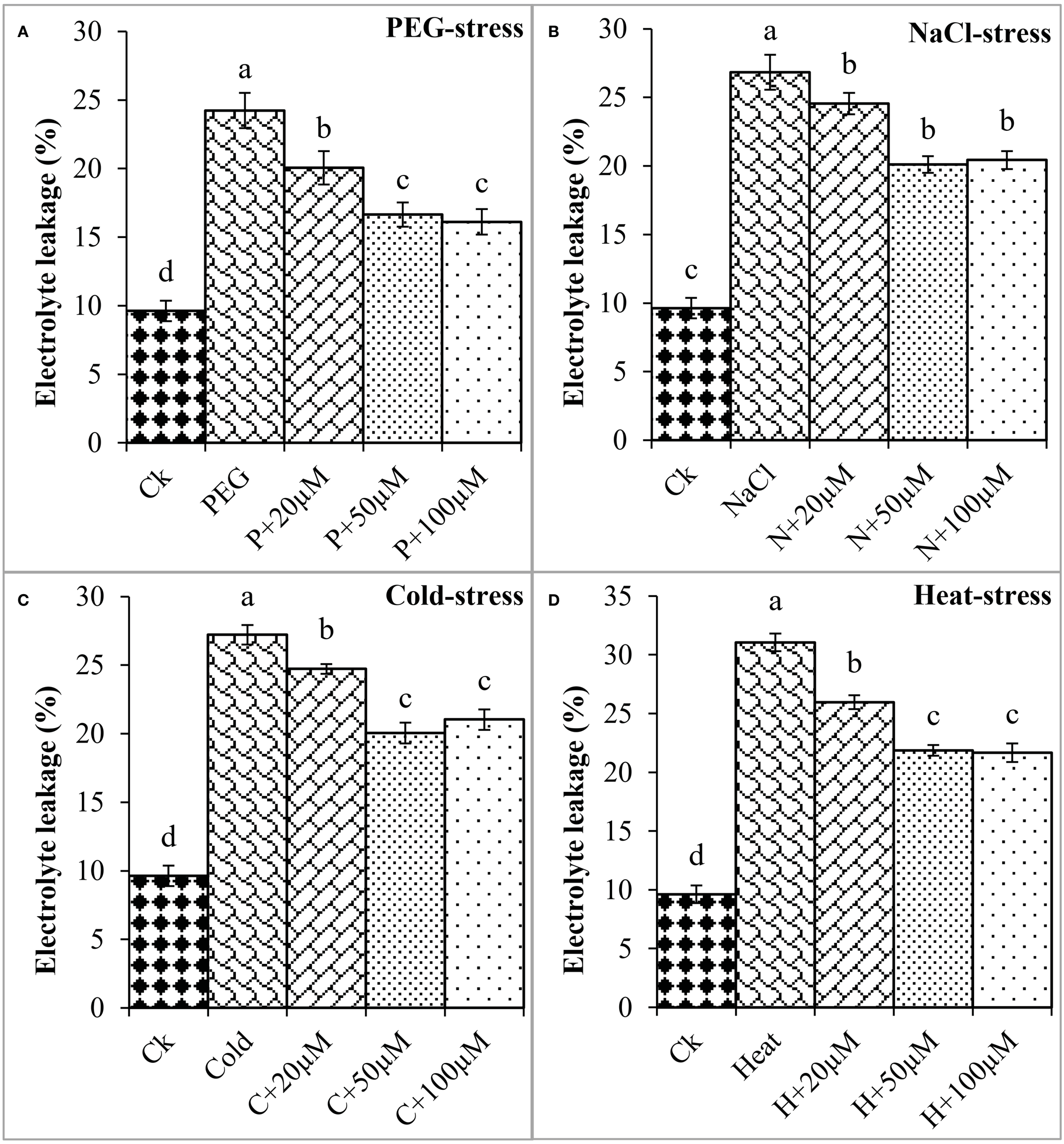


FIGURE 3
Effect of different concentrations of melatonin on the activity of Electrolyte leakage (EL) under PEG **(A)**, NaCl **(B)**, Cold **(C)**, and Heat **(D)**. Values are the means ± SD (n=3). Different letters on the bars show a statistical significance level at *$p<0.05$*. Here, μM is indicating (μmole L^{-1}).

2013; Cao et al., 2019). This is because a stressful environment leads to reduce water intake and energy supply to seeds, negative regulation of ROS, osmoprotectants, antioxidant defense system, and hormonal balance (Shu et al., 2018; Sharma and Zheng, 2019). In the same context, the present study suggested that 15% PEG, 150mM NaCl, 10°C cold, and 30°C heat strongly inhibited the germination of soybean seeds.

Besides, different plant growth regulators have been widely used to treat seeds before sowing to improve resistance against abiotic stresses and promote germination (Liang et al., 2018; Bai et al., 2020). Among them, melatonin is being widely used to enhance growth and other physiological attributes of different crops and showing remarkable results under normal stressful conditions (Wang et al., 2018). Naturally, melatonin is synthesized by L-tryptophane which is an essential amino acid and is also used for protein biosynthesis (Arnao and Hernández-Ruiz, 2014). Therefore, under normal and stressful conditions proteins stabilize the various metabolic functions of cells including plants and animals. Previous studies have reported positive and beneficial effects of melatonin under normal and stressful conditions in different plants (Sharma

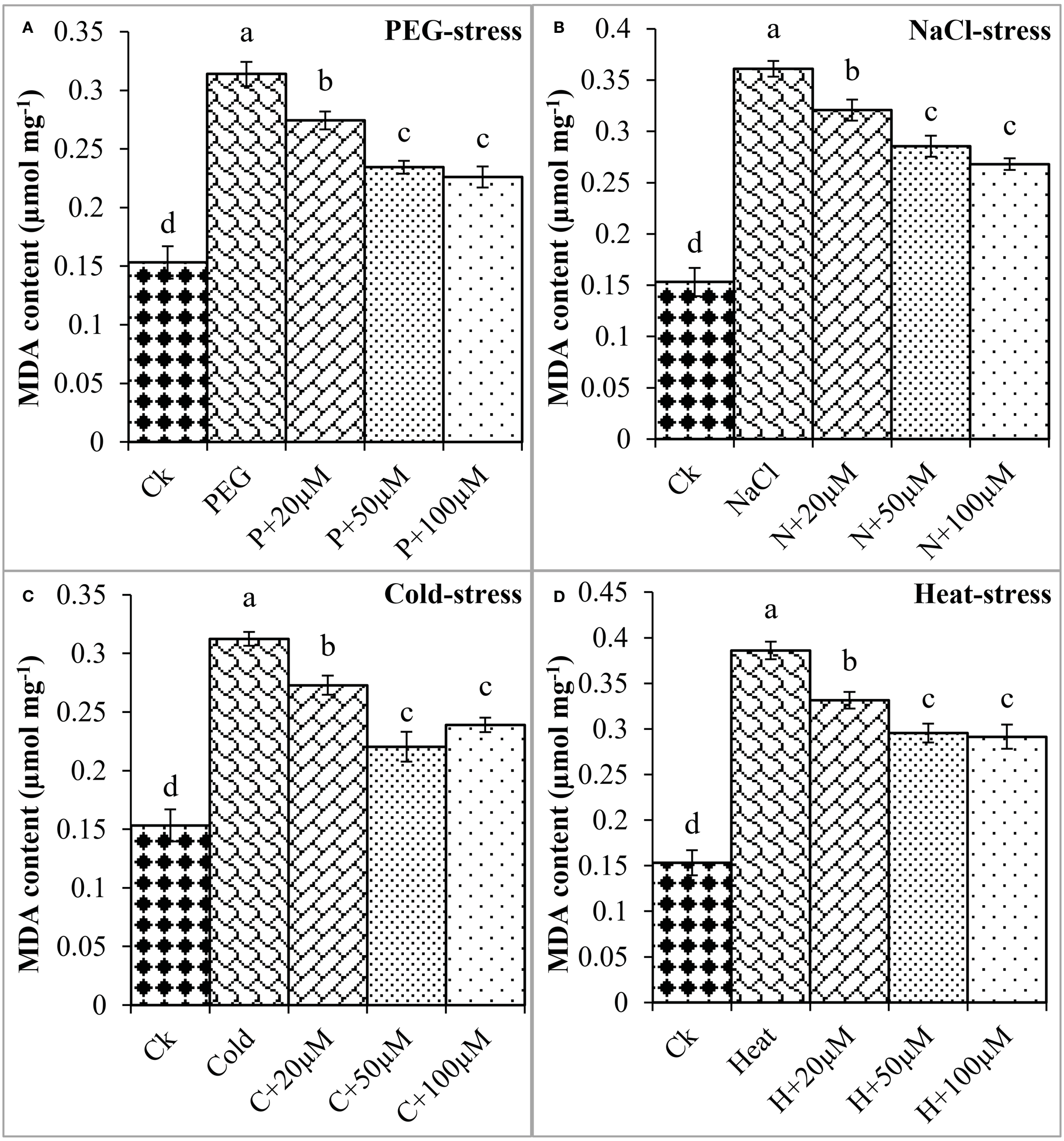


FIGURE 4
Effect of different concentrations of melatonin on the activity of MDA content under PEG **(A)**, NaCl **(B)**, Cold **(C)**, and Heat **(D)**. Values are the means ± SD (n=3). Different letters on the bars show a statistical significance level at $p<0.05$. Here, µM is indicating (µmole L^{-1}).

and Zheng, 2019). Our study depicted that lower concentrations of melatonin remarkably improved the germination potential (GP), germination rate (GR), radical length (RL), fresh weight (FW), and dry weight (DW) however; higher concentrations did not show remarkable improvements under abiotic stress conditions. Previous studies revealed that melatonin at 100 µM improved the germination indices of cotton (*Gossypium hirsutum* L.) and wheat (*Triticum aestivum* L.) under PEG and heat stress (Bai et al., 2020; Iqbal et al., 2021), 20 µM under salt stress (Chen et al., 2020), and 5-20 µM under cold stress in *Stevia rebaudiana* (Simlat et al., 2018). It is suggested that melatonin crosstalk with ABA and GA, helps in breaking seed dormancy, facilitates more water uptake, and activates secondary messengers to enhance GP, GR, and GI, and the successful establishment of seedlings under abiotic stresses (Zhang et al., 2014a; Xiao et al., 2019). Furthermore, the higher concentrations of melatonin are not beneficial at all because sometimes it becomes the cause of growth inhibition that depends upon the type of species and duration of melatonin treatment (Cao et al., 2019). Thus, the above presented literature is in line with the findings of the present study.

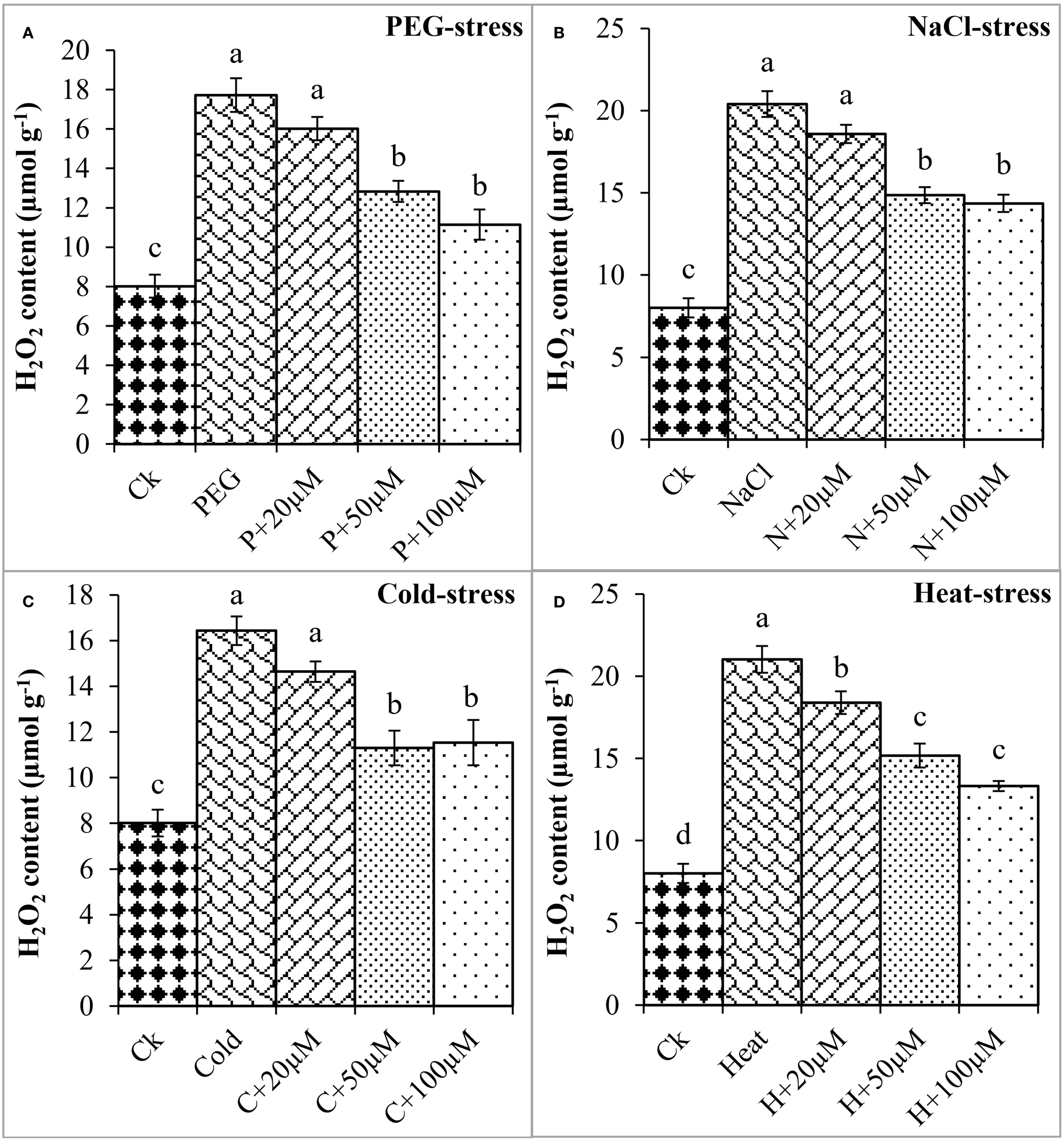


FIGURE 5
Effect of different concentrations of melatonin on the activity of H_2O_2 under PEG **(A)**, NaCl **(B)**, Cold **(C)**, and Heat **(D)**. Values are the means ± SD (n=3). Different letters on the bars show a statistical significance level at *$p<0.05$*. Here, µM is indicating (µmole L^{-1}).

Generally, PEG, NaCl, cold, and heat lead to the generation of excessive production of ROS, and MDA content as lipid peroxidation, and increase the electrolyte leakage which results in the decrease of germination and early growth of seedlings (Chen et al., 2020; Awan et al., 2021). However, melatonin has been reported to counteract H_2O_2 and reduce MDA content and EL in response to abiotic stresses (Wang et al., 2018; Seleiman et al., 2021). For example, melatonin inhibited the excessive production of ROS and MDA, and deceased the EL that cause oxidative damage in tomato (*Solanum lycopersicum* L.) and maize (*Zea mays* L.) under chilling stress (Liu et al., 2015; Cao et al., 2019), in tomato (*Solanum lycopersicum* L.) under heat stress (Jahan et al., 2019), in cotton (*Gossypium hirsutum* L.) under drought and salt stresses (Bai et al., 2020; Chen et al., 2020). It has been reported that H_2O_2 is associated with embryo elongation during seed germination and melatonin effectively and positively regulates its content directly or indirectly inside the cells (Zhang et al., 2014b; Bai et al., 2020). Basically, at the beginning of seed germination, a series of physiological processes take place including absorption of water, activation of various enzymes and hormones, membrane repair activities, and

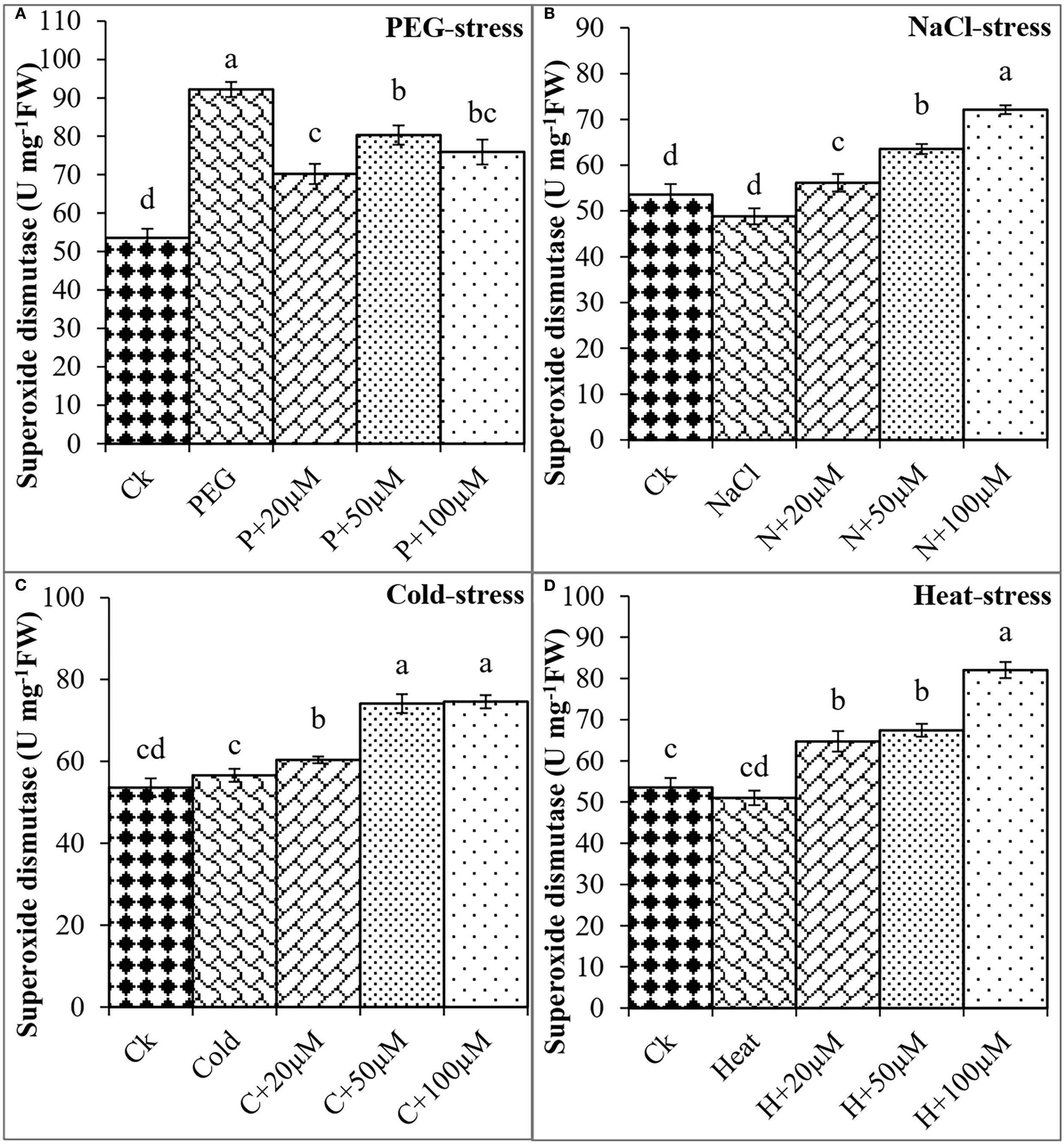


FIGURE 6
Effect of different concentrations of melatonin on the activity of superoxide dismutase under PEG **(A)**, NaCl **(B)**, Cold **(C)**, and Heat **(D)**. Values are the means ± SD (n=3). Different letters on the bars show a statistical significance level at *$p<0.05$*. Here, μM is indicating (μmole L^{-1}).

degradation of storage substances inside the seeds (Hegarty, 1978; Ma et al., 2017). So, abiotic stresses lead to inhibition or slow down these mechanisms and cause oxidative damage whereas melatonin positively regulates internal homeostasis by reducing secondary stresses caused by drought, salt, chilling and heat (Bai et al., 2020; Cao et al., 2022). Similarly, our results showed that all four stresses dramatically increased the H_2O_2, MDA, and EL whereas; melatonin at 50 μmol L^{-1} and 100 μmol L^{-1} considerably decreased the H_2O_2, MDA content, and EL under the drought, salt, cold, and heat stresses in soybean which are consistent with previous findings as in tomato (*Solanum lycopersicum* L.), wheat (*Triticum aestivum* L.), chickpea (*Cicer arietinum* L.), pepper (*Capsicum annuum* L.), rice (*Oryza sativa* L.), and cotton (*Gossypium hirsutum* L.) (Cao et al., 2019; Jahan et al., 2019; Bai et al., 2020; Buttar et al., 2020; Korkmaz et al., 2021; Raza et al., 2022) Based on these observations, an optimal level of melatonin could effectively reduce the overproduction of ROS, MDA, and decrease EL in terms of oxidative damage and provide protection by activating defensive system; however, this phenomenon might be dose-, time duration-, stage-, and species-dependent (Raza et al., 2022).

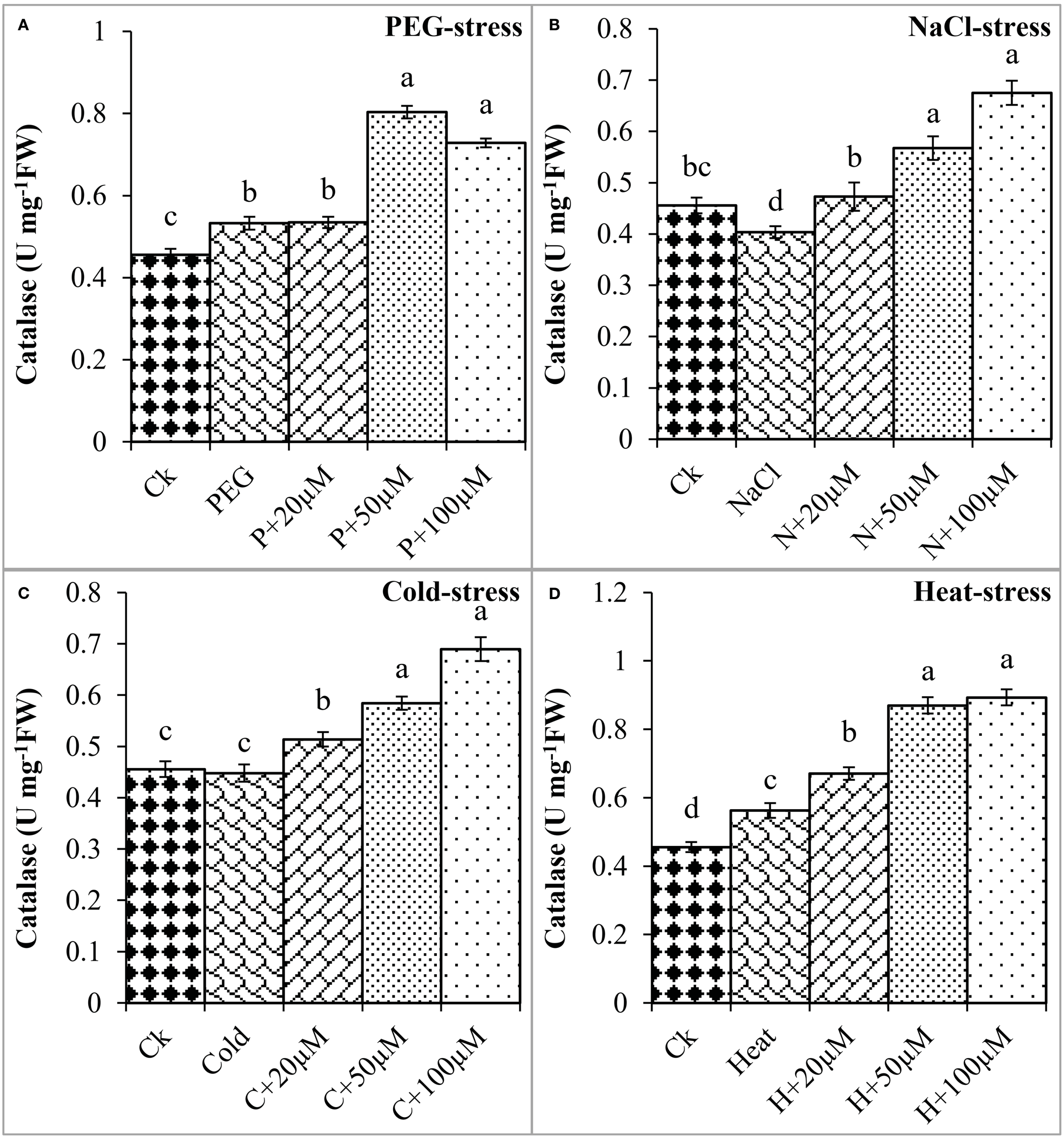


FIGURE 7
Effect of different concentrations of melatonin on the activity of Catalase (CAT) under PEG **(A)**, NaCl **(B)**, Cold **(C)**, and Heat **(D)**. Values are the means ± SD (n=3). Different letters on the bars show a statistical significance level at *p<0.05*. Here, μM is indicating (μmole L^{-1}).

The accumulation of protective substances/osmoprotectants plays a crucial role in maintaining internal stability and protecting cells from abiotic stress damage (Bai et al., 2020). Melatonin has been reported to increase the accumulation of proline in response to drought (Zhang et al., 2020), salt (Chen et al., 2020), cold (Cao et al., 2019), and heat stress (Imran et al., 2021), and enhanced the germination and early seedlings growth (Bai et al., 2020). The present study showed that abiotic stresses accelerated the level of proline whereas; the application of melatonin progressively increased the level of proline during seed germination under these stresses. These results suggested that melatonin can positively regulate the osmotic substances inside the cells and enhance the drought, salt, cold, and heat stress tolerance. Pre-treatment of melatonin at lower concentrations of 1-100 μM increased proline in cotton under drought (Bai et al., 2020), in tomato under salt (Siddiqui et al., 2019), in maize under chilling (Cao et al., 2019), and in brassica (*Brassica napus* L.) under Se-stress, and in strawberry (*Fragaria* × *ananassa*) under heat stress (Ulhassan et al., 2019). Mainly, proline is involved in ROS detoxification, protecting membrane integrity, cell organelles, and

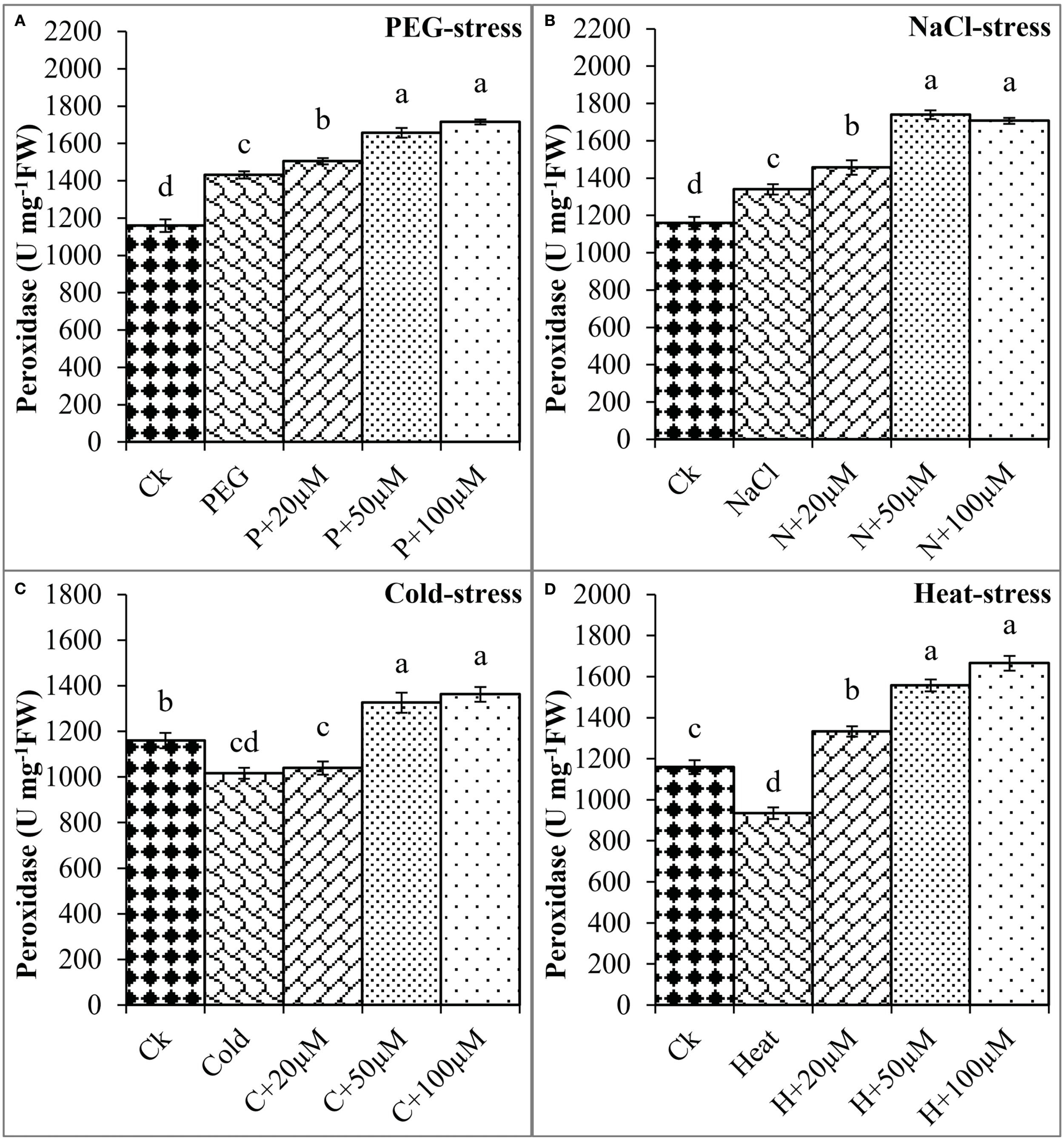


FIGURE 8
Effect of different concentrations of melatonin on the activity of Peroxidase (POD) under PEG **(A)**, NaCl **(B)**, Cold **(C)**, and Heat **(D)**. Values are the means ± SD (n=3). Different letters on the bars show a statistical significance level at *$p<0.05$*. Here, μM is indicating (μmole L^{-1}).

osmotic adjustments (Hosseinifard et al., 2022). Thus, accumulation of proline *via* pre-treatment of melatonin could increase resistance against abiotic stresses.

Furthermore, plants possess a complex defensive mechanism to inhibit the excessive production of ROS and reduce oxidative damage (Ahammed et al., 2020; Bai et al., 2020; Khan et al., 2023). This defensive system is composed of different antioxidant enzymes including SOD, CAT, POD, and APX that perform a variety of defensive functions (Khan et al., 2020). SOD is considered the first line of defense that can convert O^{2-} to H_2O_2, which is further converted to water and oxygen through CAT (Li et al., 2017; Rizwan et al., 2019). The SOD and POD are important antioxidants that can readily eliminate ROS from cells and protect cell membrane stability from lipid peroxidation (Xiao et al., 2019). Pre-treatment of melatonin has been reported to activate the defense system by increasing the activities of antioxidant enzymes that scavenge the radicals and minimize the oxidative damage in response to stressful conditions (Zhang et al., 2014a; Sharif et al., 2018; Bai et al., 2020; Mushtaq et al., 2022). In the present study, drought and heat stress decreased the activities of antioxidant

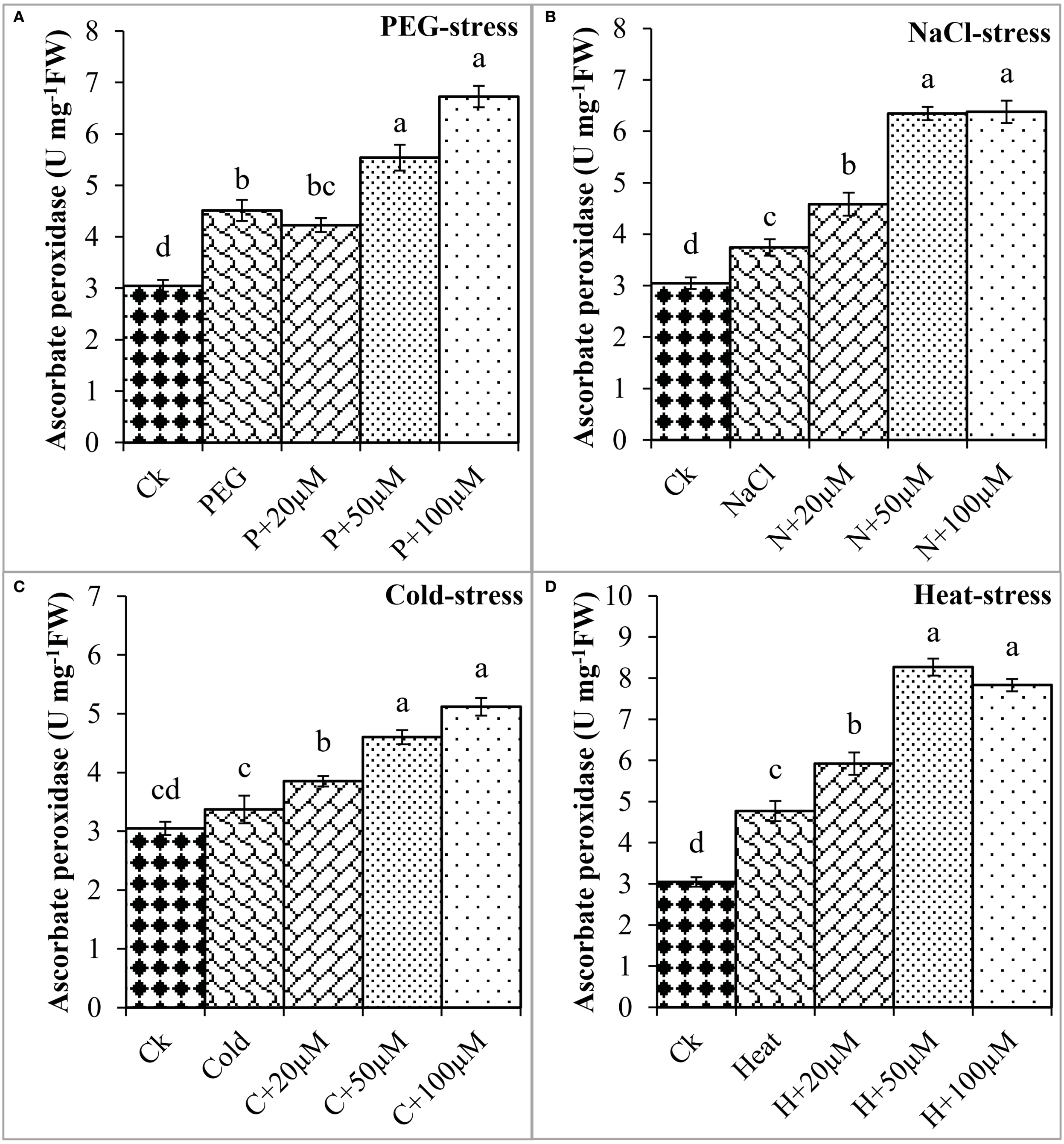


FIGURE 9
Effect of different concentrations of melatonin on the activity of Ascorbate peroxidase (APX) under PEG **(A)**, NaCl **(B)**, Cold **(C)**, and Heat **(D)**. Values are the means ± SD (n=3). Different letters on the bars show a statistical significance level at *p<0.05*. Here, μM is indicating (μmole L^{-1}).

enzymes which might be due to overproduction of ROS and strong oxidative damage as found in previous studies (Zeng et al., 2022). Moreover, pre-treatment of melatonin progressively enhanced the activities of antioxidant enzymes and maximum enhancement was observed at MT2 and MT3 in response to all stresses. These findings suggest that melatonin increases the tolerance of soybean seeds by reducing oxidative damage under drought, salt, cold, and heat stresses. Our findings are consistent with previous studies that reported the pre-treatment of melatonin at 1-100 μM improved the activities of SOD, CAT, POD, and APX in cotton under drought and salt stress (Bai et al., 2020; Chen et al., 2020), alfalfa (*Medicao sativa* L.), barley (*Hordeum Vulage* L.*)*, and corn under cold stress (Li et al., 2016; Cao et al., 2019; Irshad et al., 2021), wheat and strawberry under heat stress (Buttar et al., 2020; Manafi et al., 2022). Pieces of evidence revealed that melatonin can enter the seeds through the seed coat as a result of seed priming and regulate the various physiological mechanisms inside the seed including, activation of internal hormonal signaling, stimulation of secondary metabolites, and regulate the sugar metabolism that is responsible for early germination under normal and stressful

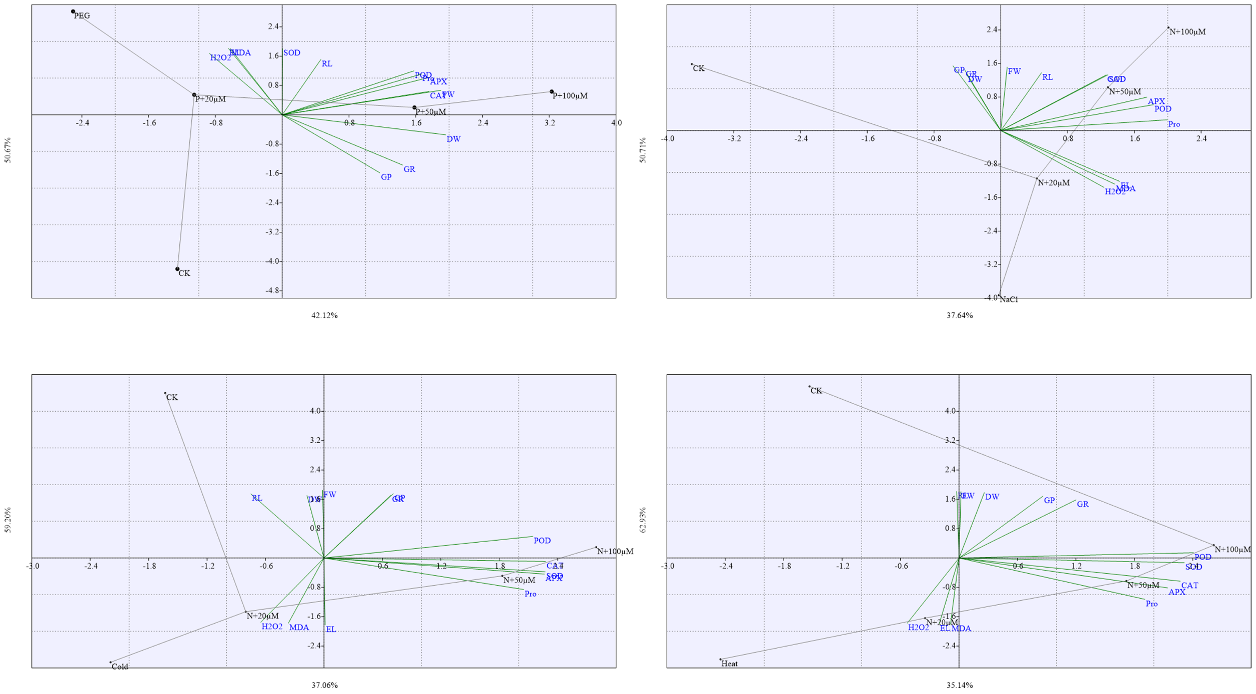


FIGURE 10
The Loading plots of principal component analysis (PCA) of different measured parameters of soybean seed germination at different concentrations of melatonin under drought **(A)**, salt **(B)**, cold **(C)**, and heat **(D)** stress. The abbreviations used in the plots are as follows; germination potential (GP), germination rate (GR), fresh weight (FW), dresh weight (DW), proline (Pro), electrolyte leakage (EL), malondialdehyde (MDA), hydrogen peroxide (H_2O_2) superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and ascorbate peroxidase (APX). Here, µM is indicating (µmole L^{-1}).

condition (Bai et al., 2020; Zhang et al., 2020). Furthermore, melatonin has been reported to positively regulate the internal ROS level of seed which is crucial to stimulate the breakage of seed dormancy and resulting in seed germination under stressful conditions (Arnao and Hernández-Ruiz, 2014; Bai et al., 2020). Based on the previous literature and findings of the present study, it is suggested that seed pre-treatment with melatonin can efficiently promote the seed germination of soybean and can potentially alleviate the deleterious effects of abiotic stresses including drought, salt, cold, and heat. Lower concentrations of melatonin at MT1, MT2, and MT3 perform significant functions in improving the germination, proline accumulation, and activities of antioxidant enzymes, and reducing the oxidative damage in terms of ROS, MDA, and EL as compared to higher concentrations under stressful condition. Similarly, present study depicted that seed priming with melatonin reduced oxidative stress in terms of H_2O_2, MDA, and EL, and enhanced the activities of SOD, CAT, POD, and APX under different abiotic stresses which promoted the early germination and improved the germination responses in soybean. In addition, the effects of melatonin on seed germination, early seedlings growth, and other physiological attributes are dependent on the type of abiotic stress, species, type of plants, growth stage, and duration of stresses (Raza et al., 2022). It can be concluded that melatonin shows variable positive effects under different types of stresses. Thus, further molecular and transcriptome studies would be required to explore the melatonin-mediated metabolic pathways and important genes that are responsible to enhance soybean tolerance against abiotic stresses during germination.

Conclusion

Abiotic stresses including drought, salt, cold, and heat lead to an increase in the oxidative damage by increasing the H_2O_2, MDA, and EL which results in membrane damage and other secondary stresses in soybean seeds during germination. Pre-treatment of soybean seeds with melatonin differentially regulated and positively improved the germination indices and other physiological processes. Pre-treatment of melatonin at 20 µmol L^{-1}, 50 µmol L^{-1}, and 100 µmol L^{-1} was found significant in improving germination potential, germination rate, radical length, fresh weight, and dry weight of soybean seed germination however, remarkable alleviation of abiotic stresses was noticed at 50 µmol L^{-1} and 100 µmol L^{-1}. Both these levels significantly increased the activities of antioxidant enzymes such as SOD, CAT, POD, and APX, osmoprotectant (proline), and decreased oxidative damage under abiotic stresses in soybean seed germination. The present study suggests that these specific concentrations of melatonin (50 µmol L^{-1} and 100 µmol L^{-1}) can positively enhance seed germination and improve tolerance against the given level of drought, salt, cold, and heat stresses. Furthermore, molecular bases with detailed regulatory networks of physiological and biochemical mechanisms in the seeds of different crop plants are needed to be further explored before the field implication. Besides, this study provides the valuable bases for the protective and successful germination of soybean with improved tolerance against abiotic stresses however; future studies are required to deeply understand the melatonin-mediated stress tolerance mechanisms in different crops during germination.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Author contributions

FY supervised the project. SA and IK performed the experiment and analyzed the data. SA and IK participated in the writing of manuscript. JG, XT, QW, and FY contributed in revising and editing the manuscript. All authors contributed to the article and approved the submitted version.

Funding

We acknowledge the National Natural Science Foundation of China (32071963); National Key Research and Development Program of China (2022YFD2300902); and Program on Industrial Technology System of National Soybean (CARS-04-PS19) on providing funding for the current study.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

Ahammed, G. J., Wang, Y., Mao, Q., Wu, M., Yan, Y., Ren, J., et al. (2020). Dopamine alleviates bisphenol a-induced phytotoxicity by enhancing antioxidant and detoxification potential in cucumber. *Environ. pollut.* 259, 113957. doi: 10.1016/j.envpol.2020.113957

Arnao, M. B., and Hernández-Ruiz, J. (2014). Melatonin: Plant growth regulator and/or biostimulator during stress? *Trends Plant Sci.* 19, 789–797. doi: 10.1016/j.tplants.2014.07.006

Awan, S. A., Khan, I., Rizwan, M., Zhang, X., Brestic, M., Khan, A., et al. (2021). Exogenous abscisic acid and jasmonic acid restrain polyethylene glycol-induced drought by improving the growth and antioxidative enzyme activities in pearl millet. *Physiologia Plantarum* 172, 809–819. doi: 10.1111/ppl.13247

Awan, S. A., Khan, I., Tariq, R., Rizwan, M., Wang, X., Zhang, X., et al. (2022). Genome-wide expression and physiological profiling of pearl millet genotype reveal the biological pathways and various gene clusters underlying salt resistance. *Front. Plant Sci.* 13, 849618. doi: 10.3389/fpls.2022.849618

Bai, Y., Xiao, S., Zhang, Z., Zhang, Y., Sun, H., Zhang, K., et al. (2020). Melatonin improves the germination rate of cotton seeds under drought stress by opening pores in the seed coat. *PeerJ* 8, e9450. doi: 10.7717/peerj.9450

Bates, L. S., Waldren, R. P., and Teare, I. (1973). Rapid determination of free proline for water-stress studies. *Plant Soil* 39, 205–207. doi: 10.1007/BF00018060

Buttar, Z. A., Wu, S. N., Arnao, M. B., Wang, C., Ullah, I., and Wang, C. (2020). Melatonin suppressed the heat stress-induced damage in wheat seedlings by modulating the antioxidant machinery. *Plants* 9, 809. doi: 10.3390/plants9070809

Cao, Q., Li, G., Cui, Z., Yang, F., Jiang, X., Diallo, L., et al. (2019). Seed priming with melatonin improves the seed germination of waxy maize under chilling stress *via* promoting the antioxidant system and starch metabolism. *Sci. Rep.* 9, 1–12. doi: 10.1038/s41598-019-51122-y

Cao, J., Xie, C., and Hou, Z. (2022). Ecological evaluation of heavy metal pollution in the soil of Pb-zn mines. *Ecotoxicology* 31, 259–270. doi: 10.1007/s10646-021-02505-3

Chaudhry, S., and Sidhu, G. P. S. (2022). Climate change regulated abiotic stress mechanisms in plants: A comprehensive review. *Plant Cell Rep.* 41, 1–31. doi: 10.1007/s00299-021-02759-5

Chen, L., Liu, L., Lu, B., Ma, T., Jiang, D., Li, J., et al. (2020). Exogenous melatonin promotes seed germination and osmotic regulation under salt stress in cotton (Gossypium hirsutum l.). *PLos One* 15, e0228241. doi: 10.1371/journal.pone.0228241

Debeaujon, I., Léon-Kloosterziel, K. M., and Koornneef, M. (2000). Influence of the testa on seed dormancy, germination, and longevity in arabidopsis. *Plant Physiol.* 122, 403–414. doi: 10.1104/pp.122.2.403

Fu, J., Wu, Y., Miao, Y., Xu, Y., Zhao, E., Wang, J.-, et al. (2017). Improved cold tolerance in elymus nutans by exogenous application of melatonin may involve ABA-dependent and ABA-independent pathways. *Sci. Rep.* 7 (1), 39865. doi: 10.3906/tar-0407-2

Gu, L., Wang, H., Wei, H., Sun, H., Li, L., Chen, P., et al. (2018). Identification, expression, and functional analysis of the group IId WRKY subfamily in upland cotton (Gossypium hirsutum l.). *Front. Plant Sci.* 9, 1684. doi: 10.3389/fpls.2018.01684

Guo, X., Shao, X., Trishna, S. M., Marinova, D., and Hossain, A. (2021). "Soybeans consumption and production in China: Sustainability perspective," in *Research anthology on food waste reduction and alternative diets for food and nutrition security* (Hershey, PA: IGI Global), 1256–1275.

Hegarty, T. (1978). The physiology of seed hydration and dehydration, and the relation between water stress and the control of germination: A review. *Plant Cell Environ.* 1, 101–119. doi: 10.1111/j.1365-3040.1978.tb00752.x

Hosseinifard, M., Stefaniak, S., Ghorbani Javid, M., Soltani, E., Wojtyla, Ł., and Garnczarska, M. (2022). Contribution of exogenous proline to abiotic stresses tolerance in plants: A review. *Int. J. Mol. Sci.* 23, 5186. doi: 10.3390/ijms23095186

Imran, M., Aaqil Khan, M., Shahzad, R., Bilal, S., Khan, M., Yun, B.-W., et al. (2021). Melatonin ameliorates thermotolerance in soybean seedling through balancing redox homeostasis and modulating antioxidant defense, phytohormones and polyamines biosynthesis. *Molecules* 26, 5116. doi: 10.3390/molecules26175116

Iqbal, N., Fatma, M., Gautam, H., Umar, S., Sofo, A., and D'ippolito, I. (2021). The crosstalk of melatonin and hydrogen sulfide determines photosynthetic performance by regulation of carbohydrate metabolism in wheat under heat stress. *Plants* 10, 1778. doi: 10.3390/plants10091778

Irshad, A., Rehman, R. N. U., Kareem, H. A., Yang, P., and Hu, T. (2021). Addressing the challenge of cold stress resilience with the synergistic effect of rhizobium inoculation and exogenous melatonin application in medicago truncatula. *Ecotoxicology Environ. Saf.* 226, 112816. doi: 10.1016/j.ecoenv.2021.112816

Jahan, M. S., Shu, S., Wang, Y., Chen, Z., He, M., Tao, M., et al. (2019). Melatonin alleviates heat-induced damage of tomato seedlings by balancing redox homeostasis and modulating polyamine and nitric oxide biosynthesis. *BMC Plant Biol.* 19, 1–16. doi: 10.1186/s12870-019-1992-7

Khan, M., Ali, S., Al Azzawi, T. N. I., Saqib, S., Ullah, F., Ayaz, A., et al. (2023). The key roles of ROS and RNS as a signaling molecule in plant–microbe interactions. *Antioxidants* 12, 268. doi: 10.3390/antiox12020268

Khan, I., Raza, M. A., Awan, S. A., Shah, G. A., Rizwan, M., Ali, B., et al. (2020). Amelioration of salt induced toxicity in pearl millet by seed priming with silver nanoparticles (AgNPs): The oxidative damage, antioxidant enzymes and ions uptake are major determinants of salt tolerant capacity. *Plant Physiol. Biocheistry* 156, 221–232. doi: 10.1016/j.plaphy.2020.09.018

Korkmaz, A., Değer, Ö., Szafrańska, K., Köklü, Ş., Karaca, A., Yakupoğlu, G., et al. (2021). Melatonin effects in enhancing chilling stress tolerance of pepper. *Scientia Hortic.* 289, 110434. doi: 10.1016/j.scienta.2021.110434

Li, H., Chang, J., Chen, H., Wang, Z., Gu, X., Wei, C., et al. (2017). Exogenous melatonin confers salt stress tolerance to watermelon by improving photosynthesis and redox homeostasis. *Front. Plant Sci.* 8, 295. doi: 10.3389/fpls.2017.00295

Li, X., Tan, D. X., Jiang, D., and Liu, F. (2016). Melatonin enhances cold tolerance in drought-primed wild-type and abscisic acid-deficient mutant barley. *J. Pineal Res.* 61, 328–339. doi: 10.1111/jpi.12350

Liang, B., Gao, T., Zhao, Q., Ma, C., Chen, Q., Wei, Z., et al. (2018). Effects of exogenous dopamine on the uptake, transport, and resorption of apple ionome under moderate drought. *Front. Plant Sci.* 9, 755. doi: 10.3389/fpls.2018.00755

Liu, J., Wang, W., Wang, L., and Sun, Y. (2015). Exogenous melatonin improves seedling health index and drought tolerance in tomato. *Plant Growth Regul.* 77, 317–326. doi: 10.1007/s10725-015-0066-6

Ma, Z., Bykova, N. V., and Igamberdiev, A. U. (2017). Cell signaling mechanisms and metabolic regulation of germination and dormancy in barley seeds. *Crop J.* 5, 459–477. doi: 10.1016/j.cj.2017.08.007

Manafi, H., Baninasab, B., Gholami, M., Talebi, M., and Khanizadeh, S. (2022). Exogenous melatonin alleviates heat-induced oxidative damage in strawberry (Fragaria× ananassa duch. cv. ventana) plant. *J. Plant Growth Regul.* 41, 52–64. doi: 10.1007/s00344-020-10279-x

Meena, R. S., Vijayakumar, V., Yadav, G. S., and Mitran, T. (2018). Response and interaction of bradyrhizobium japonicum and arbuscular mycorrhizal fungi in the soybean rhizosphere. *Plant Growth Regul.* 84, 207–223. doi: 10.1007/s10725-017-0334-8

Mushtaq, N., Iqbal, S., Hayat, F., Raziq, A., Ayaz, A., and Zaman, W. (2022). Melatonin in micro-tom tomato: Improved drought tolerance *via* the regulation of the photosynthetic apparatus, membrane stability, osmoprotectants, and root system. *Life* 12, 1922. doi: 10.3390/life12111922

Okçu, G., Kaya, M. D., and Atak, M. (2005). Effects of salt and drought stresses on germination and seedling growth of pea (Pisum sativum l.). *Turkish J. Agric. Forestry* 29, 237–242.

Papadaki, A., Kopsahelis, N., Mallouchos, A., and Mandala, I. (2019). Bioprocess development for the production of novel oleogels from soybean and microbial oils. *Food Res. Int.* 126, 108684. doi: 10.1016/j.foodres.2019.108684

Patanè, C., Saita, A., Tubeileh, A., Cosentino, S. L., and Cavallaro, V. (2016). Modeling seed germination of unprimed and primed seeds of sweet sorghum under PEG-induced water stress through the hydrotime analysis. *Acta Physiologia Plantarum* 38, 1–12. doi: 10.1007/s11738-016-2135-5

Raza, A., Charagh, S., García-Caparrós, P., Rahman, M. A., Ogwugwa, V. H., Saeed, F., et al. (2022). Melatonin-mediated temperature stress tolerance in plants. *GM Crops Food* 13, 196–217. doi: 10.1080/21645698.2022.2106111

Rizwan, M., Ali, S., Ali, B., Adrees, M., Arshad, M., Hussain, A., et al. (2019). Zinc and iron oxide nanoparticles improved the plant growth and reduced the oxidative stress and cadmium concentration in wheat. *Chemosphere* 214, 269–277. doi: 10.1016/j.chemosphere.2018.09.120

Roessali, W., Ekowati, T., and Prasetyo, E. (2019). "Economic aspects of soybean farming sustainability in central Java, Indonesia," in *IOP Conference Series: Earth and Environmental Science*, Vol. 250, No. 1. 012022 (IOP Publishing).

Seleiman, M. F., Al-Suhaibani, N., Ali, N., Akmal, M., Alotaibi, M., Refay, Y., et al. (2021). Drought stress impacts on plants and different approaches to alleviate its adverse effects. *Plants* 10, 259. doi: 10.3390/plants10020259

Sethy, S. K., and Ghosh, S. (2013). Effect of heavy metals on germination of seeds. *J. Natural Science Biology Med.* 4, 272. doi: 10.4103/0976-9668.116964

Sharif, R., Xie, C., Zhang, H., Arnao, M. B., Ali, M., Ali, Q., et al. (2018). Melatonin and its effects on plant systems. *Molecules* 23, 2352. doi: 10.3390/molecules23092352

Sharma, A., and Zheng, B. (2019). Melatonin mediated regulation of drought stress: Physiological and molecular aspects. *Plants* 8, 190. doi: 10.3390/plants8070190

Shu, K., Zhou, W., Chen, F., Luo, X., and Yang, W. (2018). Abscisic acid and gibberellins antagonistically mediate plant development and abiotic stress responses. *Front. Plant Sci.* 9, 416. doi: 10.3389/fpls.2018.00416

Siamabele, B. (2021). The significance of soybean production in the face of changing climates in Africa. *Cogent Food Agric.* 7 (1), 1933745. doi: 10.1080/23311932.2021.1933745

Siddiqui, M. H., Alamri, S., Al-Khaishany, M. Y., Khan, M. N., Al-Amri, A., Ali, H. M., et al. (2019). Exogenous melatonin counteracts NaCl-induced damage by regulating the antioxidant system, proline and carbohydrates metabolism in tomato seedlings. *Int. J. Mol. Sci.* 20, 353. doi: 10.3390/ijms20020353

Simlat, M., Ptak, A., Skrzypek, E., Warchoł, M., Morańska, E., and Piórkowska, E. (2018). Melatonin significantly influences seed germination and seedling growth of stevia rebaudiana bertoni. *PeerJ* 6, e5009. doi: 10.7717/peerj.5009

Ulhassan, Z., Huang, Q., Gill, R. A., Ali, S., Mwamba, T. M., Ali, B., et al. (2019). Protective mechanisms of melatonin against selenium toxicity in brassica napus: Insights into physiological traits, thiol biosynthesis and antioxidant machinery. *BMC Plant Biol.* 19, 1–16. doi: 10.1186/s12870-019-2110-6

Wang, Y., Reiter, R. J., and Chan, Z. (2018). Phytomelatonin: A universal abiotic stress regulator. *J. Exp. Bot.* 69, 963–974. doi: 10.1093/jxb/erx473

Wei, J., Liang, J., Liu, D., Liu, Y., Liu, G., and Wei, S. (2022). Melatonin-induced physiology and transcriptome changes in banana seedlings under salt stress conditions. *Front. Plant Sci.* 13. doi: 10.3389/fpls.2022.938262

Weitbrecht, K., Müller, K., and Leubner-Metzger, G. (2011). First off the mark: Early seed germination. *J. Exp. Bot.* 62, 3289–3309. doi: 10.1093/jxb/err030

Xiao, S., Liu, L., Wang, H., Li, D., Bai, Z., Zhang, Y., et al. (2019). Exogenous melatonin accelerates seed germination in cotton (Gossypium hirsutum l.). *PLose One* 14, e0216575. doi: 10.1371/journal.pone.0216575

Yang, F., Fan, Y., Wu, X., Cheng, Y., Liu, Q., Feng, L., et al. (2018a). Auxin-to-gibberellin ratio as a signal for light intensity and quality in regulating soybean growth and matter partitioning. *Front. Plant Sci.* 9, 56. doi: 10.3389/fpls.2018.00056

Yang, F., Feng, L., Liu, Q., Wu, X., Fan, Y., Raza, M. A., et al. (2018b). Effect of interactions between light intensity and red-to-far-red ratio on the photosynthesis of soybean leaves under shade condition. *Environ. Exp. Bot.* 150, 79–87. doi: 10.1016/j.envexpbot.2018.03.008

Yang, F., Huang, S., Gao, R., Liu, W., Yong, T., Wang, X., et al. (2014). Growth of soybean seedlings in relay strip intercropping systems in relation to light quantity and red: far-red ratio. *Field Crops Res.* 155, 245–253. doi: 10.1016/j.fcr.2013.08.011

Yang, F., Liao, D., Wu, X., Gao, R., Fan, Y., Raza, M. A., et al. (2017). Effect of aboveground and belowground interactions on the intercrop yields in maize-soybean relay intercropping systems. *Field Crops Res.* 203, 16–23. doi: 10.1016/j.fcr.2016.12.007

Yang, F., Liu, Q., Cheng, Y., Feng, L., Wu, X., Fan, Y., et al. (2020). Low red/far-red ratio as a signal promotes carbon assimilation of soybean seedlings by increasing the photosynthetic capacity. *BMC Plant Biol.* 20, 1–12. doi: 10.1186/s12870-020-02352-0

Zeng, H., Liu, M., Wang, X., Liu, L., Wu, H., Chen, X., et al. (2022). Seed-soaking with melatonin for the improvement of seed germination, seedling growth, and the antioxidant defense system under flooding stress. *Agronomy* 12, 1918. doi: 10.3390/agronomy12081918

Zhang, Y., Chen, B., Xu, Z., Shi, Z., Chen, S., Huang, X., et al. (2014b). Involvement of reactive oxygen species in endosperm cap weakening and embryo elongation growth during lettuce seed germination. *J. Exp. Bot.* 65, 3189–3200. doi: 10.1093/jxb/eru167

Zhang, M., He, S., Qin, B., Jin, X., Wang, M., Ren, C., et al. (2020). Exogenous melatonin reduces the inhibitory effect of osmotic stress on antioxidant properties and cell ultrastructure at germination stage of soybean. *PLose One* 15, e0243537. doi: 10.1371/journal.pone.0243537

Zhang, H. J., Zhang, N., Yang, R. C., Wang, L., Sun, Q. Q., Li, D. B., et al. (2014a). Melatonin promotes seed germination under high salinity by regulating antioxidant systems, ABA and GA 4 interaction in cucumber (C ucumis sativus l.). *J. Pineal Res.* 57, 269–279. doi: 10.1111/jpi.12167

Zhao, J., Wang, C., Shi, X., Bo, X., Li, S., Shang, M., et al. (2021). Modeling climatically suitable areas for soybean and their shifts across China. *Agric. Syst.* 192, 103205. doi: 10.1016/j.agsy.2021.103205

Zhao, Y., Yu, W., Hu, X., Shi, Y., Liu, Y., Zhong, Y., et al. (2018). Physiological and transcriptomic analysis revealed the involvement of crucial factors in heat stress response of rhododendron hainanense. *Gene* 660, 109–119. doi: 10.1016/j.gene.2018.03.082

Frontiers in Plant Science

TYPE Original Research
PUBLISHED 13 March 2023
DOI 10.3389/fpls.2023.1135943

OPEN ACCESS

EDITED BY
Muhammad Ahsan Altaf,
Hainan University, China

REVIEWED BY
Abolghassem Emamverdian,
Nanjing Forestry University, China
Hamza Sohail,
Huazhong Agricultural University, China
Rana Roy,
Sylhet Agricultural University, Bangladesh

*CORRESPONDENCE
Leila Pishkar
Pishkar@iiau.ac.ir
Moxian Chen
cmx2009920734@gmail.com

SPECIALTY SECTION
This article was submitted to
Plant Abiotic Stress,
a section of the journal
Frontiers in Plant Science

RECEIVED 02 January 2023
ACCEPTED 17 February 2023
PUBLISHED 13 March 2023

CITATION
Ghorbani A, Pishkar L, Saravi KV and Chen M (2023) Melatonin-mediated endogenous nitric oxide coordinately boosts stability through proline and nitrogen metabolism, antioxidant capacity, and Na^+/K^+ transporters in tomato under NaCl stress.
Front. Plant Sci. 14:1135943.
doi: 10.3389/fpls.2023.1135943

Melatonin-mediated endogenous nitric oxide coordinately boosts stability through proline and nitrogen metabolism, antioxidant capacity, and Na^+/K^+ transporters in tomato under NaCl stress

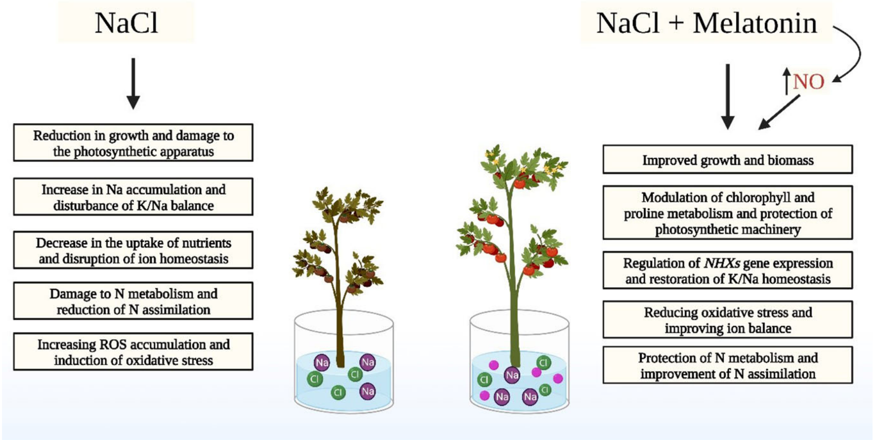
Abazar Ghorbani[1], Leila Pishkar[2]*, Kobra Valed Saravi[3] and Moxian Chen[1]*

[1]National Key Laboratory of Green Pesticide, Key Laboratory of Green Pesticide and Agricultural Bioengineering, Ministry of Education, Center for Research and Development of Fine Chemicals, Guizhou University, Guiyang, China, [2]Department of Biology, Islamshahr Branch, Islamic Azad University, Islamshahr, Iran, [3]Department of Biology, Damghan branch, Islamic Azad University, Damghan, Iran

The interactions between nitric oxide (NO) and melatonin in alleviating sodium chloride (NaCl) toxicity in plants are poorly comprehended. Here, the associations between the exogenous application of melatonin and endogenous NO levels in inducing tomato seedlings' defense response during NaCl toxicity were investigated. The results indicated that the application of melatonin (150 μM) increased height (23.7%) and biomass (32.2%), improved chlorophyll (a (137%) and b (92.8%)), and proline metabolisms, and reduced the contents of superoxide anion radicals (49.6%), hydrogen peroxide (31.4%), malondialdehyde (38%), and electrolyte leakage (32.6%) in 40-day-old tomato seedlings grown under NaCl (150 mM) treatment. Melatonin increased the antioxidant defense system in NaCl-stressed seedlings by increasing the activity of the antioxidant enzymes. Melatonin also improved N metabolism and endogenous NO content in NaCl-stressed seedlings by upregulating the activity of enzymes implicated in N assimilation. Furthermore, melatonin improved ionic balance and reduced Na content in NaCl-exposed seedlings by upregulating the expression of genes involved in K/Na ratio homeostasis (*NHX1-4*) and increasing the accumulation of mineral nutrients (P, N, Ca, and Mg). However, the addition of cPTIO (100 μM; an NO scavenger) reversed the beneficial impacts of melatonin, indicating the effective function of NO in melatonin-induced defense mechanisms in NaCl-stressed tomato seedlings. Therefore, our results revealed that melatonin improves the tolerance of tomato plants during NaCl toxicity by mediating internal NO.

KEYWORDS

melatonin, nitric oxide, NHX genes, nitrogen metabolism, NaCl stress, *Solanum lycopersicum*



GRAPHICAL ABSTRACT

1 Introduction

Tomatoes (*Solanum lycopersicum* L.) are an important industrial crop that is susceptible to a variety of environmental stresses. As one of the most important environmental stresses, water or soil salinity is increasingly a serious menace to crop production (Ghorbani et al., 2021; Hao et al., 2021). Unless effective management measures are implemented, roughly 50% of agricultural lands are anticipated to suffer from salt stress by 2050 (Wang et al., 2022). Excess sodium (Na$^+$) in irrigation water or soil inhibits photosynthesis, absorption of essential nutrients, and protein synthesis, resulting in serious disruptions in plant vital metabolism, development, and yield (Shabala and Pottosin, 2014). Salt-stressed plants maintain potassium (K$^+$) and Na$^+$ homeostasis through a variety of mechanisms. Sodium/hydrogen antiporters (NHXs) in the tonoplast regulate the homeostasis of cellular cations, modulate stomatal function, and maintain cellular pH through sequestering Na and adsorbing K into vacuoles (Bassil and Blumwald, 2014). The NHX family includes six members (NHX1-6) in maize and rice and eight members (NHX1-8) in Arabidopsis (Khan et al., 2018). NHX1–4 isoforms were specified in tomato that are implicated in the accumulation of K in vacuoles, with the NHX1 and NHX2 transporters being the most significant (Gálvez et al., 2012). Furthermore, NaCl toxicity can lead to the overproduction of reactive oxygen species (ROS) and damage to various cellular organelles. These destructive effects induced by salinity are reduced by the cellular antioxidant defense system, such as antioxidant enzymes (e.g., peroxidases, catalase, and glutathione reductase), by diminishing the accumulation of free radicals (Li et al., 2017; Ghorbani et al., 2018b).

Melatonin is a plant-synthesized indoleamine that has been discovered to have crucial functions in root and shoot development, seed germination, and circadian growth rhythms (Murch and Erland, 2021). Melatonin also improved plant adaptation to biotic and abiotic stresses such as low temperature (Qari et al., 2022), high temperature (Byeon and Back, 2014), cadmium toxicity (Kaya et al., 2019), and salinity (Ghasemi-Omran et al., 2021). It has been shown that melatonin treatment can maintain ionic homeostasis in apple and maize plants during salinity stress (Li et al., 2012; Jiang et al., 2016). In addition, melatonin has been indicated to be involved in the adjustment of phytohormones and signaling molecules such as gibberellin, hydrogen peroxide (H_2O_2), nitric oxide (NO), and abscisic acid in NaCl-exposed plants (Zhang et al., 2021; Liu et al., 2015; Liang et al., 2015). NO has a vital function in plant stress responses as a redox signaling molecule. Melatonin has been demonstrated to modulate plant adaptation to different stresses through interaction with NO metabolism (Fancy et al., 2017; Yan et al., 2020; Feng et al., 2021). Liu et al. (2015) showed that melatonin reduced the levels of H_2O_2, free toxic radicals, and malondialdehyde (MDA) and improved the activities of catalase (CAT), ascorbate peroxidase (APX), and superoxide dismutase (SOD) during sodic alkaline toxicity *via* interaction with NO. Yan et al. (2020) suggested that the application of melatonin by inducing nitrate reductase (NR)-synthesized NO up-regulated the H+-pump activity of the vacuole membrane and plasma membrane and, consequently, maintained K$^+$/Na$^+$ balance in rice under NaCl stress.

Apart from the outstanding defensive effects of NO and melatonin on the induction of plant adaptation under environmental stress, there is no accurate information on the regulatory role of melatonin treatment on NO metabolism in NaCl-stressed tomatoes and/or the function of melatonin and NO interaction in the molecular mechanisms of K/Na hemostasis in tomato plants. Accordingly, we examined the regulation of NO metabolism by melatonin as well as the cross-talk between NO and melatonin in the expression of NHXs transporters and the adjustment of K$^+$/Na$^+$ balance in tomato plants during NaCl toxicity. In addition, the effects of melatonin and NO interaction on chlorophyll and proline metabolism, the antioxidant machinery, and ionic homeostasis in tomato leaves were investigated under salinity conditions. The findings of this study may provide a new physiological basis for further elucidating the regulatory mechanisms of NaCl toxicity tolerance in tomato plants induced by the melatonin-NO interaction.

2 Material and methods

2.1 Plant materials and treatments

The surface-sterilized seeds (5% NaClO for 5 min) of tomato (*Solanum lycopersicum* var. Super 2270) were germinated in plastic trays containing autoclaved peat moss (Ghorbani et al., 2022). The 20-day-old tomato seedlings were assigned to hydroponic boxes comprising nutrient solution (1/2-strength Hoagland solution, pH 6.0) (Hoagland and Arnon, 1941). Nutrition solutions (Hoagland solution only and Hoagland solution containing NaCl, melatonin, and cPTIO treatments) were renewed every 3 days. Tomato seedlings were grown in growth chambers with a temperature of 25/22°C (14/10 h) day/night, 275 ± 25 μmol/m^2/s fluorescent light, and 60% humidity. The following treatments of NaCl (150 mM), melatonin (150 µM), and 2-(4-carboxyphenyl)-4, 4, 5, 5-tetramethylimidazoline-1-oxyl-3-oxide (cPTIO, 100 µM, as an NO scavenger) were added to 27-day-old seedlings (7 days after transfer to hydroponic medium): (i) control; only nutrient solution, (ii) melatonin 150 µM; (iii) melatonin 150 µM + cPTIO 100 µM; (iv) NaCl 150 mM; (v) NaCl 150 mM + melatonin 150 µM; (vi) NaCl 150 mM + cPTIO 100 µM; (vii) NaCl 150 mM + melatonin 150 µM + cPTIO 100 µM. NaCl concentration was obtained from the results of previous research (Ghorbani et al., 2018a), and melatonin and cPTIO concentrations were obtained based on preliminary experiments. Fourteen days after the start of various treatments, the plants were collected and held at -80°C (Ghorbani et al., 2020). Five independent replicates (three replicates for gene expression) were used for sampling for each trait.

2.2 Photosynthetic pigments and Fv/Fm index

The procedure of Lichtenthaler (1987) was applied to determine the content of chlorophyll *a*, chlorophyll *b*, and carotenoids using an acetone (80%) solution and readings at 460, 645, and 663 nm. After matching the leaves in the dark for 20 min, the Fv/Fm was detected with a PAM fluorometer (PAM 2500, Walz).

2.3 δ-Aminolevulinic acid (ALA) and proline

The leaf content of ALA was specified by estimating the production of porphobilinogen from ALA at 550 nm, as defined by Harel and Klein (1972). The Bates et al. (1973) procedure was employed to specify the contents of proline by sulfosalicylic acid and readings at 520 nm.

2.4 Hydrogen peroxide (H_2O_2), superoxide anion, and malondialdehyde (MDA)

The procedure of Velikova et al. (2000) was used to appraise the content of H_2O_2 using thiobarbituric acid (TCA, 1%) and reading at 390 nm. Superoxide anion radicals were quantified using an extraction solution containing Tris-HCl buffer (50 mM, pH 6.5), nitrobluetetrazolium (0.2 mM), NADH (0.2 mM), and sucrose (250 mM) and readings at 530 nm, as expressed by Achary et al. (2012). After homogenization of fresh tomato leaves in trichloroethanoic acid (10%) and 2-thiobarbituric acid (0.65%) and recording the optical densities at 532 and 600, the leaf content of MDA was estimated following the procedure previously explained by Heath and Packer (1968).

2.5 Determination of nitric oxide (NO) content and electrolyte leakage (EL)

The procedure of Zhou et al. (2005) was used to specify the internal level of NO in the leaves using the Griess reagent and the detection of nitrate (NO^{2-}) at 540 nm. After preparing the leaf pieces and rinsing them with distilled water, the leaf pieces were placed in tubes containing distilled water on a shaker for 24 hrs. After recording the electrical conductivity (EC1), the tubes were autoclaved at 120°C for 20 min, and then EC2 was recorded. Electrolyte leakage was achieved as per Dionisio-Sese and Tobita (1998): EL (%) = (EC1/EC2) × 100.

2.6 Extraction and assay of enzymes

An extraction solution including potassium-phosphate buffer (100 mM, pH 6.8), ethylenediaminetetraacetic acid disodium salt dehydrate (5 mM), N-vinylpyrrolidinone (2%, w/v), and 0.5% TX-100 was operated to homogenize tomato leaf. After centrifugation, the supernatants were utilized to assess the activity of enzymes (Polle et al., 1994).

The leaf activities of SOD, CAT, glutathione reductase (GR), and APX enzymes were obtained by following the procedures previously reported by Foyer and Halliwell (1976); Dhindsa and Matowe (1981); Aebi (1984), and Nakano and Asada (1981).

The leaf activities of nitrite reductase (NiR), nitrate reductase (NR), glutamyl synthetase (GOGAT), and glutamine synthetase (GS) were quantified by Sawhney and Naik (1973); Boland and Benny (1977); Planchet et al. (2005), and Washitani and Sato (1977), respectively.

The procedures formerly described by Jain and Gadre (2004); Costa et al. (2005); Sumithra et al. (2006), and Charest and Phan (1990) were employed to quantify the activities of ALA dehydratase (ALAD), chlorophyllase (Chlase), proline dehydrogenase (ProDH), and Δ1-pyrroline-5-carboxylate synthetase (P5CS) enzymes, respectively.

2.7 Chemical analyses

The PFP7 model flame photometry (Jenway, Stone, UK) was operated to measure calcium (Ca^{2+}), magnesium (Mg^{2+}), and chloride (Cl^-) ions in leaves and the concentrations of Na^+ and K^+ ions in leaves and roots. The phosphomolybdate blue (Murphy and Riley, 1962) and Kjeldahl (1883) methods were used to assess the leaf concentrations of phosphorus (P) and nitrogen (N), respectively.

2.8 Gene expression

The Qiagen RNeasy kits were employed to extract total RNA from leaves and roots, following the manufacturer's instructions. Superscript reverse transcriptase (Invitrogen) and SYBR green PCR master mix (Applied Biosystems) were utilized for cDNA synthesis and qPCR reactions, respectively. The primers for target genes (Table S1), *NHX1-4*, and *Actin* (the reference gene) were designed with the Primer3 program. The relative transcript level of the target genes was estimated as per the $2^{-\Delta\Delta ct}$ method with three technical replicates (Livak and Schmittgen, 2001).

2.9 Statistical analysis

Data analysis was achieved by SAS 9.1, and the results are displayed as mean ± SD (n = 5; n = 3 for gene expression). The data were computed employing a one-way ANOVA, and the mean differences were specified as per the LSD test ($p < 0.05$).

3 Results

3.1 Growth and photosynthetic traits

The results displayed that the application of NaCl (150 mM) remarkably decreased the height (29.2%) and the total dry weight (37.1%) compared to control plants. In the absence of NaCl treatment, melatonin (150 µM) treatment did not have a significant effect on the growth and biomass. Regardless, melatonin caused a significant enhancement in height (23.7%) and total dry weight (32.2%) of tomato plants versus plants in the presence of salinity alone. In addition, when cPTIO (100 µM, a NO scavenger) was applied in the presence of melatonin and NaCl, plant height and biomass were decreased compared to their controls (Table 1).

Salinity treatment decreased the levels of chlorophyll *a*, *b*, and carotenoids by 65.3, 59.6, and 45%, respectively, over control plants (Table 1). In NaCl-exposed plants, melatonin significantly restored the levels of chlorophyll *a*, *b*, and carotenoids by 137.2, 92.8, and 66.4%, respectively, over salinity treatment alone. Nevertheless, in plants simultaneously treated with salt and melatonin, cPTIO significantly decreased photosynthetic pigments (Table 1). NaCl and NaCl+cPTIO treatments significantly reduced Fv/Fm values by 25.9 and 27.4%, respectively, over control plants. Regardless, exogenous use of melatonin improved Fv/Fm values in seedlings exposed to NaCl and NaCl+cPTIO by 23.3 and 7.1%, respectively, over their control treatments (Table 1).

3.2 Metabolism of chlorophyll and proline

In comparison to the control, NaCl- and NaCl+cPTIO-treated plants had a significant decrease in ALA content. The greatest drop was observed at NaCl+cPTIO treatment. However, melatonin supplementation significantly raised leaf accumulation of ALA in plants treated with salinity and salinity+cPTIO by 50.5 and 18.4%, respectively, compared to their controls (Figure 1A). Compared with control plants, the application of NaCl and NaCl+melatonine treatments significantly enhanced the leaf level of proline by 4.2- and 5.6-fold, respectively. Regardless, cPTIO application diminished

TABLE 1 Height, total dry weight, chlorophyll a, chlorophyll b, carotenoids, and Fv/Fm value in tomato seedlings treated with melatonin (150 µM) and cPTIO (100 µM) under NaCl (150 mM) treatment.

	Height (cm)	Total dry weight (g)	Chlorophyll a (mg/gFW)	Chlorophyll b (mg/gFW)	Carotenoids (mg/gFW)	Fv/Fm
CT	20.70 ± 0.87^{ab}	3.40 ± 0.18^{a}	2.25 ± 0.11^{a}	0.933 ± 0.127^{a}	0.630 ± 0.046^{ab}	0.544 ± 0.014^{a}
M	21.83 ± 0.75^{a}	3.46 ± 0.13^{a}	2.23 ± 0.09^{a}	0.950 ± 0.092^{a}	0.643 ± 0.050^{a}	0.549 ± 0.012^{a}
M + cPTIO	20.00 ± 0.82^{b}	3.04 ± 0.14^{b}	2.12 ± 0.13^{a}	0.907 ± 0.084^{a}	0.650 ± 0.041^{a}	0.524 ± 0.012^{b}
S	14.63 ± 0.55^{e}	2.14 ± 0.08^{d}	0.78 ± 0.09^{d}	0.377 ± 0.035^{cd}	0.347 ± 0.035^{d}	0.403 ± 0.011^{e}
S + M	18.10 ± 0.63^{c}	2.83 ± 0.12^{c}	1.85 ± 0.09^{b}	0.727 ± 0.055^{b}	0.577 ± 0.035^{b}	0.497 ± 0.012^{c}
S + cPTIO	13.90 ± 0.40^{e}	2.00 ± 0.10^{d}	0.64 ± 0.06^{d}	0.310 ± 0.020^{d}	0.313 ± 0.025^{d}	0.395 ± 0.012^{e}
S + M + cPTIO	16.10 ± 0.36^{d}	2.20 ± 0.07^{d}	1.02 ± 0.08^{c}	0.453 ± 0.035^{c}	0.420 ± 0.031^{c}	0.423 ± 0.010^{d}

The same letters in each column display no significant differences at the $p < 0.05$ based on the LSD test (means ± SD, n = 5), CT, control; M, melatonin; S, salinity.

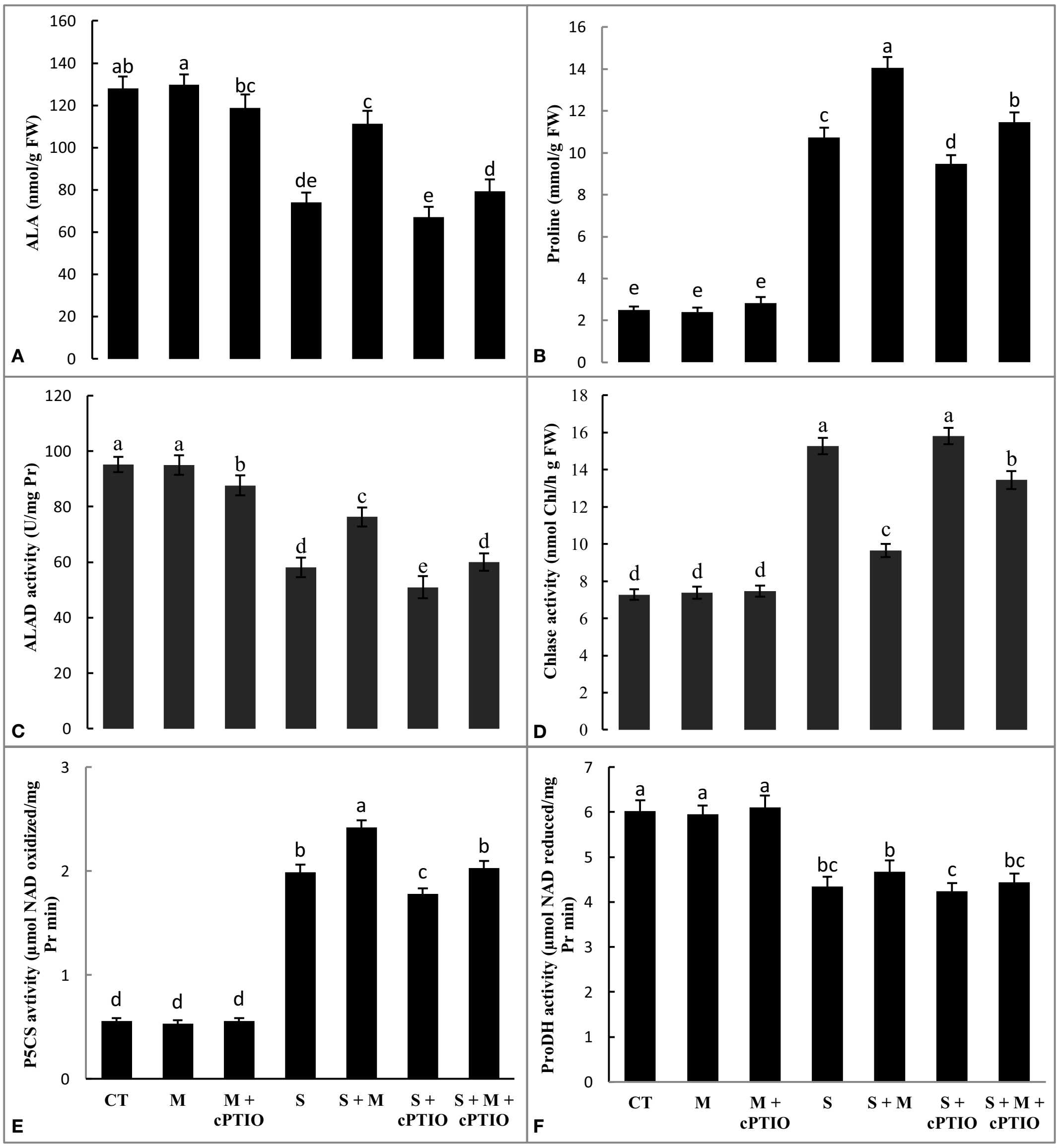


FIGURE 1
The leaf contents of minolevulinic acid (ALA, **A**) and proline **(B)**, and the leaf activities of aminolevulinic acid dehydratase (ALAD, **C**), chlorophyllase (Chlase, **D**), pyrroline-5-carboxylate synthetase (P5CS, **E**), and proline dehydrogenase (ProDH, **(F)** enzymes in tomato seedlings treated with melatonin (150 µM) and cPTIO (100 µM) under NaCl (150 mM) treatment. Different letters in each column show significant differences at the $p < 0.05$ based on the LSD test (means ± SD, n = 5), CT, control treatment; M, melatonin; S, salinity.

proline accumulation in the leaves of plants subjected to NaCl and NaCl+melatonine compared to their control (Figure 1B).

NaCl and NaCl+cPTIO treatments significantly downregulated the activity of ALAD by 39 and 46.5%, respectively, and upregulated Chlase by 2.1- and 2.2-fold, respectively. However, the exogenous application of melatonin significantly enhanced the activity of ALAD and declined the activity of Chlase in seedlings treated with NaCl+cPTIO and NaCl over their control (Figures 1C, D).

The application of NaCl and NaCl+cPTIO treatments increased the activity of P5CS and downregulated the leaf activity of ProDH over control. Regardless, melatonin enhanced the activity of both P5CS and ProDH enzymes over seedlings treated with NaCl +cPTIO and NaCl alone (Figures 1E, F).

3.3 Antioxidant defense machinery

NaCl and NaCl+cPTIO treatments significantly increased the levels of H_2O_2 by 96.5 and 109.3% and superoxide anion by 172.8 and 192%, respectively, over control plants. However, melatonin lessened the leaf accumulation of H_2O_2 by 31.4 and 12.5% and of peroxide anion by 49.6 and 11% in NaCl and NaCl+cPTIO-stressed plants, respectively, compared to their controls (Figures 2A, B). A significant enhancement was observed in the accumulation of MDA at NaCl and NaCl+cPTIO treatments over control, with the highest boost being seen under the NaCl+cPTIO treatment. In both NaCl and NaCl+cPTIO-subjected plants, the supplementation of melatonin significantly lessened MDA accumulation compared to

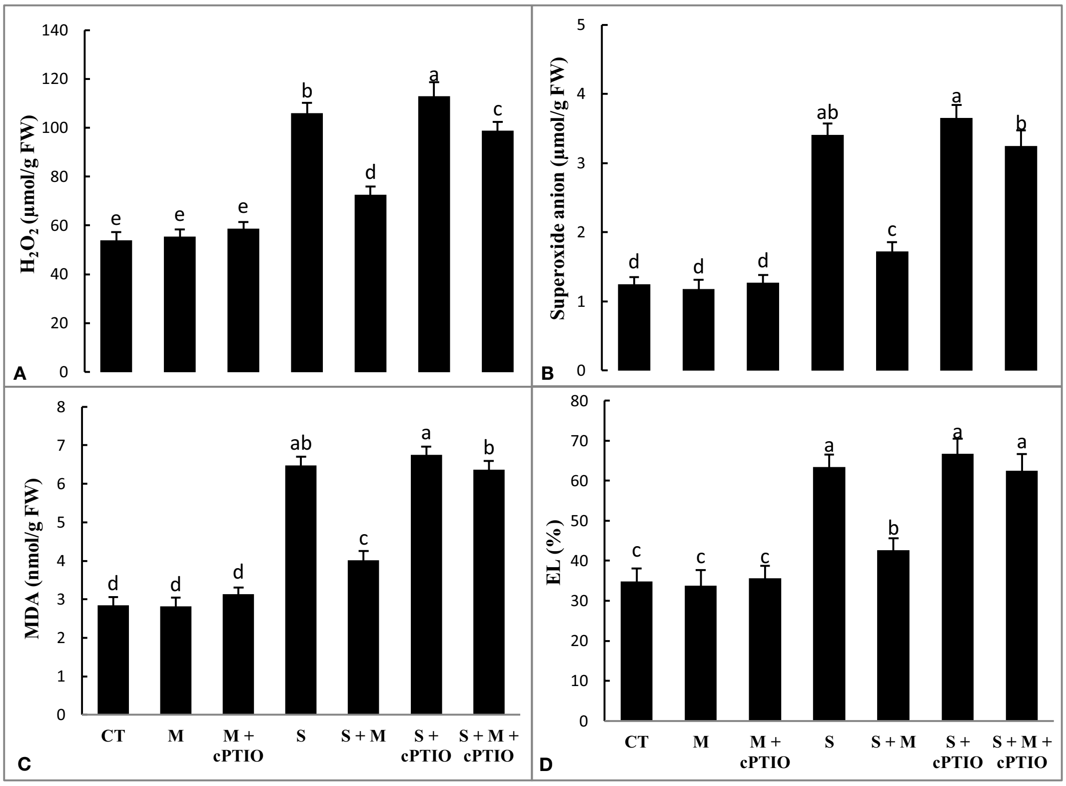


FIGURE 2
The leaf contents of hydrogen peroxide (H_2O_2, **A**), superoxide anion radical **(B)**, malondialdehyde (MDA, **C**), and electrolyte leakage (EL, **D**) in tomato seedlings treated with melatonin (150 µM) and cPTIO (100 µM) under NaCl (150 mM) treatment. Different letters in each column show significant differences at the $p < 0.05$ based on the LSD test (means ± SD, n = 5), CT, control treatment; M, melatonin; S, salinity.

their controls (Figure 2C). When tomato seedlings were treated with NaCl and NaCl+cPTIO, electrolyte leakage from leaves increased significantly by 82.2 and 91.8%, respectively, over the control. Melatonin application significantly decreased EL (32.6%) in NaCl-stressed plants but did not induce a significant reduction in NaCl+cPTIO-treated plants (Figure 2D).

When tomato seedlings were exposed to NaCl and NaCl +cPTIO treatments for 14 days, the activity of CAT, SOD, GR, and APX in the leaves was significantly upregulated over control plants. Regardless, melatonin increased the activity of antioxidant enzymes in both NaCl and NaCl+cPTIO-treated plants compared to their controls (Figures 3A, B).

3.4 Nitrogen metabolism

When tomato seedlings were subjected to NaCl toxicity, the level of NO was 46.2% higher than that of control seedlings. In NaCl-stressed plants, the exogenous use of melatonin alone and simultaneously with cPTIO enhanced NO content by 47.3 and 15.7%, respectively, compared to NaCl-exposed plants alone. Regardless, cPTIO treatment did not induce a significant effect on NO content (Figure 4A). NaCl treatment alone and simultaneously with cPTIO upregulated NR activity by 94.9 and 80.3% and NiR activity by 45.4 and 25.2%, respectively, over control plants. However, melatonin application further enhanced the activity of both NR and NiR enzymes in NaCl- and cPTIO+NaCl-treated plants compared to their control treatments (Figure 4B). The activitis of GS and GOGAT displayed significant increases under NaCl and cPTIO +NaCl treatments compared to control, with the highest boost found in NaCl-stressed seedlings. In both NaCl- and cPTIO+NaCl-treated plants, melatonin application significantly enhanced the leaf activity of GS and GOGAT enzymes over their control ones (Figures 4C, D).

3.5 Mineral nutrients

NaCl treatment alone and simultaneously with cPTIO significantly declined the leaf concentrations of P by 51.3 and 54.4%, N by 39.7 and 43.2%, Ca by 37.7 and 39.7%, and Mg by 48.6 and 50.5%, respectively, over their control. The addition of melatonin caused a further boost in the leaf concentrations of P, N, Ca, and Mg in both NaCl- and cPTIO+NaCl-treated plants (Table 2). A 100 and 40% enhancement in the leaf level of Cl was observed in NaCl- and NaCl+melatonine-treated plants, respectively, over control. However, cPTIO application caused a further enhancement in the leaf concentration of Cl in both NaCl- and NaCl+melatonin-treated seedlings over their control (Table 2).

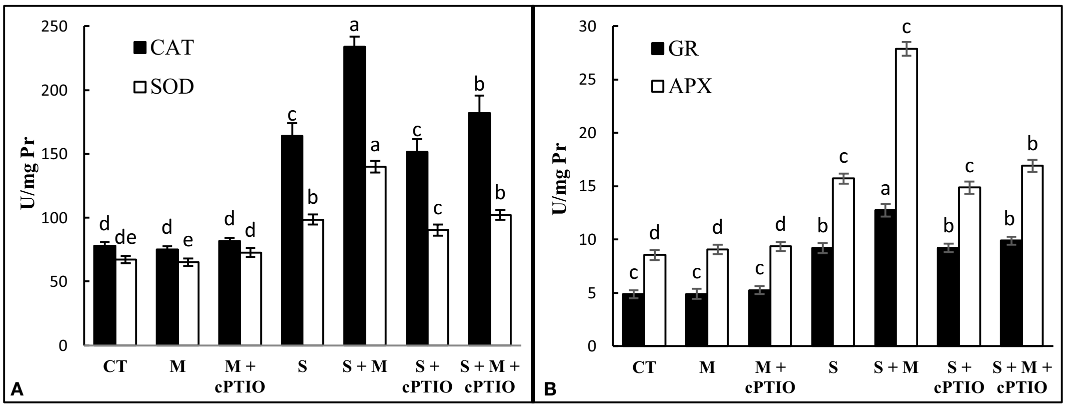


FIGURE 3
The leaf activities of catalase (CAT, **A**), superoxide dismutase (SOD, **A**), ascorbate peroxidase (APX, **B**), and glutathione reductase (GR, **B**) enzymes in tomato seedlings treated with melatonin (150 µM) and cPTIO (100 µM) under NaCl (150 mM) treatment. Different letters in each column show significant differences at the $p < 0.05$ based on the LSD test (means ± SD, n = 5), CT, control treatment; M, melatonin; S, salinity.

3.6 Expression of NHX genes

The results showed that NaCl and NaCl+cPTIO treatments significantly enhanced the relative expression of the *NHX1* gene in the leaves and roots of tomato seedlings, with the highest level of *NHX1* transcription found in NaCl-stressed seedlings. In both NaCl and NaCl +cPTIO treatments, melatonin application significantly enhanced *NHX1* mRNA levels in root and leaf tissues compared to their control treatments (Figures 5A, B). NaCl and NaCl+melatonin treatments enhanced the mRNA level of the *NHX2* gene in roots by 5.6- and 6.4-fold and in leaves by 2.3- and 5.4-fold, respectively, over control. In both root and leaf tissues, cPTIO significantly

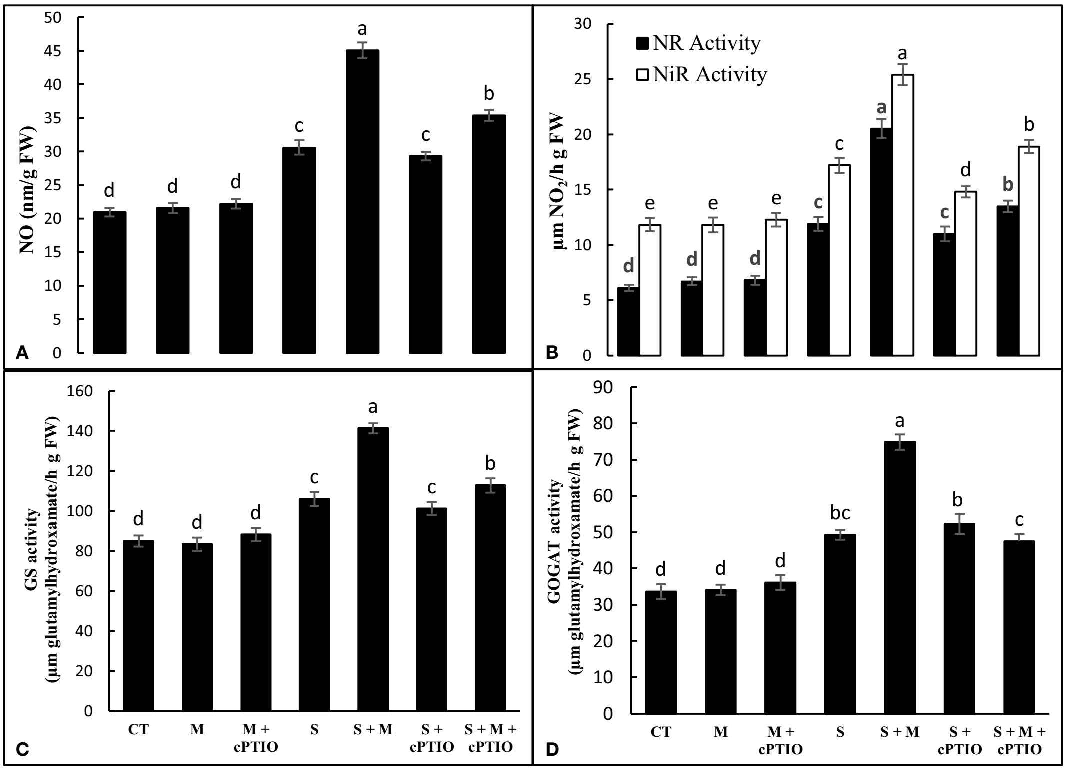


FIGURE 4
The leaf content of nitric oxide (NO, **A**) and the leaf activities of nitrate reductase (NR, **B**), nitrite reductase (NiR, **B**), glutamine synthetase (GS, **C**), and glutamyl synthetase (GOGAT, **D**) enzymes in tomato seedlings treated with melatonin (150 µM) and cPTIO (100 µM) under NaCl (150 mM) treatment. Different letters in each column show significant differences at the $p < 0.05$ based on the LSD test (means ± SD, n = 5), CT, control treatment; M, melatonin; S, salinity.

TABLE 2 The leaf concentration of mineral nutrients in tomato seedlings treated with melatonin (150 μM) and cPTIO (100 μM) under NaCl (150 mM) treatment.

	P	N	Ca	Mg	Cl
	mg/gDW				
CT	1.95 ± 0.09a	13.92 ± 0.31a	10.45 ± 0.24a	6.79 ± 0.24a	7.46 ± 0.22e
M	1.94 ± 0.12a	14.02 ± 0.27a	10.49 ± 0.27a	6.74 ± 0.24a	7.38 ± 0.22e
M + cPTIO	1.89 ± 0.10a	13.84 ± 0.27a	10.55 ± 0.28a	6.86 ± 0.31a	7.43 ± 0.19e
S	0.95 ± 0.14c	8.39 ± 0.26c	6.51 ± 0.25cd	3.49 ± 0.25cd	14.93 ± 0.31b
S + M	1.55 ± 0.11b	10.53 ± 0.27b	9.44 ± 0.31b	5.49 ± 0.30b	10.45 ± 0.19d
S + cPTIO	0.89 ± 0.12c	7.91 ± 0.18d	6.30 ± 0.27d	3.36 ± 0.23d	15.74 ± 0.24a
S + M + cPTIO	1.01 ± 0.13c	8.84 ± 0.28c	6.93 ± 0.31c	3.93 ± 0.20c	14.08 ± 0.28c

The same letters in each column display no significant differences at the $p < 0.05$ based on the LSD test (means ± SD, n = 5), CT, control treatment; M, melatonin; S, salinity.

downregulated the expression level of *NHX2* in plants treated with NaCl and NaCl+melatonin (Figures 5C, D).

The relative expression of the *NHX3* gene was significantly enhanced in the roots and leaves of seedlings exposed to NaCl and NaCl-melatonin over control. The highest upregulation was recorded during NaCl+melatonin treatment. In root and leaf, cPTIO application significantly increased the mRNA level of *NHX3* in NaCl and NaCl+melatonin treatments compared to their control (Figures 6A, B). A significant upregulation in the relative expression of the *NHX4* gene was found in the roots and leaves of tomato seedlings under NaCl treatments alone and simultaneously with cPTIO over the control. In the roots, melatonin application significantly increased the relative expression of *NHX4* in plants subjected to NaCl and NaCl

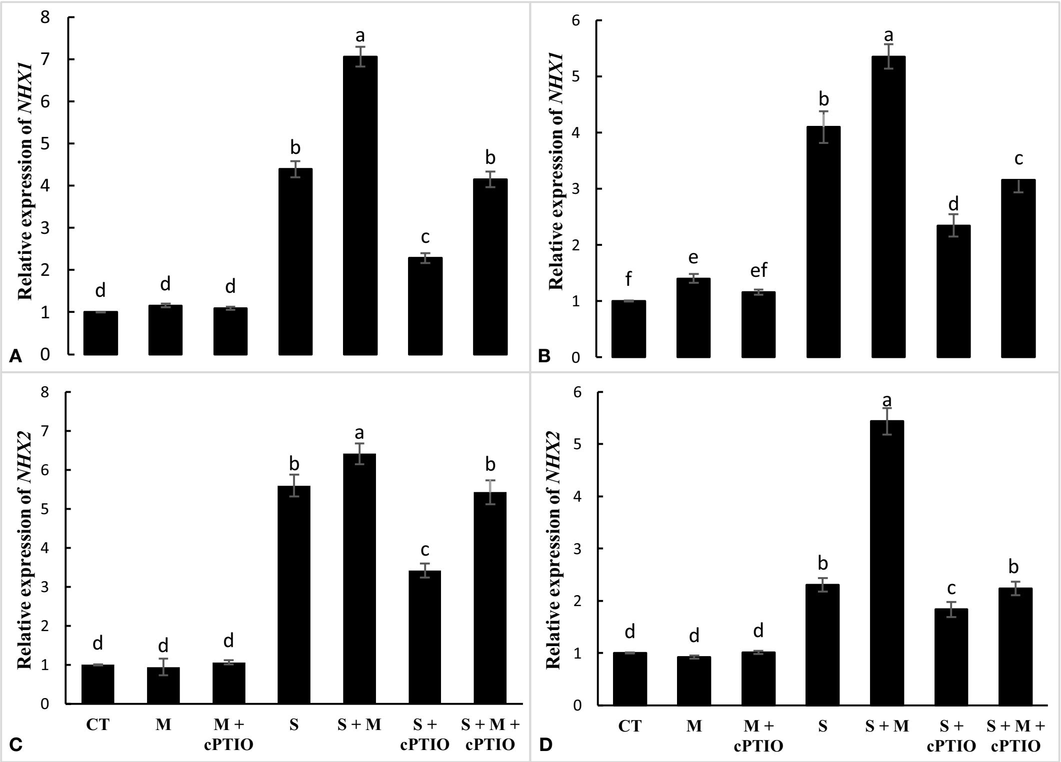


FIGURE 5
The relative expression of *NHX1* and *NHX2* genes in the root **(A, C)** and leaf **(B, D)** of tomato seedlings treated with melatonin (150 μM) and cPTIO (100 μM) under NaCl (150 mM) treatment. Different letters in each column show significant differences at the $p < 0.05$ based on the LSD test (means ± SD, n = 3), CT, control treatment; M, melatonin; S, salinity.

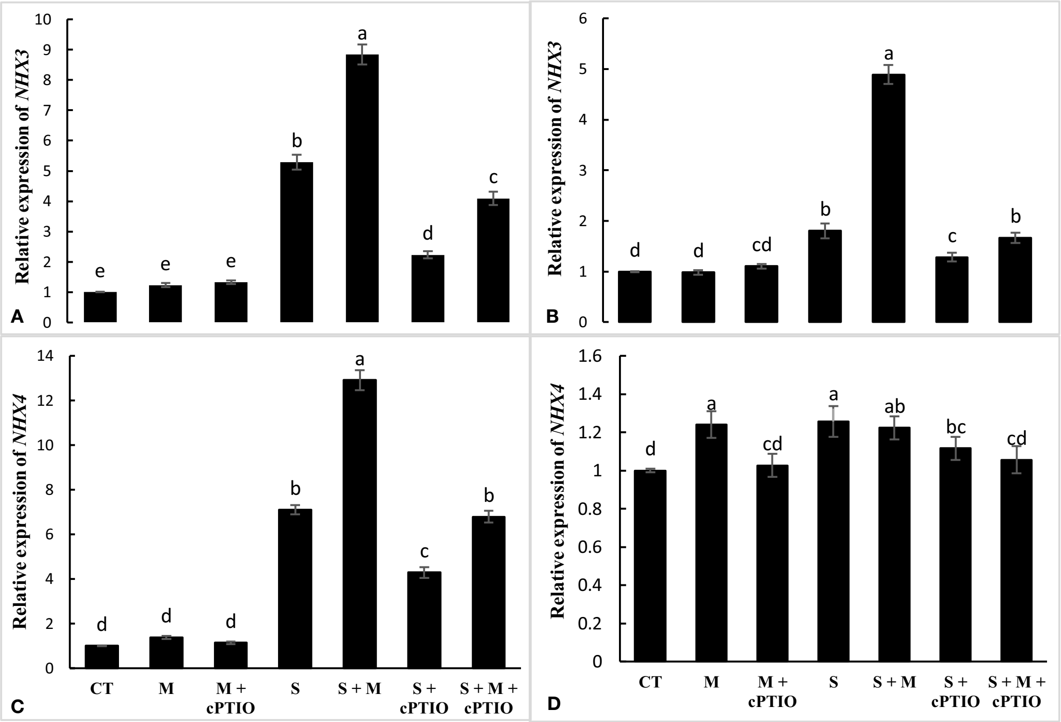


FIGURE 6
The relative expression of *NHX3* and *NHX4* genes in the root **(A, C)** and leaf **(B, D)** of tomato seedlings treated with melatonin (150 µM) and cPTIO (100 µM) under NaCl (150 mM) treatment. Different letters in each column show significant differences at the $p < 0.05$ based on the LSD test (means ± SD, n = 3), CT, control treatment; M, melatonin; S, salinity.

+cPTIO over controls. Melatonin, on the other hand, had no effect on the mRNA level of *NHX4* in NaCl- and NaCl+cPTIO treated plants (Figures 6C, D).

4 Discussion

The results showed that NaCl treatment lessened the height and biomass of tomato seedlings, which is in line with earlier declared results on tomato (Ghorbani et al., 2018a), rice (Yan et al., 2020), and cotton (Zhang et al., 2021). NaCl stress has been shown to disrupt various metabolic pathways in plants by interfering with water status and nutrient uptake (Abdelaziza et al., 2019; Ghorbani et al., 2019). Interestingly, melatonin-induced mitigation of NaCl stress was evident through endogenous NO content, verified by the use of cPTIO (a NO scavenger), indicating an inhibition of melatonin-induced impacts in the presence of NaCl toxicity. The beneficial impacts of the exogenous application of melatonin on improving the growth and biomass of various plants have already been documented (Li et al., 2017; Chen et al., 2018; Alharbi et al., 2021). Our results confirm evidence of an interaction between melatonin and NO signaling in diminishing NaCl toxicity in tomato seedlings.

The application of melatonin induced a positive impact on the metabolism of photosynthetic pigments during NaCl toxicity, as evidenced by an increase in ALA accumulation associated with a subsequent upregulation in ALAD activity. The ALAD enzyme is required for the formation of pyrrole compounds by combining two ALA molecules, which is a crucial precursor for chlorophyll production (Killiny et al., 2018). It has been shown that high levels of ALA under stressful conditions can have positive impacts on biomass, the antioxidant defense system, and osmotic regulation under environmental stress (Wu et al., 2019). Moreover, melatonin diminished the chlorophyll degradation process in NaCl-stressed plants by downregulating Chlase activity, which may have protective effects on the performance of the photosynthetic apparatus during NaCl stress. Decreased expression of the *Chlase* gene by melatonin has been earlier documented by Weeda et al. (2014). The positive effect of melatonin on chlorophyll metabolism in NaCl-stressed plants can be caused by reducing oxidative stress and improving the activity of enzymes involved in chlorophyll metabolism (Liu et al., 2020), as well as improving ionic homeostasis and providing more Mg and Fe elements (Yan et al., 2020). However, cPTIO reversed the beneficial impacts of melatonin on chlorophyll metabolism, indicating the regulatory role of melatonin-mediated NO in modulating chlorophyll metabolism during NaCl stress. Additional examinations are needed to specify the exact role of melatonin and its interaction with NO in the biosynthesis of intermediates and chlorophyll metabolism.

It has been shown that the accumulation of osmoregulatory compounds such as proline under environmental stresses maintains ROS scavenging, cellular osmolality, redox homeostasis, and the function of bio-macromoleculars in plants, and is a significant help in improving plant adaptation in stressful conditions (Ghorbani et al., 2018a). NaCl toxicity enhanced the leaf content of proline by decreasing ProDH activity and enhancing P5CS activity, which can be described by putative water stress caused by salinity, as confirmed by Jiang et al. (2021) and Ghorbani et al. (2018a). Guan et al. (2020) revealed that upregulating the expression and activity of enzymes involved in proline biosynthesis (P5CS) increases proline accumulation and, as a result, improves plant adaptation to NaCl toxicity. Melatonin, by modulating enzymes in proline metabolism, provoked a further enhancement in proline in tomato leaves under NaCl stress, which can play a key function in enhancing plant adaptation. Similarly, Jiang et al. (2021) showed that melatonin, by increasing the proline content, protected the photosynthetic apparatus and improved the biomass of the cotton plant during salinity. As a result, employing cPTIO, melatonin-mediated proline metabolism was reversed, confirming the important regulatory function of melatonin-induced NO in the regulation of proline metabolism.

One of the serious damages induced by salinity stress is the excessive production of ROS (H_2O_2 and superoxide anion) in plants, which causes serious oxidative damage to important cell components, including biomembranes and the induction of EL. Plants have machinery to balance the endogenous level of toxic free radicals. The NaCl treatment increased the accumulation of toxic compounds such as superoxide anions and H_2O_2, resulting in increased MDA and EL, which indicate the induction of oxidative stress in tomato seedlings. NaCl toxicity-induced oxidative stress in tomato (Yin et al., 2019) and stevia (Ghasemi-Omran et al., 2021) plants has also been previously documented. Salinity stress disrupts the function of the photosynthetic apparatus and induces an imbalance between the production and consumption of electrons, causing the transmission of excess electrons to the oxygen molecule and the generation of ROS (AbdElgawad et al., 2016). However, melatonin effectively increased the activity of antioxidant enzymes, thereby reducing the level of ROS and protecting the biomembranes. The helpful impacts of melatonin on the antioxidant machinery and the decline of oxidative stress under salinity stress have already been confirmed by Li et al. (2017) and Jiang et al. (2021). Sun et al. (2021) indicated that melatonin upregulated the expression of antioxidant genes, such as SOD, CAT, APX, and GR, by enhancing the internal contents of NO and, as a result, reduced oxidative stress and enhanced plant biomass under salinity, which is in accordance with the findings of this research. Arora and Bhatla (2017) indicated that melatonin-mediated antioxidant defense in sunflower plants during salinity was dependent on NO. The addition of cPTIO prevented the beneficial impacts of melatonin on the antioxidant machinery, exhibiting the critical function of melatonin-caused internal NO in augmenting the defense system of NaCl-stressed tomatoes.

Key enzymes involved in N and NO metabolism (GOGAT), GS, NiR, and NR) play a crucial function in plant adaptation during environmental stresses (Chen et al., 2021). Melatonin significantly increased the leaf activity of enzymes in N assimilation and the leaf content of NO in NaCl-exposed seedlings, which can effectively improve the tolerance of tomato seedlings. Similar results have already been documented by Talaat (2021) and Ma et al. (2021). An increase in melatonin-induced N metabolism can improve N uptake and transfer between source and sink parts, thereby accelerating the transfer of nutrients between different plant organs under salinity stress (Talaat, 2021). Melatonin was shown to upregulate the expression of *NiR*, *NR*, *GS*, and *GOGAT* genes, which significantly enhanced the adaptation of the plant during stress (Liang et al., 2018). Due to the high sensitivity of GS and GOGAT enzymes to oxidative stress, increasing their activity under melatonin treatment can be due to the melatonin-caused alleviation of oxidative stress (Bose and Howlader, 2020; Zhao et al., 2021). However, cPTIO reduced the activity of enzymes in N assimilation in melatonin-treated plants, indicating the induction of melatonin-mediated N metabolism through the internal NO content under salinity.

Depolarization of the plasma membrane resulting from the influx of Na ions into the cell causes a continuous efflux of K from the cell under salinity stress, which results in a drop in the ratio of K/Na and, consequently, serious damage to vital cell processes (Shabala et al., 2006; Munns and Tester, 2008). NaCl treatment enhanced the accumulation of Na and declined the accumulation of K in the leaf and root of tomato seedlings, which was associated with a decrease in the K/Na ratio. Similar results of reducing the K/Na ratio in tomato (Ghorbani et al., 2019) and rice (Yan et al., 2020) have already been shown. Melatonin effectively reduced Na uptake and transport to the leaves, which maintained K homeostasis and increased the K/Na ratio under NaCl stress. Yan et al. (2020) in rice and Jiang et al. (2016) in maize plants under salt stress reported similar effects of melatonin's regulatory role in diminishing Na uptake and improving the ratio of K/Na. Melatonin also diminished the translocation of Na to the leaves under salinity, which could be due to decreased Na loading in the xylem (Yan et al., 2021). A report showed that melatonin maintained K homeostasis and improved plant tolerance under salinity by alleviating oxidative stress and regulating K transporters, including HAK5 (Liu et al., 2020). NaCl treatment also reduced the concentration of nutrient minerals (P, N, Mg, and Ca) in the leaves, which could be due to oxidative stress-induced damage to biomembranes, as well as disruption of membrane potential due to the entry of excessive Na levels into the cellular cytoplasm (Ghorbani et al., 2019; Yan et al., 2021). Melatonin significantly improved the concentration of mineral nutrients in NaCl-stressed seedlings, which could result in the reduction of toxic radicals and the stabilization of cell membranes (Liu et al., 2020). The helpful impacts of melatonin on maintaining ionic homeostasis under salinity have been earlier confirmed by Zahedi et al. (2021) and Alharbi et al. (2021). Thus, melatonin improves plant growth and biomass under salinity stress by maintaining ionic homeostasis and reducing Na uptake. Numerous studies have shown that NO treatment effectively reduces Na uptake, improves K/Na, and increases the uptake of mineral nutrients in plants under salinity stress (Zhang et al., 2006; Wang et al., 2009; Hasanuzzaman et al., 2021). The inductive impacts of melatonin on the maintenance of ionic balance and the reduction of Na uptake in cPTIO-treated plants were reversed, indicating that the impacts of melatonin improvement on the maintenance of ionic homeostasis are achieved through NR-mediated NO production.

Salinity stress increases the entry of Na into the cell cytoplasm, which, by damaging cytosolic enzymes, causes a serious disruption in the vital processes of the plant (Fukuda et al., 2011). Therefore, regulating the cytosolic concentrations of Na and K and maintaining the cytosolic ratio of K/Na under salinity can play a critical function in enhancing plant adaptation to salt stress. The three main mechanisms for preventing the cytosolic accumulation of Na include: inhibiting Na influx into the cytoplasm, inducing Na efflux, and sequestering Na in vacuoles (Padan et al., 2001). The *NHX* gene family, located on the tonoplast membrane, is implicated in the compartmentalization of Na into the vacuoles, which can play an important function in reducing the potential toxicity of Na during salinity (Padan et al., 2001; Fukuda et al., 2011). Increased expression of *NHX1*, *NHX2*, *NHX3*, and *NHX4* genes has been shown to decline Na toxicity and, consequently, enhance plant growth under NaCl toxicity (Abdelaziza et al., 2019; Ghorbani et al., 2019). Tan et al. (2021) indicated that the heterologous expression of the melatonin-synthesizing gene was increased by enhancing the expression of *NHXs* and inducing an efflux of Na in the plants under NaCl toxicity, which was accompanied by increased growth and adaptation of the plant under salinity. The results showed that melatonin significantly increased the relative expression of *NHX1*, *NHX2*, *NHX3*, and *NHX4* genes in the root and leaf of tomato seedlings during NaCl toxicity, which could play an important function in Na detoxification by sequestering in vacuoles. Increased expression of *NHX* genes in the root was stronger than in the leaf, which is consistent with a decrease in Na translocation to the leaves. However, when the melatonin-subjected plants were treated with cPTIO, the inducible effects of melatonin on the expression of the *NHX* gene were inhibited, indicating that melatonin-induced upregulation of the *NHX* gene arises through NR-mediated NO during NaCl stress.

5 Conclusion

Our findings provide crucial insights into the function of exogenous melatonin and melatonin-mediated internal NO signaling in handling NaCl adaptation in tomato. The results confirmed that exogenous application of melatonin by interaction with endogenous NO increased plant growth and biomass during NaCl stress, indicating that NO as a signaling molecule may be involved downstream of the melatonin-mediated defense response in NaCl-exposed tomato seedlings. To confirm the function of NO downstream of the melatonin-induced signaling pathway, cPTIO was used as a NO scavenger, which showed that cPTIO prevented the beneficial role of melatonin in chlorophyll and proline metabolism, the antioxidant defense system, ionic homeostasis, and modulation of *NHX* gene expression. Therefore, by adjusting N metabolism and enhancing the internal content of NO, melatonin improves chlorophyll and proline metabolism, strengthens the antioxidant defense system, preserves ion balance, and modulates the expression of transporter genes involved in K/Na homeostasis, thus improving the growth and tolerance of tomato seedlings under NaCl toxicity. In the future, the interaction of other enzymes or signaling molecules in increasing the tolerance of abiotic stresses induced by melatonin should be examined.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

Author contributions

Methodology and Conceptualization, AG; Investigation and Validation, AG and LP; Resources, KVS; Analysis, AG; Writing original, AG; Review and Editing, M-XC. All authors contributed to the article and approved the submitted version.

Funding

This work was supported by the National Natural Science Foundation of China (32001452), the Program of Introducing Talent to Chinese Universities (111 Program, D20023), the Frontiers Science Center for Asymmetric Synthesis and Medicinal Molecules, Department of Education, Guizhou Province [Qianjiaohe KY (2020)004].

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2023.1135943/full#supplementary-material

References

Abdelaziza, M. E., Abdelsattar, M., Abdeldaym, E. A., Atia, M. A. M., Mahmoud, A. W. M., Saad, M. M., et al. (2019). *Piriformospora indica* alters Na^+/K^+ homeostasis, antioxidant enzymes and *LeNHX1* expression of greenhouse tomato grown under salt stress. *Sci. Hortic.* 256, 108532. doi: 10.1016/j.scienta.2019.05.059

AbdElgawad, H., Zinta, G., Hegab, M. M., Pandey, R., Asard, H., and Abuelsoud, W. (2016). High salinity induces different oxidative stress and antioxidant responses in maize seedlings organs. *Front. Plant Sci.* 7, 276. doi: 10.3389/fpls.2016.00276

Achary, V. M., Parinandi, N. L., and Panda, B. B. (2012). Aluminum induces oxidative burst, cell wall NADH peroxidase activity, and DNA damage in root cells of *Allium cepa* l. *Environ. Mol. Mutagen* 38, 550–560. doi: 10.1002/em.21719

Aebi, H. (1984). “Catalase in vitro,” in *Methods in enzymology*. Eds. S. Colowick and N. Kaplan (Florida: Elsevier), 121–126.

Alharbi, B. M., Elhakem, A. H., Alnusairi, G. S. H., Soliman, M. H., Hakeem, K. R., Hasan, M. M., et al. (2021). Exogenous application of melatonin alleviates salt stress-induced decline in growth and photosynthesis in *Glycine max* (L.) seedlings by improving mineral uptake, antioxidant and glyoxalase system. *Plant Soil Environ.* 67 (4), 208–220. doi: 10.17221/659/2020-PSE

Arora, D., and Bhatla, S. C. (2017). Melatonin and nitric oxide regulate sunflower seedling growth under salt stress accompanying differential expression of Cu/Zn SOD and Mn SOD. *Free Radic. Biol. Med.* 106, 315–328. doi: 10.1016/j.freeradbiomed.2017.02.042

Bassil, E., and Blumwald, E. (2014). The ins and outs of intracellular ion homeostasis: NHXtype cation/H^+ transporters. *Curr. Opin. Plant Biol.* 22, 1–6. doi: 10.1016/j.pbi.2014.08.002

Bates, L. S., Waldren, R. P., and Teare, I. D. (1973). Rapid determination of free proline for water-stress studies. *Plant Soil* 39, 205–207. doi: 10.1007/BF00018060

Boland, M. J., and Benny, A. G. (1977). Enzymes of nitrogen metabolism in legume nodules. purification and properties of NADH-dependent glutamate synthase from lupin nodules. *Eur. J. Biochem.* 79, 355–362. doi: 10.1111/j.1432-1033.1977.tb11816.x

Bose, S. K., and Howlader, P. (2020). Melatonin plays multifunctional role in horticultural crops against environmental stresses: a review. *Environ. Exp. Bot.* 176, 104063. doi: 10.1016/j.envexpbot.2020.104063

Byeon, Y., and Back, K. (2014). Melatonin synthesis in rice seedlings *in vivo* is enhanced at high temperatures and under dark conditions due to increased serotonin nacetyltransferase and n-acetylserotonin methyltransferase activities. *J. Pineal Res.* 56, 189–195. doi: 10.1111/jpi.12111

Charest, C., and Phan, C. T. (1990). Cold-acclimation of wheat (*Triticum aestivum*)–properties of enzymes involved in proline metabolism. *Physiol. Plant* 80, 159–168. doi: 10.1111/j.1399-3054.1990.tb04391.x

Chen, Z., Cao, X., and Niu, J. (2021). Effects of melatonin on morphological characteristics, mineral nutrition, nitrogen metabolism, and energy status in alfalfa under high-nitrate stress. *Front. Plant Sci.* 12, 694179. doi: 10.3389/fpls.2021.694179

Chen, Y. E., Mao, J. J., Sun, L. Q., Huang, B., Ding, C. B., Gu, Y., et al. (2018). Exogenous melatonin enhances salt stress tolerance in maize seedlings by improving antioxidant and photosynthetic capacity. *Physiol. Plant* 164 (3), 349–363. doi: 10.1111/ppl.12737

Costa, M. L., Civello, P. M., Chaves, A. R., and Martı´nez, G. A. (2005). Effect of ethephon and 6–benzylaminopurine on chlorophyll degrading enzymes and a peroxidase–linked chlorophyll bleaching during post-harvest senescence of broccoli (*Brassica oleracea* l.) at 20 c. *Postharvest Biol. Technol.* 35 (2), 191–199. doi: 10.1016/j.postharvbio.2004.07.007

Dhindsa, R. S., and Matowe, W. (1981). Drought tolerance in two mosses: correlated with enzymatic defence against lipid peroxidation. *J. Exp. Bot.* 32, 79–91. doi: 10.1093/jxb/32.1.79

Dionisio-Sese, M. L., and Tobita, S. (1998). Antioxidant responses of rice seedlings to salinity stress. *Plant Sci.* 135, 1–9. doi: 10.1016/S0168-9452(98)00025-9

Fancy, N. N., Bahlmann, A. K., and Loake, G. J. (2017). Nitric oxide function in plant abiotic stress. *Plant Cell Environ.* 40, 462–472. doi: 10.1111/pce.12707

Feng, Y., Fu, X., Han, L., Xu, C., Liu, C., Bi, H., et al. (2021). Nitric oxide functions as a downstream signal for melatonin-induced cold tolerance in cucumber seedlings. *Front. Plant Sci.* 12, 686545. doi: 10.3389/fpls.2021.686545

Foyer, C. H., and Halliwell, B. (1976). The presence of glutathione and glutathione reductase in chloroplasts: A proposed role in ascorbic acid metabolism. *Planta* 133, 21–25. doi: 10.1007/BF00386001

Fukuda, A., Nakamura, A., Hara, N., Toki, S., and Tanaka, Y. (2011). Molecular and functional analyses of rice NHX-type Na^+/H^+ antiporter genes. *Planta* 233, 175–188. doi: 10.1007/s00425-010-1289-4

Gálvez, F. J., Baghour, M., Hao, G., Cagnac, O., Rodríguez-Rosales, M. P., and Venema, K. (2012). Expression of *LeNHX* isoforms in response to salt stress in salt sensitive and salt tolerant tomato species. *Plant Physiol. Biochem.* 51, 109–115. doi: 10.1016/j.plaphy.2011.10.012

Ghasemi-Omran, V. O., Ghorbani, A., and Sajjadi-Otaghsara, S. A. (2021). Melatonin alleviates NaCl-induced damage by regulating ionic homeostasis, antioxidant system, redox homeostasis, and expression of steviol glycosides-related biosynthetic genes in *in vitro* cultured *Stevia rebaudiana* bertoni. *In Vitro Cell Dev. Biol-Plant* 57, 319–333. doi: 10.1007/s11627-021-10161-9

Ghorbani, A., Ghasemi Omran, V. O., Razavi, S. M., Pirdashti, H., and Ranjbar, M. (2019). *Piriformospora indica* confers salinity tolerance on tomato (*Lycopersicon esculentum* mill.) through amelioration of nutrient accumulation, K^+/Na^+ homeostasis and water status. *Plant Cell Rep.* 38, 1151–1163. doi: 10.1007/s00299-019-02434-w

Ghorbani, A., Pishkar, L., Roodbari, N., Pehlivan, N., and Wu, C. (2021). Nitric oxide could allay arsenic phytotoxicity in tomato (*Solanum lycopersicum* l.) by modulating photosynthetic pigments, phytochelatin metabolism, molecular redox status and arsenic sequestration. *Plant Physiol. Biochem.* 167, 337–348. doi: 10.1016/j.plaphy.2021.08.019

Ghorbani, A., Pishkar, L., Roodbari, N., Tavakoli, S. A., Moein Jahromi, E., and Wu, C. (2022). Nitrate reductase is needed for methyl jasmonate-mediated arsenic toxicity tolerance of rice by modulating the antioxidant defense system, glyoxalase system and arsenic sequestration mechanism. *J. Plant Growth Regul.* 42, 1107–1119. doi: 10.1007/s00344-022-10616-2

Ghorbani, A., Razavi, S. M., Ghasemi Omran, V. O., and Pirdashti, H. (2018a). *Piriformospora indica* inoculation alleviates the adverse effect of NaCl stress on growth, gas exchange and chlorophyll fluorescence in tomato (*Solanum lycopersicum* l.). *Plant Biol.* 20, 729–736. doi: 10.1111/plb.12717

Ghorbani, A., Razavi, S. M., Ghasemi Omran, V. O., and Pirdashti, H. (2018b). *Piriformospora indica* alleviates salinity by boosting redox poise and antioxidative potential of tomato. *Russ J. Plant Physiol.* 65, 898–907. doi: 10.1134/S1021443718060079

Ghorbani, A., Tafteh, M., Roudbari, N., Pishkar, L., Zhang, W., and Wu, C. (2020). *Piriformospora indica* augments arsenic tolerance in rice (*Oryza sativa*) by immobilizing arsenic in roots and improving iron translocation to shoots. *Ecotoxicol Environmen Saf.* 209, 111793. doi: 10.1016/j.ecoenv.2020.111793

Guan, C., Cui, X., Liu, H. Y., Li, X., Li, M. Q., and Zhang, Y. W. (2020). Proline biosynthesis enzyme genes confer salt tolerance to switchgrass (*Panicum virgatum* l.) in cooperation with polyamines metabolism. *Front. Plant Sci.* 11, 46. doi: 10.3389/fpls.2020.00046

Hao, S., Wang, Y., Yan, Y., Liu, Y., Wang, J., and Chen, S. (2021). A review on plant responses to salt stress and their mechanisms of salt resistance. *Horticulturae* 7, 6, 132. doi: 10.3390/horticulturae7060132

Harel, E., and Klein, S. (1972). Light dependent formation of 5-aminolevulinic acid in etiolated leaves of higher plants. *Biochem. Biophys. Res. Commun.* 49, 364–370. doi: 10.1016/0006-291X(72)90419-6

Hasanuzzaman, M., Inafuku, M., Nahar, K., Fujita, M., and Oku, H. (2021). Nitric oxide regulates plant growth, physiology, antioxidant defense, and ion homeostasis to confer salt tolerance in the mangrove species, *Kandelia obovata*. *Antioxidants (Basel)* 10 (4), 611. doi: 10.3390/antiox10040611

Heath, R. L., and Packer, L. (1968). Photoperoxidation in isolated chloroplasts. i. kinetics and stoichiometry of fatty acid peroxidation. *Arch. Biochem. Biophys.* 125, 189. doi: 10.1016/0003-9861(68)90654-1

Hoagland, D., and Arnon, D. (1941). Physiological aspects of availability of nutrients for plant growth. *Soil Sci.* 51, 431–444. doi: 10.1097/00010694-194106000-00002

Jain, M., and Gadre, R. (2004). Inhibition of chlorophyll biosynthesis by mercury in excised etiolated maize leaf segments during greening: effect of 2-oxoglutarate. *Indian J. Exp. Biol.* 42, 419–423.

Jiang, C., Cui, Q., Feng, K., Xu, D., Li, C., and Zheng, Q. (2016). Melatonin improves antioxidant capacity and ion homeostasis and enhances salt tolerance in maize seedlings. *Acta Physiol. Plant* 38, 82. doi: 10.1007/s11738-016-2101-2

Jiang, D., Lu, B., Liu, L., Duan, W., Meng, Y., Li, J., et al. (2021). Exogenous melatonin improves the salt tolerance of cotton by removing active oxygen and protecting photosynthetic organs. *BMC Plant Biol.* 21, 331. doi: 10.1186/s12870-021-03082-7

Kaya, C., Okant, M., Ugurlar, F., Alyemeni, M. N., Ashraf, M., and Ahmad, P. (2019). Melatonin-mediated nitric oxide improves tolerance to cadmium toxicity by reducing oxidative stress in wheat plants. *Chemosphere* 225, 627–638. doi: 10.1016/j.chemosphere.2019.03.026

Khan, I. U., Ali, A., and Yun, D. J. (2018). Arabidopsis NHX transporters: Sodium and potassium antiport mythology and sequestration during ionic stress. *J. Plant Biol.* 61, 292–300. doi: 10.1007/s12374-018-0244-y

Killiny, N., Hijaz, F., Nehela, Y., Hajeri, S., and Gowda, S. (2018). Effects of δ-aminolevulinic acid dehydratase silencing on the primary and secondary metabolisms of citrus. *Plant Direct* 2 (7), e00072. doi: 10.1002/pld3.72

Kjeldahl, J. (1883). Neue methode zur bestimmung des stickstoffs in organischen körpern. *Z Anal. Chem.* 22, 366–382. doi: 10.1007/BF01338151

Li, C., Wang, P., Wei, Z., Liang, D., Liu, C., Yin, L., et al. (2012). The mitigation effects of exogenous melatonin on salinity-induced stress in Malus hupehensis. *J. Pineal Res.* 53, 298–306.

Li, H., Chang, J., Chen, H., Wang, Z., Gu, X., Wei, C., et al. (2017). Exogenous melatonin confers salt stress tolerance to watermelon by improving photosynthesis and redox homeostasis. *Front. Plant Sci.* 8, 295. doi: 10.3389/fpls.2017.00295

Li, L., Li, L., Wang, X., Zhu, P., Wu, H., and Qi, S. (2017). Plant growth-promoting endophyte *Piriformospora indica* alleviates salinity stress in *Medicago truncatula*. *Plant Physiol. Biochem.* 119, 211–223. doi: 10.1016/j.plaphy.2017.08.029

Liang, C., Zheng, G., Li, W., Wang, Y., Hu, B., Wang, H., et al. (2015). Melatonin delays leaf senescence and enhances salt stress tolerance in rice. *J. Pineal Res.* 59, 91–101.

Liang, B., Ma, C., Zhang, Z., Wei, Z., Gao, T., Zhao, Q., et al. (2018). Long-term exogenous application of melatonin improves nutrient uptake fluxes in apple plants under moderate drought stress. *Environ. Exp. Bot.* 155, 650–661. doi: 10.1016/j.envexpbot.2018.08.016

Lichtenthaler, H. K. (1987). Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. *Method Enzymol.* 148, 350–382. doi: 10.1016/0076-6879(87)48036-1

Liu, N., Gong, B., Jin, Z., Wang, X., Wei, M., Yang, F., et al. (2015). Sodic alkaline stress mitigation by exogenous melatonin in tomato needs nitric oxide as a downstream signal. *J. Plant Physio.* 186–187, 68–77. doi: 10.1016/j.jplph.2015.07.012

Liu, J., Shabala, S., Zhang, J., Ma, G., Chen, D., Shabala, L., et al. (2020). Melatonin improves rice salinity stress tolerance by NADPH oxidase-dependent control of the plasma membrane k^+ transporters and k^+ homeostasis. *Plant Cell Environ.* 43 (11), 2591–2605. doi: 10.1111/pce.13759

Livak, K. J., and Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods* 25, 402–408. doi: 10.1006/meth.2001.1262

Ma, L., Huang, Z., Li, S., Ashraf, U., Yang, W., Liu, H., et al. (2021). Melatonin and nitrogen applications modulate early growth and related physio-biochemical attributes in maize under cd stress. *J. Soil Sci. Plant Nutr.* 21, 978–990. doi: 10.1007/s42729-021-00415-1

Munns, R., and Tester, M. (2008). Mechanisms of salinity tolerance. *Annu. Rev. Plant Biol.* 59, 651–681. doi: 10.1146/annurev.arplant.59.032607.092911

Murch, S. J., and Erland, L. A. E. (2021). A systematic review of melatonin in plants: An example of evolution of literature. *Front. Plant Sci.* 12, 683047. doi: 10.3389/fpls.2021.683047

Murphy, J., and Riley, J. P. (1962). A modified single solution method for the determination of phosphate in natural waters. *Anal. Chim. Acta* 27, 31–36. doi: 10.1016/S0003-2670(00)88444-5

Nakano, Y., and Asada, K. (1981). Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant Cell Physiol.* 22, 867–880.

Padan, E., Venturi, M., Gerchman, Y., and Dover, N. (2001). Na^+/H^+ antiporters. *BBA-Bioenergetics* 1505, 144–157. doi: 10.1016/S0005-2728(00)00284-X

Planchet, E., Gupta, K. J., Sonoda, M., and Kaiser, W. M. (2005). Nitric oxide emission from tobacco leaves and cell suspensions: Rate limiting factors and evidence for the involvement of mitochondrial electron transport. *Plant J.* 41, 732–743. doi: 10.1111/j.1365-313X.2005.02335.x

Polle, A., Otter, T., and Mehne-Jakobs, B. (1994). Effect of magnesium-deficiency on antioxidative systems in needles of Norway spruce [*Picea abies* (L.) karst.] grown with different ratios of nitrate and ammonium as nitrogen sources. *New Phytol.* 128, 621–628. doi: 10.1111/j.1469-8137.1994.tb04026.x

Qari, S. H., Hassan, M. U., Chattha, M. U., Mahmood, A., Naqve, M., Nawaz, M., et al. (2022). Melatonin induced cold tolerance in plants: Physiological and molecular responses. *Front. Plant Sci.* 13, 843071. doi: 10.3389/fpls.2022.843071

Sawhney, S. K., and Naik, M. S. (1973). Effect of chloramphenicol and cycloheximide on synthesis of nitrate reductase and nitrite reductase in rice leaves. *Biochem. Biophys. Res. Commun.* 51, 67–73. doi: 10.1016/0006-291X(73)90508-1

Shabala, S., Demidchik, V., Shabala, L., Cuin, T. A., Smith, S. J., Miller, A. J., et al. (2006). Extracellular Ca^{2+} ameliorates NaCl-induced k^+ loss from arabidopsis root and leaf cells by controlling plasma membrane k^+-permeable channels. *Plant Physiol.* 141, 1653–1665. doi: 10.1104/pp.106.082388

Shabala, S., and Pottosin, I. (2014). Regulation of potassium transport in plants under hostile conditions: implications for abiotic and biotic stress tolerance. *Physiol. Plant* 151, 257–279. doi: 10.1111/ppl.12165

Sumithra, K., Jutur, P. P., Carmel, B. D., and Reddy, A. R. (2006). Salinity-induced changes in two cultivars of *Vigna radiata*: Responses of antioxidative and proline metabolism. *Plant Growth Regul.* 50, 11–22. doi: 10.1007/s10725-006-9121-7

Sun, Y., Ma, C., Kang, X., Zhang, L., Wang, J., Zheng, S., et al. (2021). Hydrogen sulfide and nitric oxide are involved in melatonin-induced salt tolerance in cucumber. *Plant Physiol. Biochem.* 167, 101–112. doi: 10.1016/j.plaphy.2021.07.023

Talaat, N. B. (2021). Polyamine and nitrogen metabolism regulation by melatonin and salicylic acid combined treatment as a repressor for salt toxicity in wheat (*Triticum aestivum* l.) plants. *Plant Growth Regul.* 95, 315–329. doi: 10.1007/s10725-021-00740-6

Tan, K., Zheng, J., Liu, C., Liu, X., Liu, X., Gao, T., et al. (2021). Heterologous expression of the melatonin-related gene HIOMT improves salt tolerance in *Malus domestica*. *Int. J. Mol. Sci.* 22 (22), 12425. doi: 10.3390/ijms222212425

Velikova, V., Yordanov, I., and Edreva, A. (2000). Oxidative stress and some antioxidant systems in acid rain-treated bean plants: Protective role of exogenous polyamines. *Plant Sci.* 151, 59–66. doi: 10.1016/S0168-9452(99)00197-1

Wang, H., Liang, X., Wan, Q., Wang, X., and Bi, Y. (2009). Ethylene and nitric oxide are involved in maintaining ion homeostasis in arabidopsis callus under salt stress. *Planta* 230 (2), 293–307. doi: 10.1007/s00425-009-0946-y

Wang, W., Pang, J., Zhang, F., Sun, L., Yang, L., Fu, T., et al. (2022). Salt-responsive transcriptome analysis of canola roots reveals candidate genes involved in the key metabolic pathway in response to salt stress. *Sci. Rep.* 12, 1666. doi: 10.1038/s41598-022-05700-2

Washitani, I., and Sato, S. (1977). Studies on function of proplastids in metabolism of invitro cultured tobacco cells. 2. glutamine synthetase glutamate synthetase pathway. *Plant Cell Physiol.* 18, 505–512. doi: 10.1093/oxfordjournals.pcp.a075546

Weeda, S., Zhang, N., Zhao, X., Ndip, G., Guo, Y., Buck, G. A., et al. (2014). Arabidopsis transcriptome analysis reveals key roles of melatonin in plant defense systems. *PloS One* 9 (3), e93462. doi: 10.1371/journal.pone.0093462

Wu, Y., Liao, W. B., Dawuda, M. M., Hu, L. L., and Yu, J. H. (2019). 5-aminolevulinic acid (ALA) biosynthetic and metabolic pathways and its role in higher plants: A review. *Plant Growth Regul.* 87, 357–374. doi: 10.1007/s10725-018-0463-8

Yan, F., Wei, H., Ding, Y., Li, W., Chen, L., Ding, C., et al. (2021). Melatonin enhances Na^+/K^+ homeostasis in rice seedlings under salt stress through increasing the root h^+-pump activity and Na^+/K^+ transporters sensitivity to ROS/RNS. *Environ. Exp. Bot.* 182, 104328. doi: 10.1016/j.envexpbot.2020.104328

Yan, F., Wei, H., Li, W., Liu, Z., Tang, S., Chen, L., et al. (2020). Melatonin improves k^+ and na^+ homeostasis in rice under salt stress by mediated nitric oxide. *Ecotoxicol Environ. Saf.* 206, 111358. doi: 10.1016/j.ecoenv.2020.111358

Yin, Z., Lu, J., Meng, S., Liu, Y., Mostafa, I., Qi, M., et al. (2019). Exogenous melatonin improves salt tolerance in tomato by regulating photosynthetic electron flux and the ascorbate–glutathione cycle. *J. Plant Interact.* 14 (1), 453–463. doi: 10.1080/17429145.2019.1645895

Zahedi, S. M., Hosseini, M. S., FahadiHoveizeh, N., Gholami, R., Abdelrahman, M., and Tran, L. -S. P. (2021). Exogenous melatonin mitigates salinity-induced damage in olive seedlings by modulating ion homeostasis, antioxidant defense and phytohormone balance. *Physiologia Plantarum.* doi: 10.1111/ppl.13589

Zhang, Y., Fan, Y., Rui, C., Zhang, H., Xu, N., Dai, M., et al. (2021). Melatonin improves cotton salt tolerance by regulating ROS scavenging system and Ca^{2+} signal transduction. *Front. Plant Sci.* 12, 693690. doi: 10.3389/fpls.2021.693690

Zhang, Y., Wang, L., Liu, Y., Zhang, Q., Wei, Q., and Zhang, W. (2006). Nitric oxide enhances salt tolerance in maize seedlings through increasing activities of proton-pump and Na^+/H^+ antiport in the tonoplast. *Planta* 224, 545–555. doi: 10.1007/s00425-006-0242-z

Zhao, D., Wang, H., Chen, S., Yu, D., and Reiter, R. J. (2021). Phytomelatonin: an emerging regulator of plant biotic stress resistance. *Trends Plant Sci.* 26, 70–82. doi: 10.1016/j.tplants.2020.08.009

Zhou, B., Guo, Z., Xing, J., and Huang, B. (2005). Nitric oxide is involved in abscisic acid induced antioxidant activities in *Stylosanthes guianensis*. *J. Exp. Bot.* 56, 3223–3228. doi: 10.1093/jxb/eri319

frontiers | Frontiers in Plant Science

TYPE Original Research
PUBLISHED 23 March 2023
DOI 10.3389/fpls.2023.1151722

OPEN ACCESS

EDITED BY
Vijay Gahlaut,
Chandigarh University, India

REVIEWED BY
Sanaullah Jalil,
Zhejiang University, China
Rana Roy,
Sylhet Agricultural University, Bangladesh

*CORRESPONDENCE
Faisal Zulfiqar
✉ ch.faisal.zulfiqar@gmail.com
Anam Moosa
✉ anammoosa1@outlook.com
Kadambot H. M Siddique
✉ kadambot.siddique@uwa.edu.au

SPECIALTY SECTION
This article was submitted to
Plant Abiotic Stress,
a section of the journal
Frontiers in Plant Science

RECEIVED 26 January 2023
ACCEPTED 09 March 2023
PUBLISHED 23 March 2023

CITATION
Zulfiqar F, Moosa A, Darras A, Nafees M, Ferrante A and Siddique KHM (2023) Preharvest melatonin foliar treatments enhance postharvest longevity of cut tuberose *via* altering physio-biochemical traits. *Front. Plant Sci.* 14:1151722. doi: 10.3389/fpls.2023.1151722

Preharvest melatonin foliar treatments enhance postharvest longevity of cut tuberose *via* altering physio-biochemical traits

Faisal Zulfiqar[1]*, Anam Moosa[2]*, Anastasios Darras[3], Muhammad Nafees[1], Antonio Ferrante[4] and Kadambot H. M. Siddique[5]*

[1]Department of Horticultural Sciences, Faculty of Agriculture and Environment, The Islamia University of Bahawalpur, Bahawalpur, Pakistan, [2]Department of Plant Pathology, Faculty of Agriculture and Environment, The Islamia University of Bahawalpur, Bahawalpur, Pakistan, [3]Department of Agriculture, University of the Peloponnese, Kalamata, Greece, [4]Department of Agricultural and Environmental Sciences, Università degli Studi di Milano, Milan, Italy, [5]The University of Western Australia (UWA) Institute of Agriculture, The University of Western Australia, Perth, WA, Australia

Introduction: Melatonin (MLT) is a bioactive molecule involved in the physiological functioning of plants. Reports related to preharvest applications of melatonin on the postharvest performance of cut flowers are not available in the literature.

Materials & methods: This study evaluated the effects of different concentrations of exogenous MLT [0 mM (MT0), 0.5 mM (MT1), 0.7 mM (MT2), 1 mM (MT3)] applied preharvest on the physiological characteristics and postharvest performance of cut tuberose, a globally demanded cut flower.

Results & discussion: The results revealed that all treatments increased postharvest vase life by up to 4 d. The MT1, MT2, and MT3 treatments increased total soluble proteins (TSP) by 25%, 41%, and 17%, soluble sugars (SS) by 21%, 36%, and 33%, an+d postharvest catalase (CAT) activity by 52%, 66%, and 70%, respectively. Malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) decreased in all preharvest treatments by up to 23% and 56%, respectively. Proline concentration decreased in all treatments, particularly MT3 (38%). These findings suggest that preharvest MLT treatment is a promising strategy for improving the postharvest quality of cut tuberose.

KEYWORDS

oxidative stress, postharvest, ornamental cut flower, soluble proteins, soluble sugars, antioxidants

Introduction

Tuberose (Asparagaceae) is a herbaceous perennial tropical and subtropical ornamental geophyte native to Mexico. Its tubular, sweet-scented white flowers are economically important as cut flowers, fragrance, and aromatic oil. The inflorescence of tuberose has a spike ranging from 90–120 cm, arranged in single or paired, waxy, highly fragrant flowers (Dole and Wilkins, 2005) that can be harvested for commercial purposes or used in landscape designs.

The vase life of cut flowers is a critical quality influencing profit margins for growers in national and international markets (Zulfiqar and Ashraf, 2022). After harvest, the metabolic activities of flowers remain active in cells, performing crucial processes using stored substrates in the tissues (Jhanji et al., 2023). Enhanced vase life and delayed senescence can be attained by maintaining carbohydrate levels and water absorption and ameliorating oxidative stress produced by excessive reactive oxygen species (ROS) generation (Zulfiqar and Ashraf, 2022).

Techniques for delaying the senescence of cut flowers can significantly increase their market potential since postharvest longevity is a crucial factor for cut flower value (Olsen et al., 2015). Various postharvest treatments using growth regulators, sugars, signaling molecules, and biostimulants can inhibit postharvest senescence and increase vase life (Zulfiqar et al., 2020). However, few studies have focused on preharvest applications of these substances to enhance the postharvest performance of cut flowers. Recently, preharvest applications of biostimulants and potassium enhanced postharvest performance of gladiolus and statice cut flowers by altering postharvest physiological conditions and mitigating oxidative stress during senescence (Zulfiqar et al., 2020; Khandan-Mirkohi et al., 2021; Zulfiqar and Ashraf, 2022).

Melatonin (MLT; N-acetyl-5-methoxytrytamine) is a multi-regulatory pleiotropic molecule involved in various physiological and cellular functions in response to biotic and abiotic stresses (Zhang et al., 2018; Arnao et al., 2022). Due to its antioxidant impact, MLT can prevent oxidative stress in plants (Altaf et al., 2021a; Altaf et al., 2021b; Altaf et al., 2022). Treatments with MLT extended the postharvest quality and shelf life of various horticultural products (Luo et al., 2020; Wang et al., 2020; Lin et al., 2022) by regulating gene expression and inducing antioxidant enzyme production (Zheng et al., 2019; Aghdam et al., 2021). However, MLT application for improving floricultural products is in its infancy. A recent study on cut carnations (*Dianthus caryophyllus* L) evaluated different MLT concentrations (0.01, 0.1, and 1 mM) added to the vase solution (Lezoul et al., 2022). The optimum concentration (0.1 mM) decreased senescence and increased vase life by up to 10 d compared to the untreated controls. The authors found that postharvest MLT treatments improved water relations, lowered metabolic rate, and maintained membrane stability due to antioxidant activity (Lezoul et al., 2022).

To date, no studies have investigated the effects of preharvest MLT applications on growth, vase life traits, and oxidative stress-related characteristics in tuberose plants. Therefore, we hypothesized that exogenous MLT improves postharvest performance and delays senescence in cut tuberose. We assessed the effect of different concentrations of foliar MLT applications on the growth and ornamental traits of tuberose plants and the association between photosynthesis and postharvest flower longevity and enzyme activities that reduce oxidative stress during senescence.

Materials and methods

Experimental site and plant material

An outdoor pot trial was established in the summer of 2022 at the Floriculture Research Area of the Islamia University of Bahawalpur, Pakistan (lat. 29° 23’ 44.5956” N, long.71° 41’ 0.0024” E). The desert region of Bahawalpur is in the subtropical zone, associated with hot summers (March–August) and mild winters (December–February). The physio-chemical traits of the experimental soil were: sandy clay loam (sand 45%, silt 24%, clay 31%), pH 7.4, 2.78 dSm^{-1} electrical conductivity, and 4.04 $cmol_c$ kg^{-1} cation exchange capacity. Soil nutrients were: nitrogen (N), 79 g kg^{-1} soil; phosphorus (P), 9.03 g kg^{-1} soil; potassium (K), 152.54 g kg^{-1} soil. The soil was air-dried, ground, and sieved (2 mm pore size) ahead of filling 3 L earthen pots (19 cm and 13 cm top and base diameters, respectively).

Healthy, uniform tuberose bulbs of cv. Single (21–23 mm diameter) were acquired from a local supplier in Lahore, Pakistan. One tuberose bulb was planted per pot. There were ten replicate bulbs for each of the four treatments and four replications (total 160 plants). Basal N, P, and K fertilizers (6 g pot^{-1}) were applied manually using 46% urea, 50% muriate of potash (Fauji Fertilizer Company Limited, Pakistan), and 18% single super phosphate (Safi Chemicals and Fertilizer (PVT) Limited, Multan, Pakistan). Second and third applications of these fertilizers at 25 d and 40 d after planting. The experiment had a completely randomized design with four treatments: (1) distilled water used as the control (MT0), (2) 0.05 mM MLT (MT1), (3) 0.07 mM MLT (MT2), and (4) 1 mM MLT (MT3). The treatments were applied in the middle of the growing cycle and 5 d before inflorescence harvest by manually spraying the leaves until run-off. Before each foliar spray application, the top of each pot was concealed with polyethylene sheeting to avoid contamination. Watering was done manually every four days until harvest.

Leaf gas exchange

Leaf gas exchange traits [net CO_2 assimilation (A_s), and transpiration (E)] were measured at the onset of the flowering bud occurrence stage between 7.00 am and 8.00 am on three fully expanded leaf blades using an infrared gas analyzer (LI-COR 6400, LI-COR, Lincoln, NE, USA) at 400 µmol m^{-2} s^{-1} CO_2 and flow rate of 300 µmol m^{-2} s^{-1} on eight plants per treatment. At the same time, chlorophyll SPAD values were recorded on the lower, middle, and tip parts of four fully expanded tuberose plant leaves.

Harvest and vase life

At the initiation of the inflorescence maturity stage, uniform size and quality inflorescences were cut manually using a sterilized knife in the early morning (7:00 am to 8:00 am), placed vertically in a bucket half-filled with distilled water and transferred to the laboratory within 30 min. In laboratory, the inflorescences were re-cut at 85 cm length under running distilled water to revert vascular system blockage and air emboli. Individual inflorescences were placed into 200 mL glass vases containing deionized distilled water. The vases were covered with aluminum foil to reduce vase water evaporation and placed on laboratory bench at 26 ± 3°C, 65 ± 3% relative humidity, 12 h light period provided by white fluorescent lamps, and 12 h dark period. Ten cut inflorescences per treatment were kept for vase life determination. Vase life expiration was calculated as the number of days from harvest until the flower petals wilted and lost their visual aesthetic by color change and/or loss of turgidity. Data were documented daily for 15 d.

Soluble sugar (SS) and total soluble protein (TSP) contents in leaves

The SS content (g kg^{-1}) in 0.5 g tuberose leaves was measured 5 d before inflorescence harvest following the methodology of Frohlich and Kutscherah (1995), with absorbance measured at 620 nm. The TSP content (g kg^{-1}; fresh weight basis) of tuberose leaves was determined following the methodology of Bradford (1976).

Hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) contents

Cut flower florets (0.5 g) of the inflorescence were taken on day 5 postharvest to assess H_2O_2 and MDA contents following the methodologies of Patterson et al. (1984) and Hodges et al. (1999), respectively.

Superoxide dismutase (SOD) and catalase (CAT) activities

Fresh floret samples (0.5 g) were collected from the inflorescence on day 5 postharvest to assess SOD and CAT activities in stored supernatant following the methodologies of van Rossum et al. (1997) and Chance and Maehly (1955), respectively.

Proline content

The ninhydrin-oriented method was used to measure leaf proline concentration (Bates et al., 1973), with absorbance read at 520 nm.

Experimental lay-out and statistical analysis

Experiments were conducted in a CRD with MLT treatments as the only factor and were replicated four times. Data were subjected to one-way ANOVA using SPSS v. 21 (SPSS Inc., Chicago, IL, USA). Comparisons between treatment means were carried out using the LSD multiple range test at P = 0.05. Linear regression analysis (y = ax+b) was performed in Sigmaplot 10 (Systat software Inc. USA) to highlight the dynamic trends in MLT effectiveness.

Results

Leaf gas exchange and chlorophyll content

MLT did not affect leaf gas exchange and chlorophyll content. All differences between treatment means were not significant at P = 0.05 (Figure 1). Slight increases in *As* and *E* values were recorded compared to the untreated control plants. For example, plants treated with MT2 had *As* value of 4.28 µmol m^{-2}. sec, whereas the control plants had 3.03 µmol m^{-2}. sec (Figure 1A). Likewise, E values of the MT2 treated plants averaged at 0.97 mmol m^{-2}. sec, whereas the control plants averaged at 0.68 mmol m^{-2}. sec (Figure 1B). SPAD values of the MT2 treated plants reached 14.99, whereas the untreated control plants 12.41 (Figure 1C).

Vase life

Vase life increased only in MT3 treated plants (Figure 2). Plants treated with MT3 produced inflorescences with the longest VL of 9.6 d (increase by 41%), compared to the 5.6 d recorded for the control inflorescences (Figure 2).

Total soluble protein (TSP), soluble sugars (SS), and proline contents in leaves

Plants treated with MT2 showed a significant increase in TSP and SS (Figures 3A, B). MT2 treated plants had a TSP mean value of 0.42 g kg^{-1}, whereas the untreated controls averaged at 0.33 g kg^{-1} (Figure 3A). That was an average increase of 41%. Likewise, MT2 treated plants had a SS mean value of 4.59 g kg^{-1}, whereas the untreated controls averaged at 3.26 g kg^{-1} (Figure 3B). This increase was by up to 41% between the MT2-treated and the untreated plants. Additionally, the proline content in MT2-treated plants was significantly reduced by up to 78%, compared to the controls (Figure 3C). The MT2-treated plants had a proline content of 22.4 µmol g^{-1} FW, whereas the untreated control plants averaged at 39.8 µmol g^{-1} FW (Figure 3C).

Hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) contents

H_2O_2 and MDA contents were generally reduced by MLT treatments (Figure 4). H_2O_2 was significantly reduced in MT3 plants by up to 56% (Figure 4A). MT3-treated plants had a H_2O_2 content of

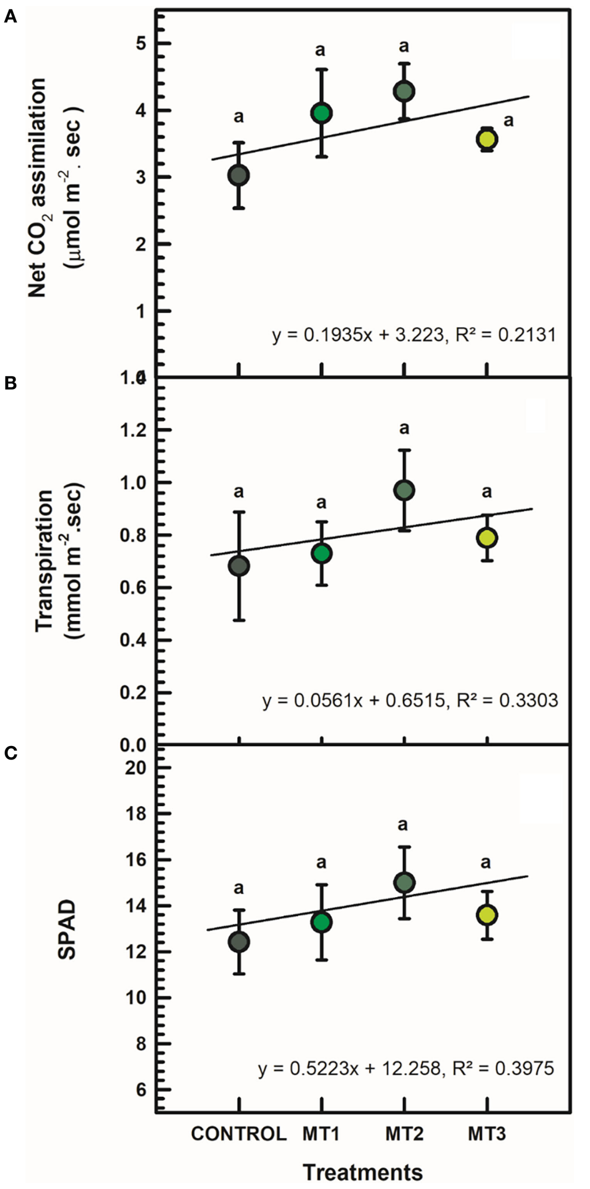


FIGURE 1
Net CO_2 assimilation (**A**; µmol m^{-2} sec), transpiration (**B**; mmol m^{-2} sec), and SPAD **(C)** of tuberose plants treated with preharvest foliar applications of distilled water (control; MT0) and three concentrations of melatonin (MLT): 0.05 mM (MT1), 0.07 mM (MT2), and 1 mM (MT3). Data are means ± SE (n = 40). Different letters above the bars indicate significant differences according to the LSD test at P = 0.05.

12 mmol kg^{-1}, whereas the untreated control had 18.8 mmol kg^{-1}. Furthermore, the MT2-treated plants showed a significantly reduced MDA content by 23% compared to the controls (Figure 4B). MT2-treated plants showed an average of 107.6 mmol kg^{-1} MDA, whereas the untreated control 132.3 mmol kg^{-1}.

Superoxide dismutase (SOD) and catalase (CAT) activities

SOD and CAT activities were significantly induced by MLT only in certain cases (Figure 5). SOD was significantly increased by up to 54% in the MT2-treated plants (Figure 5A). SOD in MT2-treated plants was 0.21 units mg^{-1} protein and 0.01 units mg^{-1} protein in the untreated control plants (Figure 5A). CAT activity was increase by up to 70% in the MT3-treated plants (Figure 5B). CAT in MT3-treated plants was 32.5 units mg^{-1} protein and 9.6 units mg^{-1} protein in the untreated controls (Figure 5B).

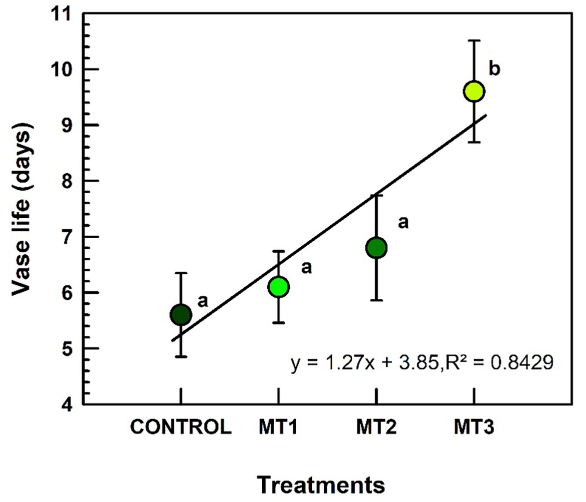


FIGURE 2
Vase life (d) of tuberose flowers harvested from the plants treated with preharvest foliar applications of distilled water (control; MT0) and three concentrations of melatonin (MLT): 0.05 mM (MT1), 0.07 mM (MT2), and 1 mM (MT3). Data are means ± SE (n = 40). Different letters above the bars indicate significant differences according to the LSD test at P = 0.05.

Discussion

Numerous studies have discovered that endogenous MLT content is involved in floral senescence. However, among different flower species, MLT levels appear to decline during development, particularly at later phases of senescence (Murch et al., 2009; Zhao et al., 2017). In the current study, preharvest MLT treatments enhanced physiological traits and had an anti-senescent effect on tuberose cut flowers, elongating vase life, particularly in MT3. The increased vase life was associated with improved biochemical characteristics, as the MLT treatments controlled antioxidant defenses postharvest for longer than the control.

Photosynthesis is the primary process for harnessing light energy to manufacture carbohydrates, and is closely linked to plant growth. The MLT treatments enhanced photosynthetic activity in tuberose, evident in the leaf gas exchange properties (Figure 1A), and in line with similar studies on cotton (Khattak et al., 2022) and tomato (Altaf et al., 2022). In contrast, Zhao et al. (2021) reported that MLT treatments did not affect leaf gas exchange in maize (*Zea mays*) under normal conditions. Furthermore, MLT has a protective impact on chlorophyll (Campos et al., 2019; Li et al., 2021; Altaf et al., 2022). The MLT-treated tuberose plants had more chlorophyll than control plants (Figure 1C), showing that exogenous MLT inhibits photosynthetic

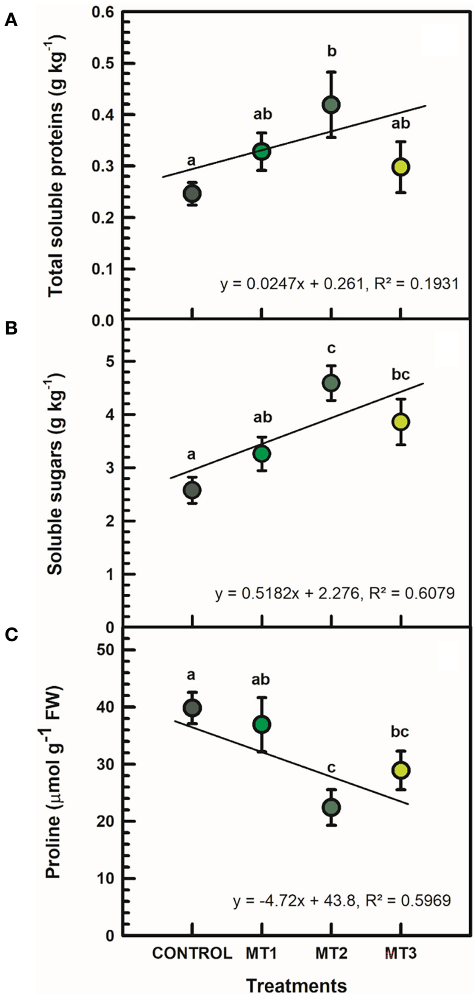


FIGURE 3
Total soluble protein (TSP) (**A**; g kg-1), soluble sugars (SS) (**B**; g kg-1), and proline contents (**C**; mmol g-1 FW) in leaves of tuberose plants treated with preharvest foliar applications of distilled water (control; MT0) and three concentrations of melatonin (MLT): 0.05 mM (MT1), 0.07 mM (MT2), and 1 mM (MT3). Data are means ± SE (n = 40). Different letters above the bars indicate significant differences according to the LSD test at P = 0.05.

machinery damage. An increased photosynthetic capability provides plants with more energy, allowing them to withstand stressors like postharvest stress (Fan et al., 2015).

This study is the first to investigate the effect of preharvest MLT on the vase life of cut tuberose flowers. The prolonged vase life with preharvest MLT is likely related to enhanced photosynthesis, soluble sugars and antixodant defense system. In addition, the increased protein content and antioxidant activity with preharvest MLT may have reduced the oxidative damage in tuberose tissues, extending the vase life. At the highest dose (MT3), this cost-effective preharvest treatment could benefit cut flower sellers, prolonging tuberose vase life. Lezoul et al. (2022) reported that MLT improved the vase life of carnation due to its antioxidative potential. Our results showed improved vase life, which also shows that MLT mitigates oxidative stress, as reported by Mazrou et al. (2022).

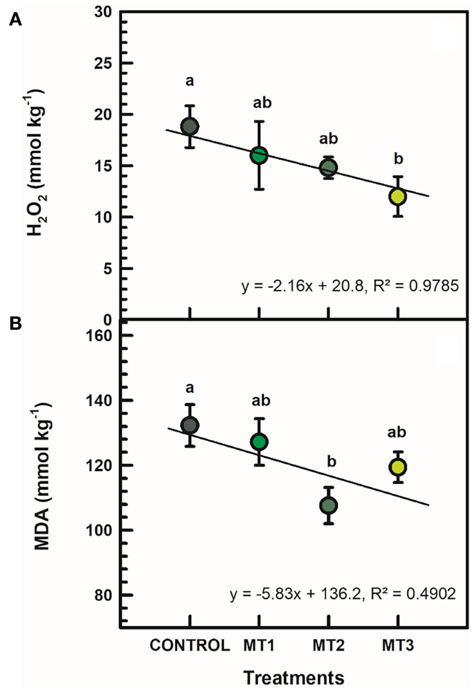


FIGURE 4
Hydrogen peroxide (H2O2) (**A**; mmol kg-1) and malondialdehyde (MDA) (**B**; mmol kg-1) contents in flowers of tuberose plants treated with preharvest foliar applications of distilled water (control; MT0) and three concentrations of melatonin (MLT): 0.05 mM (MT1), 0.07 mM (MT2), and 1 mM (MT3). Data are means ± SE (n = 40). Different letters above the bars indicate significant differences according to the LSD test at P = 0.05.

The water balance in petals is crucial for extending the vase life of cut flowers. Numerous investigations have revealed a strong connection between water balance and the capacity of cut flowers for osmotic adjustment (Hou et al., 2018; Zheng and Guo, 2019; Lu et al., 2020). In plants, the levels of osmolytes, such as soluble proteins, soluble sugars, and proline, are closely linked with the ability to modify osmotic pressure. The data increasingly indicates that exogenous substances could improve water balance by controlling osmolyte concentrations (Shan and Zhao, 2015). Shan and Zhao (2015) demonstrated that lanthanum improved water balance in *Lilium longiflorum* cut flowers by increasing soluble protein, soluble sugar, and proline contents, further enhancing the relative water content of the petals and extending vase life. We found that MLT increased soluble protein and sugar contents in tuberose leaves, consistent with Xing et al. (2021) for chrysanthemum seedlings. Proline, an osmotic adjustment chemical, in addition to the cell's antioxidant system, participates in the defense mechanism against adverse situations (Zulfiqar and Ashraf, 2022). Proline is a non-polar amino acid renowned for its numerous and significant roles in plant metabolism, particularly in response to biotic and abiotic stresses (Zulfiqar and Ashraf, 2022). Under stress, proline functions as a signaling molecule, compatible osmolyte, non-enzymatic antioxidant, molecular chaperone, and energy provider (Szepesi and Szőllősi, 2018). Studies have shown that proline content plays a vital role in the postharvest performance of cut flowers (Aghdam et al., 2019; Sukpitak and

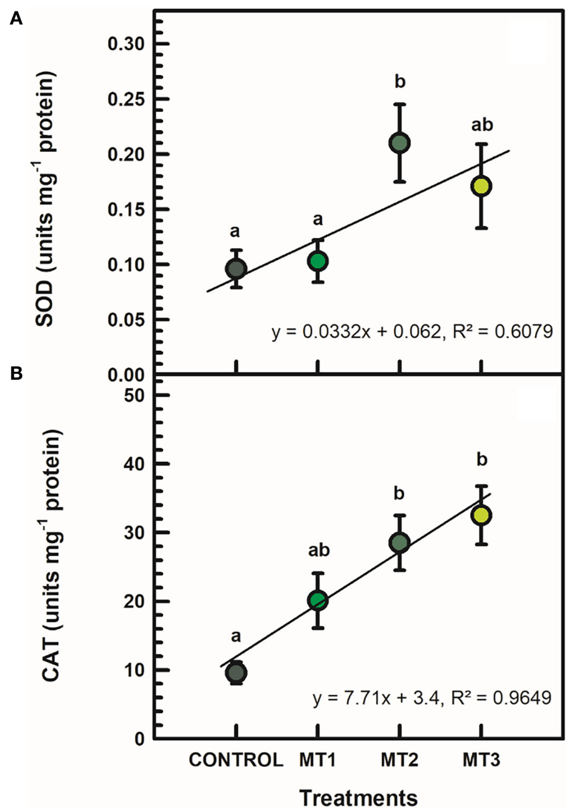


FIGURE 5
Superoxide dismutase (SOD) (**A**; units mg^{-1} protein) and catalase (CAT) (**B**; units mg^{-1} protein)activities in flowers of tuberose plants treated with preharvest foliar applications of distilled water (control; MT0) and three concentrations of melatonin (MLT): 0.05 mM (MT1), 0.07 mM (MT2), and 1 mM (MT3). Data are means ± SE (n = 40). Different letters above the bars indicate significant differences according to the LSD test at P = 0.05.

Seraypheap, 2023; Zeng et al., 2023). For example, sucrose application to rose cut flowers prolonged vase life, compared to non-treated cut flowers, which was associated with proline content (Zeng et al., 2023).

In the current study, the MLT treatments decreased MDA content, reflecting a decrease in lipid peroxidation in cut flowers, hence maintaining membrane integrity. Other studies have shown that MLT reduces lipid peroxidation while preserving the membrane stability index (Hassan et al., 2020; Zulfiqar and Ashraf, 2022).

Antioxidant enzymes such as SOD and CAT are the most important defense enzymes for ROS detoxification in plant tissues (Hasanuzzaman et al., 2020; Zulfiqar and Ashraf, 2021). The MLT treatments, especially at 0.07 and 1 mM, increased SOD and CAT activities in tuberose cut flowers. Horticultural commodities, including cut flowers, have increased antioxidant enzyme activity, reducing lipid peroxidation and H_2O_2 concentration during their postharvest (Lezoul et al., 2022; Mazrou et al., 2022). Several studies have demonstrated that MLT treatments increased antioxidant enzyme activities (e.g., SOD and CAT) in horticultural produce (Sharafi et al., 2021; Mazrou et al., 2022). In the current study, the improved antioxidant activities with MLT were related to decreased oxidative stress, as indicated by the reductions in H_2O_2 and MDA.

Conclusion

The 0.07 and 1 mM preharvest MLT treatments were the most effective in delaying the senescence of tuberose cut flowers by improving leaf gas exchange, and TSP and soluble sugar contents and decreasing proline, H_2O_2, and MDA contents. Furthermore, these treatments increased SOD and CAT activities, decreasing oxidative stress. Preharvest MLT treatments at specific concentrations could prolong the vase life of tuberose cut inflorescences.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

FZ and AM designed and executed the experiments. All authors contributed on the writing, editing and revision of the manuscript. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

Aghdam, M. S., Jannatizadeh, A., Nojadeh, M. S., and Ebrahimzadeh, A. (2019). Exogenous melatonin ameliorates chilling injury in cut anthurium flowers during low temperature storage. *Postharvest Biol. Technol.* 148, 184–191. doi: 10.1016/j.postharvbio.2018.11.008

Aghdam, M. S., Mukherjee, S., Flores, F. B., Arnao, M. B., Luo, Z., and Corpas, F. J. (2021). Functions of melatonin during postharvest of horticultural crops. *Plant Cell Physiol.* 63 (12), 1764–1786. doi: 10.1016/j.tifs.2021.07.034

Altaf, M. A., Shahid, R., Ren, M. X., Altaf, M. M., Khan, L. U., Shahid, S., et al. (2021a). Melatonin alleviates salt damage in tomato seedling: a root architecture system, photosynthetic capacity, ion homeostasis, and antioxidant enzymes analysis. *Sci. Hortic.* 285, 110145. doi: 10.1016/j.scienta.2021.110145

Altaf, M. A., Shahid, R., Ren, M. X., Mora-Poblete, F., Arnao, M. B., Naz, S., et al. (2021b). Phytomelatonin: An overview of the importance and mediating functions of melatonin against environmental stresses. *Physiol. Plant* 172 (2), 820–846. doi: 10.1111/ppl.13262

Altaf, M. A., Shahid, R., Ren, M.-X., Naz, S., Altaf, M. M., Khan, L. U., et al. (2022). Melatonin improves drought stress tolerance of tomato by modulating plant growth, root architecture, photosynthesis, and antioxidant defense system. *Antioxidants.* 11 (2), 309. doi: 10.3390/antiox11020309

Arnao, M. B., Cano, A., and Hernández-Ruiz, J. (2022). Phytomelatonin: An unexpected molecule with amazing performances in plants. *J. Exp. Bot.* 73, 5779–5800. doi: 10.1093/jxb/erac009

Bates, L. S., Waldren, R. P., and Teare, I. D. (1973). Rapid determination of free proline for water-stress studies. *Plant Soil.* 39, 205–207. doi: 10.1007/BF00018060

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72, 248–254. doi: 10.1016/0003-2697(76)90527-3

Campos, C. N., Avila, R. G., de Souza, K. R. D., Azevedo, L. M., and Alves, J. D. (2019). Melatonin reduces oxidative stress and promotes drought tolerance in young *Coffea arabica* l. plants. agric. *Water Manage.* 211, 37–47. doi: 10.1016/j.agwat.2018.09.025

Chance, B., and Maehly, A. C. (1955). Assay of catalases and peroxidases. *Methods Enzymol.* 2, 764–385 775. doi: 10.1002/9780470110171.ch14

Dole, J., and Wilkins, H. (2005). *Floriculture: Principles and species, prentice-hall, upper saddle river, NJ, USA* Pearson, NJ, USA.

Fan, H. M., Li, T., Sun, X., Sun, X. Z., and Zheng, C. S. (2015). Effects of humic acid derived from sediments on the postharvest vase life extension in cut chrysanthemum flowers. *Postharvest Biol. Technol.* 101, 82–87. doi: 10.1016/j.postharvbio.2014.09.019

Frohlich, M., and Kutscherah, U. (1995). Changes in soluble sugars and proteins during.Development of rye coleoptiles. *J. Plant Physiol.* 146, 121–125. doi: 10.1016/S0176-1617(11)81977-2

Hasanuzzaman, M., Bhuyan, M. B., Zulfiqar, F., Raza, A., Moshin, S. M., Mahmud, J. A., et al. (2020). Reactive oxygen species and antioxidant defense in plants under abiotic stress: Revisiting the crucial role of a universal defense regulator. *Antioxidants* 9 (8), 681. doi: 10.3390/antiox9080681

Hassan, F., Ali, E., and Mazrou, R. (2020). Involvement of ethylene synthetic inhibitors in regulating the senescence of cut carnations through membrane integrity maintenance. *J. Hortic. Res.* 28, 39–48. doi: 10.2478/johr-2020-0010

Hodges, D. M., DeLong, J. M., Forney, C. F., and Prange, R. K. (1999). Improving the thiobarbituric acid-reactive-substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds. *Planta.* 207, 604–611. doi: 10.1007/s004250050524

Hou, K., Bao, D., and Shan, C. (2018). Cerium improves the vase life of lilium longiflorum cut flowers through ascorbate-glutathione cycle and osmoregulation in the petals. *Sci. Hortic.* 227, 142–145. doi: 10.1016/j.scienta.2017.09.040

Jhanji, S., Kaur, G., Kaur, R., and Dhatt, U. K. (2023). Physiological and biochemical changes during flower development and senescence in chrysanthemum and gladiolus. *Acta Physiol. Plant* 45 (1), 1–13. doi: 10.1007/s11738-022-03486-4

Khandan-Mirkohi, A., Pirgazi, R., Taheri, M. R., Ajdanian, L., Babaei, M., Jozay, M., et al. (2021). Effects of salicylic acid and humic material preharvest treatments on postharvest physiological properties of statice cut flowers. *Sci. Hortic.* 283, 110009. doi: 10.1016/j.scienta.2021.110009

Khattak, W. A., He, J., Abdalmegeed, D., Hu, W., Wang, Y., and Zhou, Z. (2022). Foliar melatonin stimulates cotton boll distribution characteristics by modifying leaf sugar metabolism and antioxidant activities during drought conditions. *Physiol. Plant* 174 (1), e13526. doi: 10.1111/ppl.13526

Lezoul, N. E. H., Serrano, M., Ruiz-Aracil, M. C., Belkadi, M., Castillo, S., Valero, D., et al. (2022). Melatonin as a new postharvest treatment for increasing cut carnation (*Dianthus caryophyllus* l.) vase life. *Postharvest Biol. Technol.* 184, 111759. doi: 10.1016/j.postharvbio.2021.111759

Li, Z., Su, X. Y., Chen, Y. L., Fan, X. C., He, L. Z., Guo, J. M., et al. (2021). Melatonin improves drought resistance in maize seedlings by enhancing the antioxidant system and regulating abscisic acid metabolism to maintain stomatal opening under PEG-induced drought. *J. Plant Biol.* 64, 299–312. doi: 10.1007/s12374-021-09297-3

Lin, Y., Zhan, L., Shao, P., and Sun, P. (2022). Phase-change materials and exogenous melatonin treatment alleviated postharvest senescence of *Agaricus bisporus* by inhibiting browning and maintaining cell membrane integrity. *Postharvest Biol. Technol.* 192, 112009. doi: 10.1016/j.postharvbio.2022.112009

Lu, N., Wu, L., and Shi, M. (2020). Selenium enhances the vase life of *Lilium longiflorum* cut flower by regulating postharvest physiological characteristics. *Sci. Hortic.* 264, 109172. doi: 10.1016/j.scienta.2019.109172

Luo, S., Hu, H., Wang, Y., Zhou, H., and Li, P. (2020). The role of melatonin in alleviating the postharvest browning of lotus seeds through energy metabolism and membrane lipid metabolism. *Postharvest Biol. Technol.* 167, 111243. doi: 10.1016/j.postharvbio.2020.111243

Mazrou, R. M., Hassan, S., Yang, M., and Hassan, F. A. (2022). Melatonin preserves the postharvest quality of cut roses through enhancing the antioxidant system. *Plants.* 11 (20), 2713. doi: 10.3390/plants11202713

Murch, S. J., Alan, A. R., Cao, J., and Saxena, P. K. (2009). Melatonin and serotonin in flowers and fruits of *Datura metel* l. J. *Pineal Res.* 47 (3), 277–283. doi: 10.1111/j.1600-079X.2009.00711.x

Olsen, A., Lütken, H., Hegelund, J. N., and Müller, R. (2015). Ethylene resistance in flowering ornamental plants–improvements and future perspectives. *Hortic. Res.* 2, 15038. doi: 10.1038/hortres.2015.38

Patterson, B. D., MacRae, E. A., and Ferguson, I. B. (1984). Estimation of hydrogen peroxide in plant extracts using titanium (IV). *Analytical Biochem.* 139, 487–492. doi: 10.1016/0003-2697(84)90039-3

Shan, C., and Zhao, X. (2015). Lanthanum delays the senescence of *Lilium longiflorum* cut flowers by improving antioxidant defense system and water retaining capacity. *Sci. Hortic.* 197, 516–520. doi: 10.1016/j.scienta.2015.10.012

Sharafi, Y., Jannatizadeh, A., Fard, J. R., and Aghdam, M. S. (2021). Melatonin treatment delays senescence and improves antioxidant potential of sweet cherry fruits during cold storage. *Sci. Hortic.* 288, 110304. doi: 10.1016/j.scienta.2021.110304

Sukpitak, C., and Seraypheap, K. (2023). Postharvest transient water deficit limits longevity of cut dendrobium 'Khao sanan'orchid. *Sci. Hortic.* 309, 111637. doi: 10.1016/j.scienta.2022.111637

Szepesi, Á., and Szőllősi, R. (2018). *Mechanism of proline biosynthesis and role of proline metabolism enzymes under environmental stress in plants. in: Plant metabolites and regulation under environmental stress* (USA: Academic Press), 337–353.

van Rossum, M. W. P. C., Alberda, M., and van der Plas, L. H. W. (1997). Role of oxidative damage in tulip bulb scale micropropagation. *Plant Sci.* 130, 207–216. doi: 10.1016/S0168-9452(97)00215-X

Wang, S. Y., Shi, X. C., Wang, R., Wang, H. L., Liu, F., and Laborda, P. (2020). Melatonin in fruit production and postharvest preservation: A review. *Food Chem.* 320, 126642. doi: 10.1016/j.foodchem.2020.126642

Xing, X., Ding, Y., Jin, J., Song, A., Chen, S., Chen, F., et al. (2021). Physiological and transcripts analyses reveal the mechanism by which melatonin alleviates heat stress in chrysanthemum seedlings. *Front. Plant Sci.* 12. doi: 10.3389/fpls.2021.673236

Zeng, F., Xu, S., Geng, X., Hu, C., and Zheng, F. (2023). Sucrose+ 8-HQC improves the postharvest quality of lily and rose cut flowers by regulating ROS-scavenging systems and ethylene release. *Sci. Hortic.* 308, 111550. doi: 10.1016/j.scienta.2022.111550

Zhang, Y., Huber, D. J., Hu, M., Jiang, G., Gao, Z., Xu, X., et al. (2018). Delay of postharvest browning in litchi fruit by melatonin *via* the enhancing of antioxidative processes and oxidation repair. *J. Agric. Food Chem.* 66, 7475–7484. doi: 10.1021/acs.jafc.8b01922

Zhao, C., Guo, H., Wang, J., Wang, Y., and Zhang, R. (2021). Melatonin enhances drought tolerance by regulating leaf stomatal behavior, carbon and nitrogen metabolism, and related gene expression in maize plants. *Front. Plant Sci.* 12. doi: 10.3389/fpls.2021.779382

Zhao, D., Wang, R., Meng, J., Zhiyuan, Li, Wu, Y., and Tao, J. (2017). Ameliorative effects of melatonin on dark-induced leaf senescence in gardenia (*Gardenia jasminoides* ellis): leaf morphology, anatomy, physiology and transcriptome. *Sci. Rep.* 7, 10423. doi: 10.1038/s41598-017-10799-9

Zheng, M., and Guo, Y. (2019). Effects of neodymium on the vase life and physiological characteristics of *Lilium casa* blanca petals. *Sci. Hortic.* 256, 108553. doi: 10.1016/j.scienta.2019.108553

Zheng, H., Liu, W., Liu, S., Liu, C., and Zheng, L. (2019). Effects of melatonin treatment on the enzymatic browning and nutritional quality of fresh-cut pear fruit. *Food Chem.* 299, 125111–125116. doi: 10.1016/j.foodchem.2019.125116

Zulfiqar, F., and Ashraf, M. (2021). Bioregulators: Unlocking their potential role in regulation of the plant oxidative defense system. *Plant Mol. Biol.* 105, 11–41. doi: 10.1007/s11103-020-01077-w

Zulfiqar, F., and Ashraf, M. (2022). Proline alleviates abiotic stress induced oxidative stress in plants. *J. Plant Growth Regul.*, 1–23. doi: 10.1007/s00344-022-10839-3

Zulfiqar, F., Younis, A., Finnegan, P. M., and Ferrante, A. (2020). Comparison of soaking corms with moringa leaf extract alone or in combination with synthetic plant growth regulators on the growth, physiology and vase life of sword lily. *Plants.* 9 (11), 1590. doi: 10.3390/plants9111590

frontiers | Frontiers in Plant Science

TYPE Review
PUBLISHED 23 March 2023
DOI 10.3389/fpls.2023.1140803

Melatonin: Current status and future perspectives in horticultural plants

Jing Zhao* and Junjie Hu

School of Agricultural, Jilin Agricultural Science and Technology University, Jilin, China

OPEN ACCESS

EDITED BY
Safina Naz,
Bahauddin Zakariya University, Pakistan

REVIEWED BY
Iqra Noor,
Huazhong Agricultural University, China
Rana Roy,
Sylhet Agricultural University, Bangladesh
Sami Abou Fayssal,
University of Forestry, Sofia, Bulgaria

*CORRESPONDENCE
Jing Zhao
✉ zhaojing9951@sina.com

SPECIALTY SECTION
This article was submitted to
Plant Abiotic Stress,
a section of the journal
Frontiers in Plant Science

RECEIVED 09 January 2023
ACCEPTED 01 February 2023
PUBLISHED 23 March 2023

CITATION
Zhao J and Hu J (2023) Melatonin: Current status and future perspectives in horticultural plants.
Front. Plant Sci. 14:1140803.
doi: 10.3389/fpls.2023.1140803

Global warming in this century increases incidences of various abiotic stresses, restricting plant growth and productivity and posing a severe threat to global food production and security. Different phytohormones are produced by plants to mitigate the adverse effects of these stresses. One such phytohormone is melatonin (MEL), which, being a potential bio-stimulator, helps to govern a wide array of functions in horticultural crops. Recent advancements have determined the role of MEL in plants' responses to abiotic stresses. MEL enhances physiological functions such as seed germination, growth and development, seedling growth, root system architecture, and photosynthetic efficiency. The potential function of MEL in stressful environments is to regulate the enzymatic and non-enzymatic antioxidant activity, thus playing a role in the substantial scavenging of reactive oxygen species (ROS). Additionally, MEL, as a plant growth regulator and bio-stimulator, aids in promoting plant tolerance to abiotic stress, mainly through improvements in nutrient uptake, osmolyte production, and cellular membrane stability. This review, therefore, focuses on the possible functions of MEL in the induction of different abiotic stresses in horticultural crops. Therefore, this review would help readers learn more about MEL in altered environments and provide new suggestions on how this knowledge could be used to develop stress tolerance.

KEYWORDS

root architecture, ion homeostasis, melatonin, redox balance, horticultural crops

Introduction

Plants, being sessile organisms, face a variety of environmental stresses (low and high temperature, metal stress, salinity, and drought stress) (Hassan et al., 2022), which have detrimental impacts on their performance in terms of growth and development (Rasheed et al., 2021; Altaf et al., 2023). It has been projected that about 90% of arable land is susceptible to one of the above-mentioned stresses (Dos Reis et al., 2012). Due to the devastating impact on the growth and productivity of agricultural crops, global attention has been diverted to these abiotic stresses. Various developmental functions and processes of plants, including morphological, physiological, and biochemical, are disrupted by these abiotic stresses (Marino, 2021; Arnao et al., 2023). Further, environmental stresses cause significant yield losses through excessive production of reactive oxygen species (ROS), nutrient deficiencies, decrease in photosynthetic efficiency, reduction in root growth, and osmolyte over-accumulation (Figure 1) (Ayyaz et al., 2022; Imran et al., 2022). With the ongoing changes in climate, these abiotic stresses are getting intensified, thus calling for the need for appropriate controlling measures (Gao et al., 2007; Andreotti, 2020; Shahid et al., 2021). In horticultural crops such as tomato, potato, pepper, and cucumber, around 70% of total yield losses are due to effects caused by environmental stresses at different growth phases (Martinez et al., 2018; Zörb et al., 2018). To promote sustainable agriculture, different management strategies have been introduced for achieving the targets (Ahammed and Li, 2023). Such techniques include plant growth regulators, different osmolyte syntheses, and accumulation to protect against stress-induced damages for maintaining cellular homoeostasis and optimum plant growth (Nawaz et al., 2017; Koza et al., 2022; Peng et al., 2023).

One of the essential plant growth regulators in stressed environments is melatonin (MEL), which is a small molecule acting as a powerful antioxidant, thus enhancing the stress resistance of plants against many environmental stressors (Hoque et al., 2021) (Table 1). This pleotropic molecule is found in various plant parts of several plant species, such as broccoli, coriander, mango, cabbage, tobacco, cucumber, and orange (Badria, 2002; Posmyk et al., 2009; Johns et al., 2013; Aguilera et al., 2015). MEL is also involved in the regulation of seed germination, seedling growth, photosynthetic efficiency, root system architecture, leaf senescence, fruit ripening, stomatal opening, and redox homeostasis (Figure 2) (Jan et al., 2022). Further, MEL has been well defined as an anti-stress promoter and growth bio-stimulator for horticultural plants, particularly in adverse environmental situations, such as cold, heat, heavy metals, salinity, drought, acidic rain, and UV stress (Sharif et al., 2018; Aghdam et al., 2021; Wu et al., 2021; Zhao et al., 2022). Plants are protected against stressful environments by the regulation of gene expressions mediated by MEL, such as the plants' "antioxidant defense system" activation (Jahan et al., 2020) which places MEL among vital bio-stimulants to improve crop productivity in stress conditions. In stressful environments, MEL helps to trigger the antioxidant defense system, which favors the scavenging of ROS and thus acts as a stress protector (Moustafa-Farag et al., 2020). Due to this function, MEL is a promising molecule that can be applied exogenously to alleviate stress. The current review aims to explore the biochemical and physiological functioning of MEL in abiotic stress environments, along with its possible mechanism of action. Further, the future aspect of MEL-regulated stress tolerance of horticultural crops is also discussed for a detailed overview of the research.

Function of melatonin in horticultural crops

MEL promotes plant growth and development *via* different functions, most of which are related to different abiotic stressors such as drought, temperature fluctuation, heavy metals, and salinity (Arnao and Hernández-Ruiz, 2006; Altaf et al., 2022a). MEL functions as a growth regulator, bio-stimulator, and potential antioxidant compound (Arnao and Hernández-Ruiz, 2014) (Figure 3). A primary function attributed to MEL in plants is to act as an antioxidant, providing protection against environmental agents (Nawaz et al., 2016; Tiwari et al., 2022). However, one important function of MEL may be the scavenging of free radicals, thereby protecting plants against oxidative damage (Paredes et al., 2009). MEL is significantly involved in the process of leaf senescence (Arnao and Hernández-Ruiz, 2009). A range of different functions of MEL have been investigated in horticultural plants, some more thoroughly than others, but in all cases the data are scarce (Table 2).

Melatonin as abiotic stress regulator in horticultural crops

Salinity

Salinity has been declared a significant hazard in modern horticulture, as it impairs and inhibits the growth and development

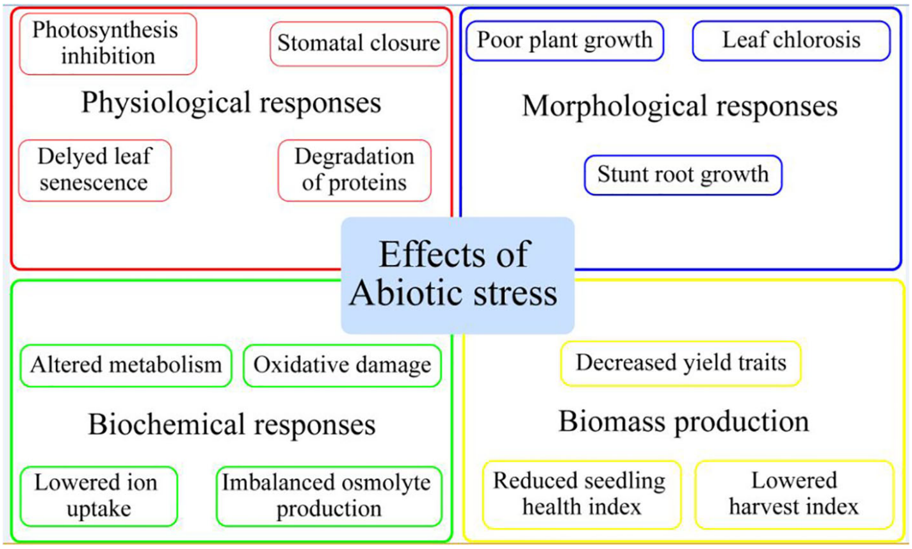


FIGURE 1
Effect of abiotic stress on horticultural plants.

TABLE 1 Exogenous melatonin enhanced abiotic stress tolerance in horticultural plants.

Stress type	MEL levels	Scientific name	Reference
Cold	5 µM	*Capsicum annum* L.	Korkmaz et al. (2021)
	100 µM	*Solanum lycopersicum* L.	Ding et al. (2017)
	200 µM	*Cucumis sativus* L.	Zhao et al. (2017)
	150 µM	*Citrullus lanatus* L.	Li et al. (2017b)
	5 µM	*Solanum melongena* L.	Yakuboğlu et al. (2022)
	200 µM	*Cucumis melo* L.	Zhang et al. (2017c)
	100 µM	*Prunus persica*	Gao et al. (2018)
Heavy metal	100 µM	*Capsicum annum* L.	Kaya et al. (2022)
	100 µM	*Fragaria* × *ananassa* Duch.	Wu et al. (2021)
	0.1 µM	*Citrullus lanatus* L.	Nawaz et al. (2018)
	100 µM	*Nicotiana tabacum* L.	Wang et al. (2019)
	100 µM	*Spinacia oleracea* L.	Asif et al. (2020)
Salinity	1 µM	*Solanum lycopersicum* L.	Ali et al. (2021)
	150 µM	*Citrullus lanatus* L.	Li et al. (2017a)
	50–150 µM	*Cucumis sativus* L.	Wang et al. (2016)
	100 and 200 µM	*Fragaria* × *ananassa* Duch.	Zahedi et al. (2020)
	0.1 µM	*Malus hupehensis*	Li et al. (2012)
	50 µM	*Vitis vinifera* L.	Xu et al. (2019)
Drought	100 µM	*Cucumis sativus* L.	Zhang et al. (2013)
	100 µM	*Solanum lycopersicum* L.	Liu et al. (2015a)
	100 µM	*Actinidia chinensis*	Liang et al. (2019)
	100 µM	*Carya cathayensis*	Sharma et al. (2020)
	0.2 µM	*Vitis vinifera* L.	Meng et al. (2014)
	100 µM	Camellia sinensis L.	Li et al. (2019)
Heat	100 µM	*Solanum lycopersicum* L.	Ahammed et al. (2018)
	100 µM	*Cucumis sativus* L.	Zhang et al. (2013)
	100 µM	Apium Graveolens L.	Li et al. (2022b)
	100 µM	*Fragaria* × *ananassa* Duch.	Manafi et al. (2022)

of plants, mainly through disruption of the soil's osmotic and ionic balances (Abdelaal et al., 2020; Zulfiqar et al., 2022). With increments in soil salt levels, an osmotic stress condition develops, which leads to declining water levels in the soil, thus less water is available for plant uptake, causing a conditional physiological drought in plants (Chang et al., 2014). Recently, MEL has emerged as an effective plant growth regulator, playing a significant role in the development of abiotic stress resistance in horticultural crops. Resultantly, several studies highlighted the stress-mitigating functions of MEL in horticultural crops under salt stress (Bose and Howlader, 2020). Plants' antioxidant system has been reported to improve, and photosynthetic capacity is seen to get protected with exogenously applied MEL under NaCl stress in peanut (ElSayed et al., 2020), orange (Hu et al., 2022), watermelon (Li et al., 2017a), pistachio (Kamiab, 2020), and tomato (Liu et al., 2019). According to Li et al. (2012), the photosynthetic capacity of plants is maintained by the exogenous application of MEL (0.1 µM) under salinity stress, which leads to significant alleviation of growth inhibition. Further, the oxidative damage caused by the scavenging of ROS was also decreased by MEL, and antioxidant enzymes' activity was improved, including catalase, peroxidase, and ascorbate. Salinity exerts its negative impact irrespective of the growth stage of the plants, and its effects range from seed germination to plant senescence and occur throughout the life cycle. Seed germination and plant growth are severely affected by saline stress (Nawaz et al., 2016; Zhan et al., 2019). In tomato, under salinity stress, the MEL applied exogenously reduced the uptake of sodium (Na^+), hydrogen peroxide (H_2O_2) content, and malonaldehyde (MDA) content, while enhancing enzyme activity, relative water content (RWC), membrane stability index, gas exchange parameters, and growth attributes (Ali et al., 2021). Furthermore, MEL pre-treatment

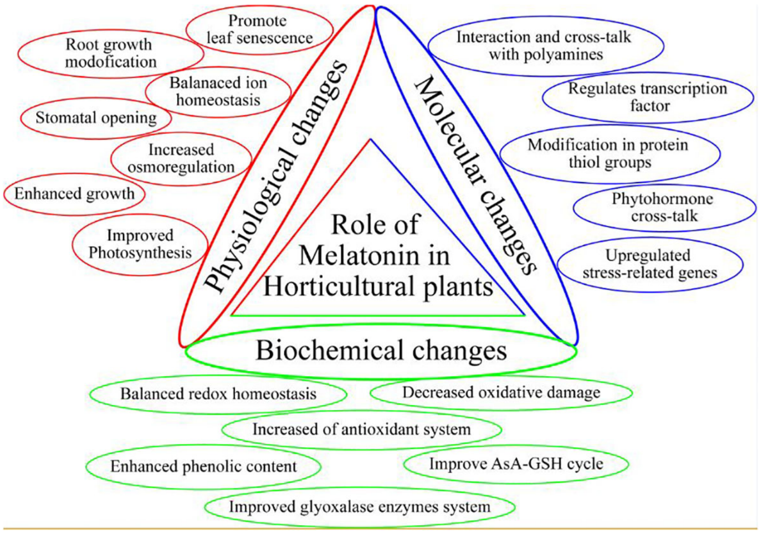


FIGURE 2
Role of melatonin in horticultural plants.

of cucumber seeds showed an increase in seed germination rate and seedling growth, along with a 5-fold increase in antioxidant enzyme activity under salinity stress (Zhang et al., 2014).

MEL supplementation improved growth traits and reduced the levels of MDA, ROS, and EL (electrolyte leakage), mainly through upregulating the enzymatic and non-enzymatic antioxidant enzyme activity. Moreover, in strawberries, MEL improved the phenolic and photosynthetic content (Zahedi et al., 2020). Importantly, Hu et al. (2021) revealed efficient reductions in the levels of MDA and ROS, increases in antioxidant activities, endogenous levels of MEL, proline, and pigment content, stomatal conductance, and the upregulation of genes related to redox, salt tolerance, and MEL biosynthesis. In addition, MEL was seen to escalate the ion homeostasis under high-NaCl stress in *Malus hupehensis* (Li et al., 2012). MEL further reduced ion toxicity by suppressing the accumulation of Na$^+$ and Cl$^-$ ions (Liu et al., 2019). The tomato seedling growth showed significant improvements with the supplementation of MEL under NaCl toxicity. Additionally, MEL effectively reduced the activity of glycolate oxidase, chlorophyll degradation, and ROS levels and caused increases in antioxidant enzyme activity, proline content, and glycine betaine levels (Siddiqui et al., 2020a). According to Zhang et al. (2017a), the uniformity of seeds and germination rate of cucumber seeds increased with the regulation of energy production with the application of MEL under salinity stress. Additionally, MEL protects the photosynthetic apparatus from oxidative damage induced by NaCl stress (Zhang et al., 2020). MEL increases the antioxidant enzyme, thus leading to a decline in the accumulation of ROS in the leaves of salt-sensitive cucumber plants. In cucumber, MT was also reported to suppress the alleviation in maximum quantum efficiency of photosystem II (PSII) and net photosynthetic rate and to protect the total chlorophyll content under salinity stress (Wang et al. 2016).

Drought

Global climate change has intensified drought stress episodes, which are emerging as a serious threat to crop growth and productivity

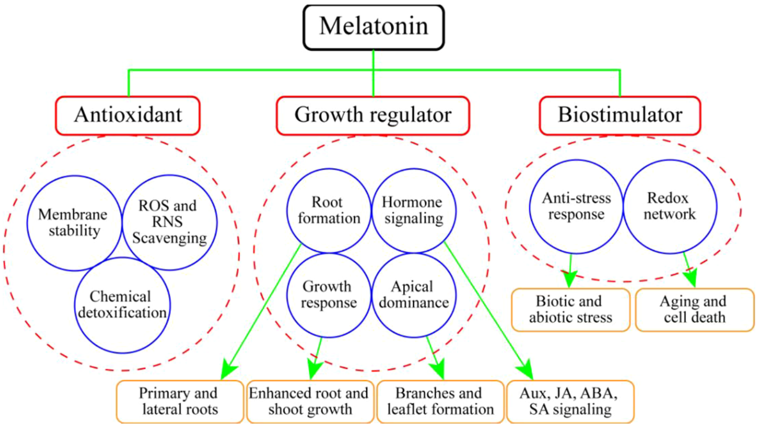


FIGURE 3
Action of melatonin inhorticultural plants as an antioxidant, growth regulator and bio-stimulator.

TABLE 2 Function of melatonin in horticultural plants.

Functions	Reference
Improved seed germination	Zhang et al. (2013)
Regulation of circadian rhythms	Kolář and Macháčková (2005)
Modification of root system architecture	Nawaz et al. (2018)
Vegetative development	Tan et al. (2013)
Regulation of photosynthetic machinery	Jahan et al. (2021)
Role as a potential growth regulator	Murch and Saxena (2002)
Enhanced seedling health index	Liu et al. (2015a)
Protection against environmental stresses	Ali et al. (2021)
Balanced of mineral nutrient homeostasis	Sarafi et al. (2017)
Reproductive development	Arnao and Hernández-Ruiz (2006)
Fruit ripening	Arnao and Hernández-Ruiz (2020)
Maintenance of ROS homeostasis	Siddiqui et al. (2020b)
Cell protection	Ahammed et al. (2020)
Regulation of antioxidant enzymes pool	Zamani et al. (2020)
Retardation of leaf senescence	Shi et al. (2015)
Modulation of flowering development	Paredes et al. (2009)
Inhibition of root elongation	Park and Back (2012)

worldwide. Horticultural crops are very vulnerable to drought stress (Gao et al., 2022; Muhammad et al., 2022; Toscano et al., 2023). Drought stress is reported to cause abnormalities in the physiological and morphological states of plants (Hanaka et al., 2021). Reduced root system architecture, cellular membrane integrity, damaged photosynthetic apparatus, and imbalanced mineral and nutrient accumulation are highlighted as some of the most important abnormalities that lead to the complete devastation of a plant facing drought stress (Tabassum et al., 2021). Melatonin protects horticultural crops by preventing damage to the root architecture system, photosynthetic machinery, inducing the antioxidative defense system, regulating oxidative stress, and some other defense mechanisms (Tiwari et al., 2020). MEL pretreatment of tomato seedlings grown in field conditions under drought stress showed effective results, and the detrimental effects of drought stress were significantly reduced (Liu et al., 2015a). MEL-supplementation effectively improved seedling growth, photosynthetic efficiency, activity of antioxidant enzymes, and decreased oxidative damage (Liu et al., 2015a). Under drought stress, exogenous MEL application improved the growth, photosynthetic apparatus, and antioxidant enzyme systems of Chinese hickory plants (Sharma et al., 2020). In addition, Karaca and Cekic (2019) observed that MEL supplementation efficiently improved chlorophyll content, antioxidant enzyme systems, and reduced MDA content in *Solanum lycopersicum* L. under drought stress.

The photosynthetic machinery of tomato seedlings under drought stress showed significant improvements with the application of MEL (Ding et al., 2018; Ibrahim et al., 2020). Similar kinds of studies that indicate the impact of melatonin treatments on minimizing drought-induced photosynthetic damage have been performed on fenugreek (Zamani et al., 2020), kiwifruit (Xia et al., 2020), and grapes (Meng et al., 2014). Exogenous melatonin treatment showed several positive phenomena, such as preventing chloroplast photosynthetic damage (Wang et al., 2012). Further, MEL supplementation led to improved turgor pressure and water content of leaves, along with enacting the spongy tissue (Meng et al., 2014). The antioxidant defense system of plants gets triggered, and the scavenging of ROS is efficiently enhanced by the application of MEL. In horticultural plants, the mechanisms related to these phenomena are well examined, such as the scavenging of H_2O_2 by the regulation of the ascorbate-glutathione cycle (Li et al., 2019; Ibrahim et al., 2020). In cucumber, the seed germination rate was improved by the exogenous application of MEL (Zhang et al., 2013). In *Moringa oleifera*, the foliar application of MEL under drought stress showed a beneficial impact on the dry and fresh weight of shoots and leaves, number of leaves/plants, plant height, and foliage yield (Sadak et al., 2020). (Sadak et al., 2020). In *Coffea arabica* seedlings, the leaf area was reduced by drought stress, which was alleviated by the supplementation of MEL (Campos et al., 2019). Plant growth is maintained by the application of MT under drought stress conditions, mainly by the maintenance of homeostatic balance and vegetative tissues' growth (Sharma and Zheng, 2019). The protective roles of MEL in plants under salinity and drought stress are summarized in Table 3.

Cold

Plant growth is significantly influenced by temperature. Low temperature stress lies amid the most detrimental environmental conditions for plants, resulting in yield and productivity losses (Malhotra, 2017). Cold stress can negatively affect seedling growth, root morphology, photosynthetic efficiency, seed germination, and pigment content (Marino, 2021). Low-temperature stress also has a negative impact on the metabolic capacity of plants. Plants' metabolic capacity is also affected by low-temperature stress. The levels and activity of enzymes involved in important metabolic pathways are usually altered in cold-stressed plants, and as a result, the plant metabolome is completely changed (Khan et al., 2015; Liang et al., 2020). The development of commercial crop cultivars that are cold-resistant has been recently focused on by plant scientists. The use of MEL significantly reduced the adverse effects of cold stress on a diverse range of plant genera. Wang et al. (2020) reported that MEL application remarkably improved photosynthesis, metabolites, and tomato seedling growth under cold stress. MEL application efficiently improved photosynthetic performance in pepper under chilling stress (Korkmaz et al., 2021). In tomato seedlings, the application of MEL under cold stress caused significant increases in chlorophyll fluorescence parameters, pigment content, gas exchange elements, and growth characteristics (Zhou et al., 2020). Furthermore, melatonin significantly imparted cold tolerance in *Citrullus lanatus* stemically by regulating antioxidant capacity and the expression of defense genes (Li et al., 2017b). In a recent study, Li et al. (2022a) reported that MT supplementation efficiently enhanced root growth, antioxidant enzymes, the photosynthetic system, and reduced oxidative damage in pepper under cold stress.

MEL supplementation increased seedling growth and cold stress tolerance by balancing redox homeostasis, stomatal opening, leaf photosynthetic activity, mineral nutrient accumulation, osmolytes

TABLE 3 Melatonin enhanced salinity and drought stress tolerance in horticultural crops.

Species name	Stress treatment	MEL level	Observation	References
Salinity stress				
Strawberry	0, 40, and 80 mM	100 and 200 µM	Enhanced strawberry fruit production, phenolics content, photosynthetic pigment, decreased oxidative stress biomarkers, and boost antioxidant enzymes system.	Zahedi et al. (2020)
Orange	150 mM	0, 50, 100, 150 µM	Better plant growth, enhanced photosynthetic efficiency, and pigment content	Hu et al. (2022)
Cucumber	150 mM	50, 100, 200, 300 µM	Improved photosynthesis, growth, carotenoids, and antioxidant enzymes; reduced MDA, EL, and H_2O_2 level	Zhang et al. (2020)
Pistachio	25, 50, 100, 150 mM	0, 25, 50, 75, 100, 125, 150 µM	Enhanced shoot and root growth, chlorophyll content, polyamine level, proline content, nutrient uptake, and antioxidant enzymes activity	Kamiab (2020)
Tomato	160 mM	1.0 µmol	Increased RWC, growth traits, gas exchange characteristics, pigments content, and antioxidant enzymes system; reduced MDA and H2O2 level; decreased Na^+ accumulation	Ali et al. (2021)
Peanut	150 mM	50, 100, 150 µM	Reduced ROS, and MDA level, increased enzymatic and non-enzymatic antioxidant system and proline content	ElSayed et al. (2020)
Watermelon	300 mM	50, 150, and 500 µM	Reduced oxidative damage, balanced redox homeostasis, protect photosynthesis, and upregulate antioxidant enzyme system	Li et al. (2017a)
Drought				
Kiwifruit	9 days	50, 100, and 200 µM	Modified root system architecture, decreased MDA level, enhanced osmoregulation content, increased photosynthesis	Liang et al. (2019)
Fenugreek	7 days	50, 100, 300, and 500 µM	Improved growth parameters, Enhanced antioxidant enzymes and proline content; reduced H_2O_2 and MDA level	Zamani et al. (2020)
Cucumber	–	100 µM	Reduced ROS production, decreased chlorophyll degradation, increased net photosynthetic rate	Zhang et al. (2013)
Tomato	–	100 µM	Promoted growth, chlorophyll content, proline level, soluble sugar content, and antioxidant enzymes system	Ibrahim et al. (2020)
Tobacoo	14 days	200 µM	Promote root system architecture, enhanced nutrient uptake and antioxidant enzymes system, reduced H2O2 and MDA level	Liu et al. (2021)

production, and primary and secondary metabolites, as well as improvements in antioxidant activities and ROS scavenging (Qari et al., 2022). MEL alleviates cold-induced adverse effects on tea plants. MEL pretreatment in tea plants significantly improved growth traits, photosynthesis, antioxidant profile, and balanced redox homeostasis under chilling stress (Li et al., 2018). Root pretreatment with MEL reduced aerial cold-induced suppression of photosystem II and oxidative damage in *C. lanatus* (Chang et al., 2020). Cao et al. (2016) revealed that in peach fruit, chilling injury was very effectively reduced by the application of MEL at a dose of 100 µM. In tomato plants under cold stress, the pretreatment of MEL showed higher levels of non-enzymatic antioxidants, greater activities of antioxidant enzymes, and reduced MDA content and EL (Ding et al., 2017). Furthermore, pretreatment of MEL declined the harmful impact of cold stress and accelerated the plants' recovery, primarily by improving photosynthesis and antioxidant enzyme capacity in the leaves of melon (Zhang et al., 2017b). Several reports revealed that MEL positively modulates the growth of cucumber (Marta et al., 2016), tomato (Yang et al., 2018), and watermelon (Li et al., 2017a). MEL application considerably reduced the adverse effect of cold stress on eggplant seedlings. MEL supplementation enhanced leaf area, biomass production, photosynthetic mechanism, activity of antioxidant enzymes, proline content, and reduced MDA and H_2O_2 levels of eggplant seedlings under chilling conditions (Yakuboğlu et al., 2022).

Heat

The production of horticultural crops is severely and significantly hindered by heat stress driven by climate change. High temperature stress is a major environmental stress that limits plant growth, metabolism, and productivity worldwide. Temperature affects many of the biochemical reactions that are important for the steady growth and development of plants (Hasanuzzaman et al., 2013). High temperatures are becoming an important concern for sustainable crop production (Wahid et al., 2012). A few researchers have investigated the possible role and defensive mechanism of MEL under heat stress in plants. Recently, Ahammed et al. (2018) revealed that MEL has been declared a universal regulator of abiotic stresses, which can possibly increase the heat resistance of plants. MEL-pretreated tomato plants under heat stress showed improved root growth, chlorophyll content, activities of enzymatic and non-enzymatic antioxidants, and decreased oxidative damage. Further, Jahan et al. (2019) described that MEL efficiently increased polyamine content and considerably declined levels of MDA and EL in tomatoes. MEL supplementation effectively reduced the accumulation of ROS and increased the antioxidant profile in tomato seedlings in a high-temperature environment (Martinez et al., 2018). Ahammed et al. (2019) revealed that MEL supplementation significantly reduced

MDA and EL levels and increased the antioxidant enzyme system in tomatoes under heat stress. Table 4 shows how MEL protects plants from heat and cold stress. Research to date has demonstrated the vital functions of MEL for plant survival and higher productivity of horticultural crops in high-temperature stress conditions. Yet, extensive investigations are needed to confirm the possible mechanisms of heat stress amelioration by MEL in plants.

Heavy metals

Globally, the pollution caused by heavy metals is getting worse with time, causing a wide range of toxic impacts on horticultural crop production (Shakoor et al., 2017; Behera et al., 2022). Plants are probably universally tolerant of heavy metal stress. Regardless of other stresses, the production of horticultural crops is significantly and negatively affected by heavy metal stressors, which is becoming a major concern (Noor et al., 2022). Hitherto work shows that heavy metal (lead, boron, cadmium, nickel, arsenic, and vanadium) stress remarkably reduces horticultural crop production (Dodangeh et al., 2018; Bhat et al., 2019).

One of the recently emerging potential stress-alleviating hormones is MEL, which may aid in coping mechanisms against metal-induced toxicity in plants. MEL application remarkably increased growth characteristics, root morphology, pigment content, and net photosynthetic rate. Additionally, under vanadium stress, MEL application in watermelon caused significant reductions in oxidative damage, increases in antioxidant enzymes, and levels of relevant gene expression (Nawaz et al., 2018). Under nickel toxicity, MEL application in tomato reduced the MDA and EL levels and increased the secondary metabolite content, proline level, leaf photosynthesis, and antioxidant defense mechanism (Jahan et al., 2020). MEL application considerably increased the root architecture of cucumber seedlings under copper toxicity (Cao et al., 2019), the growth status of red cabbage plants under copper toxicity (Posmyk et al., 2008), the photosynthetic efficiency of radish seedlings under aluminum toxicity (Tang et al., 2016), the antioxidant enzyme system in roses under cadmium toxicity (Nabaei and Amooaghaie, 2019), the mineral nutrient content of strawberry seedlings under cadmium toxicity (Wu et al., 2021), and lowered the MDA and EL levels in tomato seedlings under cadmium toxicity (Hasan et al., 2015). Moreover, previous findings suggested that MEL significantly reduced iron accumulation from root to shoot in cucumber, watermelon, and tomato (Ahammed et al., 2020).

Hasan et al. (2018) revealed that under low sulfur conditions, MEL efficiently enhanced different growth traits, chlorophyll fluorescence parameters, gas exchange elements, and pigment molecules, as well as declining the MDA and H_2O_2 levels in tomato seedlings. Siddiqui et al. (2019) observed that MEL application significantly improved the photosynthesis and growth of tomato seedlings under lanthanum toxicity. Further, MEL supplementation significantly improved aerial biomass production, carotenoid content, chlorophyll content, and carbohydrate levels in spinach under boron toxicity. Selenium (Se) toxicity impaired rapeseed growth and biomass production, decreased photosynthesis, and lowered photosynthetic pigment content. All these parameters were remarkably alleviated by MEL application. MEL significantly reduced cellular membrane damage and ROS formation. MEL effectively improved proline level, metabolite content, antioxidant enzymes, and their gene expression levels in *Brassica napus* under Se toxicity (Ulhassan et al., 2019). In another study, MEL treatment considerably enhanced net photosynthetic rate, growth traits, leaf gas

TABLE 4 Action of melatonin in cold and heat stress tolerance.

Species name	Stress treatment	MEL level	Observation	References
Cold stress				
Pepper	10/4°C D/N	5 µM	Improved chlorophyll content, gas exchange characteristics	Altaf et al. (2022b)
Peach	4°C	100 µM	Reduced H_2O_2 level and enhanced antioxidant enzymes system	Cao et al. (2018)
Cucumber	15/8°C D/N	200 µM	Decreased ROS production and upregulated AsA-GSH cycle,	Zhao et al. (2016)
Tomato	15/6°C D/N	100 µM	Enhanced photosynthetic machinery, reduced MDA accumulation	Yang et al. (2018)
Melon	12/6°C D/N	200 µM	Improved chlorophyll content and gas exchange parameters, lowered MDA level and increased antioxidant enzymes system	Zhang et al. (2017c)
Banana	4°C	100 µM	Enhanced ETR, improved antioxidant enzymes system; reduced MDA and H_2O_2, O_2 accumulation	Liu et al. (2022)
Heat stress				
Tomato	42°C	100 µM	Lowered ROS accumulation, reduced MDA production, enhanced enzymatic and non-enzymatic antioxidant system	Jahan et al. (2019)
Kiwifruit	45°C	200 µM	Enhanced AsA-GSH cycle, proline content antioxidant enzymes system, and reduced oxidative damage	Liang et al. (2018)
Radish	35/30°C D/N	29.0 mg	Enhanced biomass yield, antioxidant enzymes, carotenoids content	Jia et al. (2020)
Celery	38°C	100 µmol	Reduced EL and MDA level; enhanced chlorophyll content	Li et al. (2022b)

exchange elements, and maintained macro- and micro-nutrient content in pepper leaves under B toxicity (Sarafi et al., 2017). MEL application improved antioxidant enzyme activities and root growth and reduced the MDA and proline levels in the roots of melon seedlings under Cu toxicity (Hu et al., 2020). In addition, MEL further enhanced the antioxidant enzyme system, along with a decline in the ROS level in spinach seedlings (Moussa and Algamal, 2017). MEL positively regulates the growth of tobacco under lead stress (Kobylińska et al., 2017), radish under Cd toxicity (Xu et al., 2020), and pepper under boron stress (Sarafi et al., 2017). Table 5 provides an overview of the protective functions of MEL in horticultural crops under heavy metal toxicity.

Acid rain, sodic alkaline toxic, and chemical stress

The protective role of MEL against acid rain, toxic chemicals, and sodic alkaline stresses has also been proven. Under acid rain conditions, the application of MEL on tomato plants leads to significant reductions in levels of MDA and H_2O_2, repairing the chloroplast's grana lamella, along with escalations in growth parameters, antioxidant enzymes, pigment molecules, phenolic, flavonoids, and proline content (Debnath et al., 2018a). Debnath et al. (2018b) revealed that under stimulated acid-rain stress, the application of MEL to tomato plants exhibited significant improvements in yield attributes and quality traits of fruit. Moreover, pretreatment with MEL led to an increase in antioxidant enzymes' activities, total soluble solids, soluble proteins, flavonoids, phenolics, and carotenoid content, along with remarkable reductions in MDA levels. MEL application remarkably improved the activity of antioxidant enzymes, chlorophyll content, and growth, and decreased the production of H_2O_2 and MDA in fenugreek under lead and acid rain stress (Xalxo and Keshavkant, 2019). Furthermore, Liu et al. (2015b) revealed that application of MEL under alkaline stress caused reductions in Na^+ levels and enhanced the levels of K^+ in tomato leaves, exhibiting the role of MEL in the maintenance of ion homeostasis and thus increasing the stress resistance of tomato plants to alkaline stress. The positive role of MEL in the stress resistance of plants to alkaline stress is due to the regulation of enzyme activity, and polyamine biosynthesis (Gong et al., 2017). MEL enhanced the photosynthetic pigment content of pea plants under paraquat stress (Szafrańska et al., 2017). In cucumber seedlings, the application of MEL under cinnamic acid stress increases plant stress resistance, mainly by integrating the morphology, mineral nutrient contents, and signaling crosstalk of plant hormones (Li et al., 2017c). The growth of cucumber plants is boosted by the application of MEL, which modulates mineral nutrient composition and nitrogen metabolism under nitrate stress (Zhang et al., 2017b).

Future perspectives

The review will also help and encourage plant researchers to deeply examine the mechanism of stress tolerance mediated by MEL. Exogenously applied MEL is declared a potential growth regulator for plants, which aids under abiotic stress conditions by increasing plants' growth, yield, and quality. Various functions of plants are found to be associated with MEL, such as the regulation of physiological functions, including seed germination and seedling growth, along with the functions of MEL in stress resistance under environmental stressors. The harmful effects of abiotic stresses are alleviated by the application of MEL, either directly by scavenging ROS or indirectly by improving the photosynthetic machinery, enhancing the activities of antioxidant enzymes, regulating metal transport and growth regulators in plants, as well as increasing osmotic metabolites. Although MEL has attracted the interest of plant researchers recently and progress can be seen on the topic, there are still unexplored MEL signaling pathways that, though

TABLE 5 Melatonin mitigates heavy metals toxicity in horticultural plants.

Species name	Stress treatment	MEL level	Observation	References
Watermelon	40 mg vanadium	0.1 µM	Higher chlorophyll content, reduced MDA content, enhanced antioxidant enzymes, low vanadium uptake from root to shoot	Nawaz et al. (2018)
Cucumber	80 µM copper	10 nM	Promoted seedling growth, improved antioxidant enzymes. Inhibited ROS accumulation,	Cao et al. (2019)
Radish	200 mg lead	50 µM	Enhanced growth, reduced oxidative damage lowered lead accumulation	Tang et al. (2021)
Strawberry	100 µM cadmium	100 µM	Enhanced seedling growth, antioxidant enzymes, anthocyanin, and chlorophyll content; decreased oxidative stress biomarkers	Wu et al. (2021)
Tomato	50 µM nickel	100 µM	Improved photosynthesis, metabolites content, antioxidant enzymes; reduced oxidative damage and lowered Ni accumulation from root to shoot	Jahan et al. (2020)
Pepper	100 µM boron	1 µM	Enhanced mineral nutrient content, and carotenoids content, lowered metal uptake from root to shoot	Sarafi et al. (2017)
Fava bean	5 µM arsenic	50 µM	Enhanced pigment content, gas exchange parameters, reduced ROS production and MDA level	Siddiqui et al. (2020b)
Eggplant	10 mg cadmium	150 µmol	Improved water use efficiency, gas exchange characteristics	Tang et al. (2015)
Cucumber	90 mg iron	100 µmol	Improved leaf photosynthetic efficiency, growth characteristics, and antioxidant enzymes, reduced ROS, MDA and EL level	Ahammed et al. (2020)

complicated, need to be investigated under abiotic stresses. There exists a major gap in the literature about understanding the regulation of pathways by MEL and associated genes. Moreover, there is a need to address several major problems. For instance, there is a lack of understanding regarding the mechanisms of HM uptake, sequestration, and transportation as regulated by MEL. Future research should thus aim to deeply investigate the functions of MEL and its underlying mechanisms to sustain crop production under abiotic stress environments.

Author contributions

JZ: Conceptualization, literature survey, figure designing, writing major original draft, review structure. JH: Literature survey, writing—review and editing. All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

Abdelaal, K. A., EL-Maghraby, L. M., Elansary, H., Hafez, Y. M., Ibrahim, E. I., El Banna, M., et al. (2020). Treatment of sweet pepper with stress tolerance-inducing compounds alleviates salinity stress oxidative damage by mediating the physio-biochemical activities and antioxidant systems. *Agronomy* 10, 26. doi: 10.3390/agronomy10010026

Aghdam, M. S., Mukherjee, S., Flores, F. B., Arnao, M. B., Luo, Z., and Corpas, F. J. (2021). Functions of melatonin during postharvest of horticultural crops. *Plant Cell Physiol.* 0, 1–23. doi: 10.1093/pcp/pcab175

Aguilera, Y., Herrera, T., Benítez, V., Arribas, S. M., Lopez De Pablo, A. L., and Esteban, R. M. (2015). Estimation of scavenging capacity of melatonin and other antioxidants: contribution and evaluationing germinated seeds. *Food Chem.* 170, 203–211. doi: 10.1016/j.foodchem.2014.08.071

Ahammed, G. J., and Li, X. (2023). Dopamine-induced abiotic stress tolerance in horticultural plants. *Sci. Hortic.* 307, 111506. doi: 10.1016/j.scienta.2022.111506

Ahammed, G. J., Wu, M., Wang, Y., Yan, Y., Mao, Q., Ren, J., et al. (2020). Melatonin alleviates iron stress by improving iron homeostasis, antioxidant defense and secondary metabolism in cucumber. *Sci. Hortic.* 265, 109205. doi: 10.1016/j.scienta.2020.109205

Ahammed, G. J., Xu, W., Liu, A., and Chen, S. (2018). COMT1 silencing aggravates heat stress-induced reduction in photosynthesis by decreasing chlorophyll content, photosystem II activity, and electron transport efficiency in tomato. *Front. Plant Sci.* 9, 998. doi: 10.3389/fpls.2018.00998

Ahammed, G. J., Xu, W., Liu, A., and Chen, S. (2019). Endogenous melatonin deficiency aggravates high temperature-induced oxidative stress in *Solanum lycopersicum* l. *Environ. Exp. Bot.* 161, 303–311. doi: 10.1016/j.envexpbot.2018.06.006

Ali, M., Kamran, M., Abbasi, G. H., Saleem, M. H., Ahmad, S., Parveen, A., et al. (2021). Melatonin-induced salinity tolerance by ameliorating osmotic and oxidative stress in the seedlings of two tomato (*Solanum lycopersicum* l.) cultivars. *J. Plant Growth Regul.* 40, 2236–2248. doi: 10.1007/s00344-020-10273-3

Altaf, M. A., Hao, Y., He, C., Mumtaz, M. A., Shu, H., Fu, H., et al. (2022a). Physiological and biochemical responses of pepper (*Capsicum annuum* l.) seedlings to nickel toxicity. *Front. Plant Sci.* 13, 950392. doi: 10.3389/fpls.2022.950392

Altaf, M. A., Sharma, N., Singh, J., Samota, M. K., Sankhyan, P., Singh, B., et al. (2023). Mechanistic insights on melatonin-mediated plant growth regulation and hormonal cross-talk process in solanaceous vegetables. *Sci. Hortic.* 308, 111570. doi: 10.1016/j.scienta.2022.111570

Altaf, M. A., Shu, H., Hao, Y., Mumtaz, M. A., Lu, X., and Wang, Z. (2022b). Melatonin affects the photosynthetic performance of pepper (*Capsicum annuum* l.) seedlings under cold stress. *Antioxidants* 11, 2414. doi: 10.3390/antiox11122414

Andreotti, C. (2020). Management of abiotic stress in horticultural crops: Spotlight on biostimulants. *Agronomy* 10, 1514. doi: 10.3390/agronomy10101514

Arnao, M. B., Giraldo-Acosta, M., Castejón-Castillejo, A., Losada-Lorán, M., Sánchez-Herrerías, P., El Mihyaoui, A., et al. (2023). Melatonin from microorganisms, algae, and plants as possible alternatives to synthetic melatonin. *Metabolites* 13, 72. doi: 10.3390/metabo13010072

Arnao, M. B., and Hernández-Ruiz, J. (2006). The physiological function of melatonin in plants. *Plant Signal. Behav.* 1, 89–95. doi: 10.4161/psb.1.3.2640

Arnao, M. B., and Hernández-Ruiz, J. (2009). Protective effect of melatonin against chlorophyll degradation during the senescence of barley leaves. *J. Pineal Res.* 46, 58–63. doi: 10.1111/j.1600-079X.2008.00625.x

Arnao, M. B., and Hernández-Ruiz, J. (2014). Melatonin: plant growth regulator and/or biostimulator during stress? *Trends Plant Sci.* 19, 789–797. doi: 10.1016/j.tplants.2014.07.006

Arnao, M. B., and Hernández-Ruiz, J. (2020). Melatonin in flowering, fruit set and fruit ripening. *Plant Reprod.* 11, 77–87. doi: 10.1007/s00497-020-00388-8

Asif, M., Pervez, A., Irshad, U., Mehmood, Q., and Ahmad, R. (2020). Melatonin and plant growth-promoting rhizobacteria alleviate the cadmium and arsenic stresses and increase the growth of *Spinacia oleracea* l. *Plant Soil Environ.* 66, 234–241. doi: 10.17221/135/2020-PSE

Ayyaz, A., Shahzadi, A. K., Fatima, S., Yasin, G., Zafar, Z. U., Athar, H. U. R., et al. (2022). Uncovering the role of melatonin in plant stress tolerance. *Theor. Exp. Plant Physiol.* 34, 335–346. doi: 10.1007/s40626-022-00255-z

Badria, F. A. (2002). Melatonin, serotonin, and tryptamine in some Egyptian food and medicinal plants. *J. Medicinal Food.* 5, 153–157. doi: 10.1089/10966200260398189

Behera, B., Kancheti, M., Raza, M. B., Shiv, A., Mangal, V., Rathod, G., et al. (2022). Mechanistic insight on boron-mediated toxicity in plant vis-a-vis its mitigation strategies: a review. *Int. J. Phyto.*, 1–18. doi: 10.1080/15226514.2022.2049694

Bhat, J. A., Shivaraj, S. M., Singh, P., Navadagi, D. B., Tripathi, D. K., Dash, P. K., et al. (2019). Role of silicon in mitigation of heavy metal stresses in crop plants. *Plants* 8, 71. doi: 10.3390/plants8030071

Bose, S. K., and Howlader, P. (2020). Melatonin plays multifunctional role in horticultural crops against environmental stresses: A review. *Environ. Exp. Bot.* 176, 104063. doi: 10.1016/j.envexpbot.2020.104063

Campos, C. N., Ávila, R. G., de Souza, K. R. D., Azevedo, L. M., and Alves, J. D. (2019). Melatonin reduces oxidative stress and promotes drought tolerance in young *Coffea arabica* l. plants. *Agric. Water Manage.* 211, 37–47. doi: 10.1016/j.agwat.2018.09.025

Cao, Y. Y., Qi, C. D., Li, S., Wang, Z., Wang, X., Wang, J., et al. (2019). Melatonin alleviates copper toxicity *via* improving copper sequestration and ROS scavenging in cucumber. *Plant Cell Physiol.* 60, 562–574. doi: 10.1093/pcp/pcy226

Cao, S., Shao, J., Shi, L., Xu, L., Shen, Z., Chen, W., et al. (2018). Melatonin increases chilling tolerance in postharvest peach fruit by alleviating oxidative damage. *Sci. Rep.* 8, 1–11. doi: 10.1038/s41598-018-19363-5

Cao, S., Song, C., Shao, J., Bian, K., Chen, W., and Yang, Z. (2016). Exogenous melatonin treatment increases chilling tolerance and induces defense response in harvested peach fruit during cold storage. *J. Agricul Food Chem.* 64, 5215–5222. doi: 10.1021/acs.jafc.6b01118

Chang, J., Guo, Y., Zhang, Z., Wei, C., Zhang, Y., Ma, J., et al. (2020). CBF-responsive pathway and phytohormones are involved in melatonin-improved photosynthesis and redox homeostasis under aerial cold stress in watermelon. *Acta Physiol. Plant* 42, 159. doi: 10.1007/s11738-020-03147-4

Chang, B., Yang, L., Cong, W., Zu, Y., and Tang, Z. (2014). The improved resistance to high salinity induced by trehalose is associated with ionic regulation and osmotic adjustment in *Catharanthus roseus*. *Plant Physiol. Biochem.* 77, 140–148. doi: 10.1016/j.plaphy.2014.02.001

Debnath, B., Hussain, M., Irshad, M., Mitra, S., Li, M., Liu, S., et al. (2018a). Exogenous melatonin mitigates acid rain stress to tomato plants through modulation of leaf ultrastructure, photosynthesis and antioxidant potential. *Molecules* 23, 388. doi: 10.3390/molecules23020388

Debnath, B., Hussain, M., Li, M., Lu, X., Sun, Y., and Qiu, D. (2018b). Exogenous melatonin improves fruit quality features, health promoting antioxidant compounds and yield traits in tomato fruits under acid rain stress. *Molecules* 23, 1868. doi: 10.3390/molecules23081868

Ding, F., Liu, B., and Zhang, S. (2017). Exogenous melatonin ameliorates cold-induced damage in tomato plants. *Sci. Hortic.* 219, 264–271. doi: 10.1016/j.scienta.2017.03.029

Ding, F., Wang, G., Wang, M., and Zhang, S. (2018). Exogenous melatonin improves tolerance to water deficit by promoting cuticle formation in tomato plants. *Molecules* 23, 1605. doi: 10.3390/molecules23071605

Dodangeh, H., Rahimi, G., Fallah, M., and Ebrahimi, E. (2018). Investigation of heavy metal uptake by three types of ornamental plants as affected by application of organic and chemical fertilizers in contaminated soils. *Environ. Earth Sci.* 77, 1–15. doi: 10.1007/s12665-018-7620-2

Dos Reis, S. P., Lima, A. M., and De Souza, C. R. B. (2012). Recent molecular advances on downstream plant responses to abiotic stress. *Int. J. Mol. Sci.* 13, 8628–8647. doi: 10.3390/ijms13078628

ElSayed, A. I., Boulila, M., Rafudeen, M. S., Mohamed, A. H., Sengupta, S., Rady, M., et al. (2020). Melatonin regulatory mechanisms and phylogenetic analyses of melatonin biosynthesis related genes extracted from peanut under salinity stress. *Plants* 9, 854. doi: 10.3390/plants9070854

Gao, J. P., Chao, D. Y., and Lin, H. X. (2007). Understanding abiotic stress tolerance mechanisms: recent studies on stress response in rice. *J. Integr. Plant Biol.* 49, 742–750. doi: 10.1111/j.1744-7909.2007.00495.x

Gao, H., Lu, Z., Yang, Y., Wang, D., Yang, T., Cao, M., et al. (2018). Melatonin treatment reduces chilling injury in peach fruit through its regulation of membrane fatty acid contents and phenolic metabolism. *Food Chem.* 245, 659–666. doi: 10.1016/j.foodchem.2017.10.008

Gao, J., Shahid, R., Ji, X., and Li, S. (2022). Climate change resilience and sustainable tropical agriculture: Farmers' perceptions, reactive adaptations and determinants of reactive adaptations in hainan, China. *Atmosphere* 13, 955. doi: 10.3390/atmos13060955

Gong, X., Shi, S., Dou, F., Song, Y., and Ma, F. (2017). Exogenous melatonin alleviates alkaline stress in malus hupehensis rehd. by regulating the biosynthesis of polyamines. *Molecules* 22, 1542. doi: 10.3390/molecules22091542

Hanaka, A., Ozimek, E., Reszczyńska, E., Jaroszuk-Ściseł, J., and Stolarz, M. (2021). Plant tolerance to drought stress in the presence of supporting bacteria and fungi: An efficient strategy in horticulture. *Horticulturae* 7, 390. doi: 10.3390/horticulturae7100390

Hasan, M., Ahammed, G. J., Yin, L., Shi, K., Xia, X., Zhou, Y., et al. (2015). Melatonin mitigates cadmium phytotoxicity through modulation of phytochelatins biosynthesis, vacuolar sequestration, and antioxidant potential in *Solanum lycopersicum* l. *Front. Plant Sci.* 6, 601. doi: 10.3389/fpls.2015.00601

Hasan, M. K., Liu, C. X., Pan, Y. T., Ahammed, G. J., Qi, Z. Y., and Zhou, J. (2018). Melatonin alleviates low-sulfur stress by promoting sulfur homeostasis in tomato plants. *Sci. Rep.* 8, 10182. doi: 10.1038/s41598-018-28561-0

Hasanuzzaman, M., Nahar, K., Alam, M. M., Roychowdhury, R., and Fujita, M. (2013). Physiological, biochemical, and molecular mechanisms of heat stress tolerance in plants. *Int. J. Mol. Sci.* 14, 9643–9684. doi: 10.3390/ijms14059643

Hassan, M. U., Mahmood, A., Awan, M. I., Maqbool, R., Aamer, M., Alhaithloul, H. A., et al. (2022). Melatonin-induced protection against plant abiotic stress: mechanisms and prospects. *Front. Plant Sci.* 13, 902694. doi: 10.3389/fpls.2022.902694

Hoque, M. N., Tahjib-Ul-Arif, M., Hannan, A., Sultana, N., Akhter, S., Hasanuzzaman, M., et al. (2021). Melatonin modulates plant tolerance to heavy metal stress: morphological responses to molecular mechanisms. *Int. J. Mol. Sci.* 22, 11445. doi: 10.3390/ijms222111445

Hu, Z., Fu, Q., Zheng, J., Zhang, A., and Wang, H. (2020). Transcriptomic and metabolomic analyses reveal that melatonin promotes melon root development under copper stress by inhibiting jasmonic acid biosynthesis. *Hortic. Res.* 7, 79. doi: 10.1038/s41438-020-0293-5

Hu, E., Liu, M., Zhou, R., Jiang, F., Sun, M., Wen, J., et al. (2021). Relationship between melatonin and abscisic acid in response to salt stress of tomato. *Sci. Hortic.* 285, 110176. doi: 10.1016/j.scienta.2021.110176

Hu, C. H., Zheng, Y., Tong, C. L., and Zhang, D. J. (2022). Effects of exogenous melatonin on plant growth, root hormones and photosynthetic characteristics of trifoliate orange subjected to salt stress. *Plant Growth Regul.* 1-8. doi: 10.1007/s10725-022-00814-z

Ibrahim, M. F., Elbar, O. H. A., Farag, R., Hikal, M., El-Kelish, A., El-Yazied, A. A., et al. (2020). Melatonin counteracts drought induced oxidative damage and stimulates growth, productivity and fruit quality properties of tomato plants. *Plants* 9, 1276. doi: 10.3390/plants9101276

Imran, K., Zafar, H., Chattha, M. U., Mahmood, A., Maqbool, R., and Athar, F. (2022). Seed priming with different agents mitigate alkalinity induced oxidative damage and improves maize growth. *Not. Bot. Horti Agrobot. Cluj Napoca* 50, 12615–12615. doi: 10.15835/nbha50112615

Jahan, M. S., Guo, S., Baloch, A. R., Sun, J., Shu, S., Wang, Y., et al. (2020). Melatonin alleviates nickel phytotoxicity by improving photosynthesis, secondary metabolism and oxidative stress tolerance in tomato seedlings. *Ecotoxicol. Environ. Saf.* 197, 110593. doi: 10.1016/j.ecoenv.2020.110593

Jahan, M. S., Shu, S., Wang, Y., Chen, Z., He, M., Tao, M., et al. (2019). Melatonin alleviates heat-induced damage of tomato seedlings by balancing redox homeostasis and modulating polyamine and nitric oxide biosynthesis. *BMC Plant Biol.* 19, 1–16. doi: 10.1186/s12870-019-1992-7

Jahan, M. S., Guo, S., Sun, J., Shu, S., Wang, Y., Abou El-Yazied, A., et al. (2021). Melatonin-mediated photosynthetic performance of tomato seedlings under high-temperature stress. *Plant Physiology and Biochemistry* 167, 309–320.

Jan, S., Singh, B., Bhardwaj, R., Singh, R., Mansoor, S., and Ahmad, P. (2022). Recent advances on the pragmatic roles of phytomelatonin and its exogenous application for abiotic stress management in plants. *J. Plant Growth Regul.*, 1–16. doi: 10.1007/s00344-022-10766-3

Jia, C., Yu, X., Zhang, M., Liu, Z., Zou, P., Ma, J., et al. (2020). Application of melatonin-enhanced tolerance to high-temperature stress in cherry radish (*Raphanus sativus* l. var. radculus pers). *J. Plant Growth Regul.* 39, 631–640. doi: 10.1007/s00344-019-10006-1

Johns, N. P., Johns, J., Porasuphatana, S., Plaimee, P., and Sae-Teaw, M. (2013). Dietary intake of melatonin from tropical fruit altered urinary excretion of 6- sulfatoxymelatonin in healthy volunteers. *J. Agricul. Food Chem.* 61, 913–919. doi: 10.1021/jf300359a

Kamiab, F. (2020). Exogenous melatonin mitigates the salinity damages and improves the growth of pistachio under salinity stress. *J. Plant Nutr.* 43, 1468–1484. doi: 10.1080/01904167.2020.1730898

Karaca, P., and Cekic, F. O. (2019). Exogenous melatonin-stimulated defense responses in tomato plants treated with polyethylene glycol. *Int. J. Veg. Sci.* 25, 601–609. doi: 10.1080/19315260.2019.1575317

Kaya, C., Sarıoglu, A., Ashraf, M., Alyemeni, M. N., and Ahmad, P. (2022). The combined supplementation of melatonin and salicylic acid effectively detoxifies arsenic toxicity by modulating phytochelatins and nitrogen metabolism in pepper plants. *Environ. Poll.* 297, 118727. doi: 10.1016/j.envpol.2021.118727

Khan, T. A., Fariduddin, Q., and Yusuf, M. (2015). *Lycopersicon esculentum* under low temperature stress: an approach toward enhanced antioxidants and yield. *Environ. Sci. Poll. Res.* 22, 14178–14188. doi: 10.1007/s11356-015-4658-5

Kobylińska, A., Reiter, R. J., and Posmyk, M. M. (2017). Melatonin protects cultured tobacco cells against lead-induced cell death *via* inhibition of cytochrome c translocation. *Front. Plant Sci.* 8, 1560. doi: 10.3389/fpls.2017.01560

Kolář, J., and Macháčková, I. (2005). Melatonin in higher plants: occurrence and possible functions. *J. Pineal Res.* 39, 333–341. doi: 10.1111/j.1600-079X.2005.00276.x

Korkmaz, A., DEĞER, Ö., SZAFRAŃSKA, K., KÖKLÜ, Ş., KARACA, A., YAKUPOĞLU, G., et al. (2021). Melatonin effects in enhancing chilling stress tolerance of pepper. *Sci. Hortic.* 289, 110434. doi: 10.1016/j.scienta.2021.110434

Koza, N. A., Adedayo, A. A., Babalola, O. O., and Kappo, A. P. (2022). Microorganisms in plant growth and development: Roles in abiotic stress tolerance and secondary metabolites secretion. *Microorganisms* 10, 1528. doi: 10.3390/microorganisms10081528

Li, H., Chang, J., Chen, H., Wang, Z., Gu, X., Wei, C., et al. (2017a). Exogenous melatonin confers salt stress tolerance to watermelon by improving photosynthesis and redox homeostasis. *Front. Plant Sci.* 8, 295. doi: 10.3389/fpls.2017.00295

Li, H., Chang, J., Zheng, J., Dong, Y., Liu, Q., Yang, X., et al. (2017b). Local melatonin application induces cold tolerance in distant organs of *Citrullus lanatus* l. *Via* long distance transport. *Sci. Rep.* 7, 40858. doi: 10.1038/srep40858

Li, J., Li, Y., Tian, Y., Qu, M., Zhang, W., and Gao, L. (2017c). Melatonin has the potential to alleviate cinnamic acid stress in cucumber seedlings. *Front. Plant Sci.* 8, 1193.

Li, C., Wang, P., Wei, Z., Liang, D., Liu, C., Yin, L., et al. (2012). The mitigation effects of exogenous melatonin on salinity-induced stress in malus hupehensis. *J. Pineal Res.* 53, 298–306. doi: 10.1111/j.1600-079X.2012.00999.x

Li, X., Wei, J. P., Scott, E. R., Liu, J. W., Guo, S., Li, Y., et al. (2018). Exogenous melatonin alleviates cold stress by promoting antioxidant defense and redox homeostasis in *Camellia sinensis* l. *Molecules* 23, 165. doi: 10.3390/molecules23010165

Li, J., Xie, J., Yu, J., Lyv, J., Zhang, J., Ding, D., et al. (2022a). Melatonin enhanced low-temperature combined with low-light tolerance of pepper (*Capsicum annuum* l.) seedlings by regulating root growth, antioxidant defense system, and osmotic adjustment. *Front. Plant Sci.* 13, 998293. doi: 10.3389/fpls.2022.998293

Li, J., Yang, Y., Sun, K., Chen, Y., Chen, X., and Li, X. (2019). Exogenous melatonin enhances cold, salt and drought stress tolerance by improving antioxidant defense in tea plant (*Camellia sinensis* (L.) o. kuntze). *Molecules* 24, 1826. doi: 10.3390/molecules24091826

Li, M., Zhou, J., Du, J., Li, X., Sun, Y., Wang, Z., et al. (2022b). Comparative physiological and transcriptomic analyses of improved heat stress tolerance in celery (*Apium graveolens* l.) caused by exogenous melatonin. *Int. J. Mol. Sci.* 23, 11382. doi: 10.3390/ijms231911382

Liang, D., Gao, F., Ni, Z., Lin, L., Deng, Q., Tang, Y., et al. (2018). Melatonin improves heat tolerance in kiwifruit seedlings through promoting antioxidant enzymatic activity and glutathione s-transferase transcription. *Molecules* 23 (3), 584. doi: 10.3390/molecules23030584

Liang, S. M., Kuang, J. F., Ji, S. J., Chen, Q. F., Deng, W., Min, T., et al. (2020). The membrane lipid metabolism in horticultural products suffering chilling injury. *Food Qual. Safety.* 4, 9–14. doi: 10.1093/fqsafe/fyaa001

Liang, D., Ni, Z., Xia, H., Xie, Y., Lv, X., Wang, J., et al. (2019). Exogenous melatonin promotes biomass accumulation and photosynthesis of kiwifruit seedlings under drought stress. *Sci. Hortic.* 246, 34–43. doi: 10.1016/j.scienta.2018.10.058

Liu, N., Jin, Z., Wang, S., Gong, B., Wen, D., Wang, X., et al. (2015b). Sodic alkaline stress mitigation with exogenous melatonin involves reactive oxygen metabolism and ion homeostasis in tomato. *Sci. Hortic.* 181, 18–25. doi: 10.1016/j.scienta.2014.10.049

Liu, L., Li, D., Ma, Y., Shen, H., Zhao, S., and Wang, Y. (2021). Combined application of arbuscular mycorrhizal fungi and exogenous melatonin alleviates drought stress and improves plant growth in tobacco seedlings. *J. Plant Growth Regul.* 40, 1074–1087. doi: 10.1007/s00344-020-10165-6

Liu, D. D., Sun, X. S., Liu, L., Shi, H. D., Chen, S. Y., and Zhao, D. K. (2019). Overexpression of the melatonin synthesis-related gene SlCOMT1 improves the resistance of tomato to salt stress. *Molecules* 24, 1514. doi: 10.3390/molecules24081514

Liu, J., Wang, W., Wang, L., and Sun, Y. (2015a). Exogenous melatonin improves seedling health index and drought tolerance in tomato. *Plant Growth Regul.* 77, 317–326. doi: 10.1007/s10725-015-0066-6

Liu, J., Wu, H., Wang, B., Zhang, Y., Wang, J., Cheng, C., et al. (2022). Exogenous melatonin enhances cold resistance by improving antioxidant defense and cold-responsive genes' expression in banana. *Horticulturae* 8, 260. doi: 10.3390/horticulturae8030260

Malhotra, S. K. (2017). Horticultural crops and climate change: A review. *Indian J. Agricul Sci.* 87, 12–22.

Manafi, H., Baninasab, B., Gholami, M., Talebi, M., and Khanizadeh, S. (2022). Exogenous melatonin alleviates heat-induced oxidative damage in strawberry (*Fragaria× ananassa* duch. cv. ventana) plant. *J. Plant Growth Regul.* 41, 52–64. doi: 10.1007/s00344-020-10279-x

Marino, S. (2021). Horticultural crop response to different environmental and nutritional stress. *Horticulturae* 7, 240. doi: 10.3390/horticulturae7080240

Marta, B., Szafra ´nska, K., and Posmyk, M. M. (2016). Exogenous melatonin improves antioxidant defense in cucumber seeds (*Cucumis sativus* l.) germinated under chilling stress. *Front. Plant Sci.* 7, 575. doi: 10.3389/fpls.2016.00575

Martinez, V., Nieves-Cordones, M., Lopez-Delacalle, M., Rodenas, R., Mestre, T. C., Garcia-Sanchez, F., et al. (2018). Tolerance to stress combination in tomato plants: New insights in the protective role of melatonin. *Molecules* 23, 535. doi: 10.3390/molecules23030535

Meng, J. F., Xu, T. F., Wang, Z. Z., Fang, Y. L., Xi, Z. M., and Zhang, Z. W. (2014). The ameliorative effects of exogenous melatonin on grape cuttings under water-deficient stress: antioxidant metabolites, leaf anatomy, and chloroplast morphology. *J. Pineal Res.* 57, 200–212. doi: 10.1111/jpi.12159

Moussa, H. R., and Algamal, S. M. A. (2017). Does exogenous application of melatonin ameliorate boron toxicity in spinach plants? *Int. J. Vegetable Sci.* 23, 233–245. doi: 10.1080/19315260.2016.1243184

Moustafa-Farag, M., Mahmoud, A., Arnao, M. B., Sheteiwy, M. S., Dafea, M., Soltan, M., et al. (2020). Melatonin-induced water stress tolerance in plants: Recent advances. *Antioxidants* 9, 809. doi: 10.3390/antiox9090809

Muhammad, F., Raza, M. A. S., Iqbal, R., Zulfiqar, F., Aslam, M. U., Yong, J. W. H., et al. (2022). Ameliorating drought effects in wheat using an exclusive or Co-applied rhizobacteria and ZnO nanoparticles. *Biology* 11, 1564. doi: 10.3390/biology11111564

Murch, S. J., and Saxena, P. K. (2002). Melatonin: a potential regulator of plant growth and development? *In Vitro Cell. Dev. Biology-Plant* 38, 531–536. doi: 10.1079/IVP2002333

Nabaei, M., and Amooaghaie, R. (2019). Nitric oxide is involved in the regulation of melatonin-induced antioxidant responses in *Catharanthus roseus* roots under cadmium stress. *Botany* 97, 681–690. doi: 10.1139/cjb-2019-0107

Nawaz, M. A., Jiao, Y., Chen, C., Shireen, F., Zheng, Z., Imtiaz, M., et al. (2018). Melatonin pretreatment improves vanadium stress tolerance of watermelon seedlings by reducing vanadium concentration in the leaves and regulating melatonin biosynthesis and antioxidant-related gene expression. *J. Plant Physiol.* 220, 115–127. doi: 10.1016/j.jplph.2017.11.003

Nawaz, F., Shabbir, R. N., Shahbaz, M., Majeed, S., Raheel, M., Hassan, W., et al. (2017). Cross talk between nitric oxide and phytohormones regulate plant development during abiotic stresses. phytohormones: signaling mechanisms and crosstalk in plant development and stress responses. *InTech Rijeka*, 117–141. doi: 10.5772/intechopen.69812

Nawaz, M. A., Huang, Y., Bie, Z., Ahmed, W., Reiter, R. J., Niu, M., et al. (2016). Melatonin: current status and future perspectives in plant science. *Front. Plant Sci.* 6, 1230.

Noor, I., Sohail, H., Sun, J., Nawaz, M. A., Li, G., Hasanuzzaman, M., et al. (2022). Heavy metal and metalloid toxicity in horticultural plants: Tolerance mechanism and remediation strategies. *Chemosphere* 303, 135196. doi: 10.1016/j.chemosphere.2022.135196

Paredes, S. D., Korkmaz, A., Manchester, L. C., Tan, D. X., and Reiter, R. J. (2009). Phytomelatonin: a review. *J. Exp. Botany.* 60, 57–69. doi: 10.1093/jxb/ern284

Park, S., and Back, K. (2012). Melatonin promotes seminal root elongation and root growth in transgenic rice after germination. *J. Pineal Res.* 53, 385–389. doi: 10.1111/j.1600-079X.2012.01008.x

Peng, X., Wang, N., Sun, S., Geng, L., Guo, N., Liu, A., et al. (2023). Reactive oxygen species signaling is involved in melatonin-induced reduction of chlorothalonil residue in tomato leaves. *J. Hazardous Mater.* 443, 130212. doi: 10.1016/j.jhazmat.2022.130212

Posmyk, M. M., Balabusta, M., and Wieczorek, M. (2009). Melatonin applied to cucumber (*Cucumis sativus* l.) seeds improves germination during chilling stress. *J. Pineal Res.* 46, 214–223. doi: 10.1111/j.1600-079X.2008.00652.x

Posmyk, M. M., Kuran, H., Marciniak, K., and Janas, K. M. (2008). Pre sowing seed treatment with melatonin protects red cabbage seedlings against toxic copper ion concentrations. *J. Pineal Res.* 45, 24–31. doi: 10.1111/j.1600-079X.2007.00552.x

Qari, S. H., Hassan, M. U., Chattha, M. U., Mahmood, A., Naqve, M., Nawaz, M., et al. (2022). Melatonin induced cold tolerance in plants: Physiological and molecular responses. *Front. Plant Sci.* 13, 843071. doi: 10.3389/fpls.2022.843071

Rasheed, A., Gill, R. A., Hassan, M. U., Mahmood, A., Qari, S., and Zaman, Q. U. (2021). A critical review: recent advancements in the use of CRISPR/Cas9 technology to enhance crops and alleviate global food crises. *Curr. Issues Mol. Biol.* 43, 1950–1976. doi: 10.3390/cimb43030135

Sadak, M. S., Abdalla, A. M., Abd Elhamid, E. M., and Ezzo, M. I. (2020). Role of melatonin in improving growth, yield quantity and quality of *Moringa oleifera* l. plant under drought stress. *Bull. Natl. Res. Cent.* 44, 1–13. doi: 10.1186/s42269-019-0259-7

Sarafi, E., Tsouvaltzis, P., Chatzissavvidis, C., Siomos, A., and Therios, I. (2017). Melatonin and resveratrol reverse the toxic effect of high boron (B) and modulate biochemical parameters in pepper plants (*Capsicum annuum* l.). *Plant Physiol. Biochem.* 112, 173–182. doi: 10.1016/j.plaphy.2016.12.018

Shahid, R., Shijie, L., Shahid, S., Altaf, M. A., and Shahid, H. (2021). Determinants of reactive adaptations to climate change in semi-arid region of Pakistan. *J. Arid Environ.* 193, 104580. doi: 10.1016/j.jaridenv.2021.104580

Shakoor, A., Abdullah, M., Sarfraz, R., Altaf, M. A., and Batool, S. (2017). A comprehensive review on phytoremediation of cadmium (Cd) by mustard (*Brassica juncea* l.) and sunflower (*Helianthus annuus* l.). *J. Biodiversity Environ. Sci.* 10, 88–98.

Sharif, R., Xie, C., Zhang, H., Arnao, M. B., Ali, M., Ali, Q., et al. (2018). Melatonin and its effects on plant systems. *Molecules* 23, 2352. doi: 10.3390/molecules23092352

Sharma, A., Wang, J., Xu, D., Tao, S., Chong, S., and Yan, D. (2020). Melatonin regulates the functional components of photosynthesis, antioxidant system, gene expression, and metabolic pathways to induce drought resistance in grafted *Carya cathayensis* plants. *Sci. Total Environ.* 713, 136675. doi: 10.1016/j.scitotenv.2020.136675

Sharma, A., and Zheng, B. (2019). Melatonin mediated regulation of drought stress: Physiological and molecular aspects. *Plants* 8, 190. doi: 10.3390/plants8070190

Shi, H., Reiter, R. J., Tan, D. X., and Chan, Z. (2015). INDOLE-3-ACETIC ACID INDUCIBLE 17 positively modulates natural leaf senescence through melatonin-mediated pathway in *Arabidopsis*. *J. Pineal Res.* 58, 26–33. doi: 10.1111/jpi.12188

Siddiqui, M. H., Alamri, S., Alsubaie, Q. D., and Ali, H. M. (2020a). Melatonin and gibberellic acid promote growth and chlorophyll biosynthesis by regulating antioxidant and methylglyoxal detoxification system in tomato seedlings under salinity. *J. Plant Growth Regul.* 39, 1488–1502. doi: 10.1007/s00344-020-10122-3

Siddiqui, M. H., Alamri, S., Alsubaie, Q. D., Ali, H. M., Ibrahim, A. A., and Alsadon, A. (2019). Potential roles of melatonin and sulfur in alleviation of lanthanum toxicity in tomato seedlings. *Ecotoxicol. Environ. Saf.* 180, 656–667. doi: 10.1016/j.ecoenv.2019.05.043

Siddiqui, M. H., Alamri, S., Khan, M. N., Corpas, F. J., Al-Amri, A. A., Alsubaie, Q. D., et al. (2020b). Melatonin and calcium function synergistically to promote the resilience through ROS metabolism under arsenic-induced stress. *J. Hazardous Mat.* 398, 122882. doi: 10.1016/j.jhazmat.2020.122882

Szafrańska, K., Reiter, R. J., and Posmyk, M. M. (2017). Melatonin improves the photosynthetic apparatus in pea leaves stressed by paraquat *via* chlorophyll breakdown regulation and its accelerated *de novo* synthesis. *Front. Plant Sci.* 8, 878. doi: 10.3389/fpls.2017.00878

Tabassum, S., Ossola, A., Marchin, R. M., Ellsworth, D. S., and Leishman, M. R. (2021). Assessing the relationship between trait-based and horticultural classifications of plant responses to drought. *Urban Forestry Urban Greening* 61, 127109. doi: 10.1016/j.ufug.2021.127109

Tan, D. X., Manchester, L. C., Liu, X., Rosales-Corral, S. A., Acuna-Castroviejo, D., and Reiter, R. J. (2013). Mitochondria and chloroplasts as the original sites of melatonin synthesis: a hypothesis related to melatonin's primary function and evolution in eukaryotes. *J. Pineal Res.* 54, 127–138. doi: 10.1111/jpi.12026

Tang, Y., Lei, F., Zhang, E., and Li, H. (2016). "Soaking seeds with exogenous melatonin improve photosynthetic characteristics of radish under aluminum stress," in *2016 2nd International Conference on Advances in Energy, Environment and Chemical Engineering (AEECE 2016)*. 64–67 (Atlantis Press).

Tang, Y., Li, J., and Li, H. (2015). "Effects of exogenous melatonin on photosynthetic characteristics of eggplant (*Solanum melongena* l.) under cadmium stress," in *2015 6th International Conference on Manufacturing Science and Engineering*. 656–659 (Atlantis Press).

Tang, M., Xu, L., Wang, Y., Dong, J., Zhang, X., Wang, K., et al. (2021). Melatonin-induced DNA demethylation of metal transporters and antioxidant genes alleviates lead stress in radish plants. *Hortic. Res.* 8, 124. doi: 10.1038/s41438-021-00561-8

Tiwari, R. K., Kumar, R., Lal, M. K., Kumar, A., Altaf, M. A., Devi, R., et al. (2022). Melatonin-polyamine interplay in the regulation of stress responses in plants. *J. Plant Growth Regul.*, 1–17. doi: 10.1007/s00344-022-10717-y

Tiwari, R. K., Lal, M. K., Naga, K. C., Kumar, R., Chourasia, K. N., Subhash, S., et al. (2020). Emerging roles of melatonin in mitigating abiotic and biotic stresses of horticultural crops. *Sci. Hortic.* 272, 109592. doi: 10.1016/j.scienta.2020.109592

Toscano, S., Franzoni, G., and Álvarez, S. (2023). Drought stress in horticultural plants. *Horticulturae* 9, 7. doi: 10.3390/horticulturae9010007

Ulhassan, Z., Huang, Q., Gill, R. A., Ali, S., Mwamba, T. M., Ali, B., et al. (2019). Protective mechanisms of melatonin against selenium toxicity in Brassica napus: insights into physiological traits, thiol biosynthesis and antioxidant machinery. *BMC Plant Biol.* 19, 1–16.

Wahid, A., Farooq, M., Hussain, I., Rasheed, R., and Galani, S. (2012). "Responses and management of heat stress in plants," in *Environmental adaptations and stress tolerance of plants in the era of climate change*. 135–157 (New York, NY: Springer).

Wang, M., Duan, S., Zhou, Z., Chen, S., and Wang, D. (2019). Foliar spraying of melatonin confers cadmium tolerance in nicotiana tabacum l. *Ecotoxicol. Environ. Safety.* 170, 68–76. doi: 10.1016/j.ecoenv.2018.11.127

Wang, P., Yin, L., Liang, D., Li, C., Ma, F., and Yue, Z. (2012). Delayed senescence of apple leaves by exogenous melatonin treatment: toward regulating the ascorbate-glutathione cycle. *J. Pineal Res.* 53, 11–20. doi: 10.1111/j.1600-079X.2011.00966.x

Wang, M., Zhang, S., and Ding, F. (2020). Melatonin mitigates chilling-induced oxidative stress and photosynthesis inhibition in tomato plants. *Antioxidants* 9, 218. doi: 10.3390/antiox9030218

Wang, L., Liu, J., Wang, W., and Sun, Y. (2016). Exogenous melatonin improves growth and photosynthetic capacity of cucumber under salinity-induced stress. *Photosynthetica* 54, 19–27.

Wu, X., Ren, J., Huang, X., Zheng, X., Tian, Y., Shi, L., et al. (2021). Melatonin: Biosynthesis, content, and function in horticultural plants and potential application. *Sci. Hortic.* 288, 110392. doi: 10.1016/j.scienta.2021.110392

Wu, S., Wang, Y., Zhang, J., Gong, X., Zhang, Z., Sun, J., et al. (2021). Exogenous melatonin improves physiological characteristics and promotes growth of strawberry seedlings under cadmium stress. *Hortic. Plant J.* 7, 13–22. doi: 10.1016/j.hpj.2020.06.002

Xalxo, R., and Keshavkant, S. (2019). Melatonin, glutathione and thiourea attenuates lead and acid rain-induced deleterious responses by regulating gene expression of antioxidants in *Trigonella foenum graecum* l. *Chemosphere* 221, 1–10. doi: 10.1016/j.chemosphere.2019.01.029

Xia, H., Ni, Z., Hu, R., Lin, L., Deng, H., Wang, J., et al. (2020). Melatonin alleviates drought stress by a non-enzymatic and enzymatic antioxidative system in kiwifruit seedlings. *Int. J. Mol Sci.* 21, 852. doi: 10.3390/ijms21030852

Xu, L., Xiang, G., Sun, Q., Ni, Y., Jin, Z., Gao, S., et al. (2019). Melatonin enhances salt tolerance by promoting MYB108A-mediated ethylene biosynthesis in grapevines. *Hortic. Res.* 6, 114. doi: 10.1038/s41438-019-0197-4

Xu, L., Zhang, F., Tang, M. J., Wang, Y., and Dong, J. H. (2020). Melatonin confers cadmium tolerance by modulating critical heavy metal chelators and transporters in radish plants. *J. Pineal Res.* 69, e12659. doi: 10.1111/jpi.12659

Yakuboğlu, G., Ardıç, Ş.K., Cuci, Y., and Korkmaz, A. (2022). Mitigation of chilling stress effects on eggplant seedlings by exogenous application of melatonin. *Turkish J. Agriculture-Food Sci. Technol.* 10, 562–568. doi: 10.24925/turjaf.v10i4.562-568.4601

Yang, X. L., Xu, H., Li, D., Gao, X., Li, T. L., and Wang, R. (2018). Effect of melatonin priming on photosynthetic capacity of tomato leaves under low temperature stress. *Photosynthetica* 56, 884–892. doi: 10.1007/s11099-017-0748-6

Zahedi, S. M., Hosseini, M. S., Abadía, J., and Marjani, M. (2020). Melatonin foliar sprays elicit salinity stress tolerance and enhance fruit yield and quality in strawberry (*Fragaria× ananassa* duch.). *Plant Physiol. Biochem.* 149, 313–323. doi: 10.1016/j.plaphy.2020.02.021

Zamani, Z., Amiri, H., and Ismaili, A. (2020). Improving drought stress tolerance in fenugreek (*Trigonella foenum-graecum*) by exogenous melatonin. *Plant Biosystems-An Int. J. Dealing Aspects Plant Biol.* 154 (5), pp.643–pp.655. doi: 10.1080/11263504.2019.1674398

Zhan, H., Nie, X., Zhang, T., Li, S., Wang, X., Du, X., et al. (2019). Melatonin: a small molecule but important for salt stress tolerance in plants. *Int. J. Mol. Sci.* 20, 709. doi: 10.3390/ijms20030709

Zhang, T., Shi, Z., Zhang, X., Zheng, S., Wang, J., and Mo, J. (2020). Alleviating effects of exogenous melatonin on salt stress in cucumber. *Sci. Hortic.* 262, 109070. doi: 10.1016/j.scienta.2019.109070

Zhang, Y. P., Xu, S., Yang, S. J., and Chen, Y. Y. (2017b). Melatonin alleviates cold-induced oxidative damage by regulation of ascorbate–glutathione and proline metabolism in melon seedlings (*Cucumis melo* l.). *J. Horticul. Sci. Biotechnol.* 92, 313–324. doi: 10.1080/14620316.2016.1266915

Zhang, Y. P., Yang, S. J., and Chen, Y. Y. (2017c). Effects of melatonin on photosynthetic performance and antioxidants in melon during cold and recovery. *Biol. Plant* 61, 571–578. doi: 10.1007/s10535-017-0717-8

Zhang, N., Zhang, H. J., Sun, Q. Q., Cao, Y. Y., Li, X., Zhao, B., et al. (2017a). Proteomic analysis reveals a role of melatonin in promoting cucumber seed germination under high salinity by regulating energy production. *Sci. Rep.* 7, 1–15. doi: 10.1038/s41598-017-00566-1

Zhang, H. J., Zhang, N., Yang, R. C., Wang, L., Sun, Q. Q., and Li, D. B. (2014). Melatonin promotes seed germination under high salinity by regulating antioxidant systems, ABA and GA4 interaction in cucumber (*Cucumis sativus* l.). *J. Pineal Res.* 57, 269–279. doi: 10.1111/jpi.12167

Zhang, N., Zhao, B., Zhang, H. J., Weeda, S., Yang, C., Yang, Z. C., et al. (2013). Melatonin promotes water-stress tolerance, lateral root formation, and seed germination in cucumber (*Cucumis sativus* l.). *J. Pineal Res.* 54, 15–23. doi: 10.1111/j.1600-079X.2012.01015.x

Zhao, C., Nawaz, G., Cao, Q., and Xu, T. (2022). Melatonin is a potential target for improving horticultural crop resistance to abiotic stress. *Sci. Hortic.* 291, 110560. doi: 10.1016/j.scienta.2021.110560

Zhao, H., Ye, L., Wang, Y., Zhou, X., Yang, J., Wang, J., et al. (2016). Melatonin increases the chilling tolerance of chloroplast in cucumber seedlings by regulating photosynthetic electron flux and the ascorbate-glutathione cycle. *Front. Plant Sci.* 7, 1814. doi: 10.3389/fpls.2016.01814

Zhao, H., Zhang, K., Zhou, X., Xi, L., Wang, Y., Xu, H., et al. (2017). Melatonin alleviates chilling stress in cucumber seedlings by up-regulation of CsZat12 and modulation of polyamine and abscisic acid metabolism. *Sci. Rep.* 7, 1–12. doi: 10.1038/s41598-017-05267-3

Zhou, R., Wan, H., Jiang, F., Li, X., Yu, X., Rosenqvist, E., et al. (2020). The alleviation of photosynthetic damage in tomato under drought and cold stress by high CO_2 and melatonin. *Int. J. Mol. Sci.* 21, 5587. doi: 10.3390/ijms21155587

Zörb, C., Geilfus, C., and Dietz, K. (2018). Salinity and crop yield. *Plant Biol.* 21, 31–38. doi: 10.1111/plb.12884

Zulfiqar, F., Nafees, M., Chen, J., Darras, A., Ferrante, A., Hancock, J. T., et al. (2022). Chemical priming enhances plant tolerance to salt stress. *Front. Plant Sci.* 13, 946922. doi: 10.3389/fpls.2022.946922

frontiers | Frontiers in Plant Science

TYPE Original Research
PUBLISHED 27 May 2023
DOI 10.3389/fpls.2023.1193666

OPEN ACCESS

EDITED BY
Vijay Gahlaut,
Chandigarh University, India

REVIEWED BY
Anis Ali Shah,
University of Education Lahore, Pakistan
Md. Kamrul Hasan,
Sylhet Agricultural University, Bangladesh
Shah Fahad,
The University of Haripur, Pakistan
Ravinder Kumar,
Central Potato Research Institute (ICAR),
India

*CORRESPONDENCE
Xun Bo Zhou
✉ xunbozhou@gmail.com

RECEIVED 25 March 2023
ACCEPTED 04 July 2023
PUBLISHED 27 May 2023

CITATION
Jahan MS, Zhao CJ, Shi LB, Liang XR, Jabborova D, Nasar J and Zhou XB (2023) Physiological mechanism of melatonin attenuating to osmotic stress tolerance in soybean seedlings.
Front. Plant Sci. 14:1193666.
doi: 10.3389/fpls.2023.1193666

Physiological mechanism of melatonin attenuating to osmotic stress tolerance in soybean seedlings

Mohammad Shah Jahan[1,2], Chang Jiang Zhao[1], Li Bo Shi[3], Xiu Ren Liang[4], Dilfuza Jabborova[5], Jamal Nasar[1] and Xun Bo Zhou[1]*

[1]Guangxi Key Laboratory of Agro-environment and Agro-products Safety, Key Laboratory of Crop Cultivation and Physiology, College of Agriculture, Guangxi University, Guangxi, Nanning, China, [2]Department of Horticulture, Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh, [3]MAP Division (Shandong) of Sinochem Agriculture Holdings, Jinan, China, [4]Guangxi Ecoengineering Vocational and Technical College, Liuzhou, China, [5]Laboratory of Medicinal Plants Genetics and Biotechnology, Institute of Genetics and Plant Experimental Biology, Academy of Sciences of Uzbekistan, Tashkent, Uzbekistan

Drought is one of the most significant abiotic stress threatening to crop production worldwide. Soybean is a major legume crop with immense economic significance, but its production is highly dependent on optimum rainfall or abundant irrigation. As the global climate changes, it is more important to find solutions to make plants more resilient to drought. The prime aimed of the study is to investigate the effect of melatonin on drought tolerance in soybean and its potential mechanisms. Soybean seedlings were treated with 20% polyethylene glycol 6000 (PEG 6000) and subjected to osmotic stress (14 days) with or without 100 μM melatonin treatment. Our results revealed that melatonin supplementation significantly mitigated PEG-induced growth retardation and increased water absorption ability. Foliar application of melatonin also increased gas exchange and the chlorophyll fluorescence attributes by the mitigation of the osmotic-induced reduction of the reaction activity of photosystems I and II, net photosynthetic rate (Pn), stomatal conductance (Gs), transpiration rate (Tr), electron transport activity, and photosynthetic efficiency. In addition, PEG-induced elevated production of reactive oxygen species (ROS) and malondialdehyde (MDA) content were significantly reversed by melatonin treatment. Equally important, melatonin boosted the antioxidant activities of soybean plants. Moreover, osmotic stress substantially increased abscisic acid (ABA) accumulation in roots and leaves, while melatonin-received plant leaves accumulated less ABA but roots content higher ABA. Similarly, melatonin significantly suppressed ABA biosynthesis and signaling gene expression in soybean exposed to drought stress. Furthermore, osmotic stress significantly suppressed plasmalemma (*GmPIPs*) and tonoplast aquaporin (*GmTIPs*) genes expression, and their transcript abundance was upregulated by melatonin co-addition. Taken together, our results indicated that melatonin potentially improves drought tolerance of soybean through the regulation of ABA and aquaporin gene expression, increasing photosynthetic efficiency as well as enhancing water uptake efficiency.

KEYWORDS

Aquaporins, ABA metabolism, soybean, drought, climate change, water use efficiency

1 Introduction

Soybean (*Glycine max* L.) is an important oil seed legume and forage crop, rich in protein (40%) and edible oil (20%), cultivated globally for its valuable seed composition (Sharmin et al., 2020). The annual global soybean production in 2019 was estimated to exceed 333 million tonnes (FAOSTAT, 2019). Soybean is mainly grown in tropical, subtropical, and temperate regions (FAO, 2021). It is very sensitive to water scarcity and suffers greatly in terms of growth and development under water deficit conditions (Bortoluzzi et al., 2020). Consequently, global warming and changes in precipitation patterns pose a significant threat to soybean production, especially in areas lacking of rainwater or irrigation (Cotrim et al., 2021). It is well known that soybean yield can be reduced under dry conditions (>50%), resulting in substantial economic losses for farmers and growers (Soltani et al., 2017). Hence, drought is a significant climatic risk requiring effective mitigation strategies to sustain the global soybean supply. Over the past two decades, surface water and groundwater have been rapidly declining in China, approaching the threshold of internationally recognized water resources (Chai et al., 2014). Osmotic stress has profound negative effect on plants growth and development as well as it substantially decrease the pigment contents (Altaf et al., 2022). Malondialdehyde (MDA) is a product of membrane peroxidation and is commonly used as a prototypical symbol of membrane damage in stressed cells (Wang et al., 2018). The key antioxidant enzymes superoxide dismutase (SOD), peroxidase (POD), and glutathione-s-transferase (GST) are actively participated against stress environments (Alharby and Fahad, 2020; Alam et al., 2022). The increased antioxidant enzyme activity can decrease MDA accumulation and reduce stress-induced cellular structural damage (Ahmad et al., 2014). Osmotic regulation is an essential physiological process for plants in response to water deprivation (Iqbal et al., 2018). The most important osmolytes in plants are soluble saccharides and proline, which are required to maintain stable cellular outcomes under osmotic stress conditions (Dutta et al., 2019). Under abiotic stress conditions, increased production of reactive oxygen species (ROS) changes the phytohormone proteome and regulates the gene expression (Dalal et al., 2018). Drought stress can cause an imbalance in intracellular ROS production, leading to modulates oxidative stress responses (Raja et al., 2017).

Multiple endogenous hormones and metabolic components collaborate to govern the growth and development of plants (Dong et al., 2019). Abscisic acid is an important phytohormone that plays a key role in stress responses in different plants, generally elevates in plants exposed to drought stress which controls stomatal opening and closing and enhances the hydrophobic water holding capacity (Hossain et al., 2015). Hence, decrease in stomatal opening causes lowered CO_2 assimilation and reduced photosynthesis activity, which imparts the growth and development of plants (Mutava et al., 2015; Cohen et al., 2021). Aquaporins (AQPs) are tiny integral membrane proteins that enable water transport across cells and contribute to water homeostasis in plants. Numerous physiological and genetic studies have demonstrated that AQPs are responsible for short-term alterations in root hydraulics and leaf water relations (Shivaraj et al., 2021). Among the recognized AQP subfamilies, members of the plasma membrane intrinsic protein (*PIP*) and the tonoplast intrinsic protein (*TIP*) are widely reported for their significant role under stress conditions (Shivaraj et al., 2021; Sudhakaran et al., 2021). Arabidopsis plants exposed to water deficit condition have shown down regulation of *TIPs* expression in leaves but only one *PIP (PIP2;5)* showed higher expression (Alexandersson et al., 2010). The expression of the strawberry *FvPIP2;1* and *FvPIP2;2* genes was observed both in leaves and roots, where, *FvPIP1;1* expression was only found in roots under drought stress conditions (Šurbanovski et al., 2013). Unlike *PIPs*, barley leaves exposed to 14 days of drought stress has shown differential expression of *TIPs* (Kurowska et al., 2019). Interestingly, PIPs and TIPs are widely known aquaporins involved in transporting H_2O_2 in a stressful environment (Carvalho, 2008). The efficacy of *AtTIP1;1*, *AtTIP1;2*, and *AtTIP2;3* to transport H_2O_2 has been investigated by applying a yeast experiment (Bienert et al., 2007). In another study, *CsTIP2; 1* overexpressed tobacco plants increased drought tolerance, and it's strongly associated with the restriction of H_2O_2 production. These investigations show that *TIPs*, are involved in the implication of H_2O_2 into the vacuoles or strengthen the antioxidative system to quench the excess ROS, ultimately offering drought resilience to plants.

Many techniques, such as candidate genes, breeding, and multi-omics approaches, could be used to develop transgenic soybean lines which are more tolerant to drought stress (Dubey et al., 2018). Despite the strategies mentioned above, the application of exogenous hormones and/or biostimulants offers a simpler and more cost-effective strategy to improve the resilience of plants to adverse consequences of different abiotic stresses, including drought stress (Khan et al., 2019; Shahzad et al., 2023). Among them, melatonin is a ubiquitous master-class plant growth regulator, playing significant role in the regulation of plant growth and development under stressful environments (Tiwari et al., 2021). Melatonin promotes root and shoot formation, inhibits chlorophyll breakdown/degradation, slows the senescence process, and reduces ROS generation (Arnao and Hernández-Ruiz, 2019). Melatonin markedly improved the ability of tomato seedlings to withstand drought conditions by improving stomatal conductivity, photosynthetic rate, quantum PSII (Fv/Fm), transpiration rate, and electron transport (Liu et al., 2015). Besides this, melatonin increases the expression of abscisic acid (ABA) catabolism genes (two *CYP707* monooxygenases) while decreasing the expression of *NCED*, a vital enzyme in ABA production, leading to sharp reduction in ABA concentrations under stressful circumstances (Zhang et al., 2014).

The implication of melatonin on aquaporin gene expression has also been studied in plants, but its findings are very limited. Though aquaporins are one of the core transporters controlling the hydraulic conductance of roots (Deshmukh et al., 2017), and melatonin assists in mitigating osmotic stress, so melatonin and aquaporins are inextricably linked with each other (Rajora et al., 2022). Supplementation with melatonin improved the efficiency of root water absorption in rice through the regulation of plasma membrane intrinsic proteins (PIPs). Another study on maize showed that melatonin application enhances entire hydraulic conductance in particular roots through upregulation of *PIPs* expression (Qiao et al., 2020; Tiwari et al., 2021). However, in-depth studies on the dynamics of aquaporin expression under osmotic stress in the presence of melatonin are inadequate. Abscisic acid generally plays an essential function in AQP modulation and root water uptake in plants confronting diverse nitrogen forms and/or water deficit conditions (Parent et al., 2009). Melatonin's impact on ABA dynamics, including how ABA content controls AQP expression and water uptake in plants during drought stress, is still not fully understood. The current study sought to investigate the potential functions of melatonin in enhancing drought tolerance, and the mechanism of melatonin-induced enhanced resilience to drought. We assumed that melatonin could facilitate higher accumulation of ABA in roots than leaves in soybean and trigger the expression of AQPs under drought stress conditions and also justified the dynamics functions of melatonin in regulating ABA and AQPs under drought stress condition.

2 Materials and methods

2.1 Plant materials and growth conditions

Soybean seeds were procured from Guangxi University, followed by sterilization in 20% ethanol, and placed in an incubator for germination. The germinated seeds were sown in plastic trays (32 holes) filled with growth substrates and cultured at the Guangxi University greenhouse. The flow rate, humidity, and temperature in the chamber were kept constant at 250 mol m^{-2} s^{-1}, 65%, and 26°C, respectively. At the second true leaf stage seedlings were transplanted in new pots (the diameter of top and bottom were in 24 cm and 23 cm, respectively; 9 cm in height; water outlet at the bottom) containing 3.5 kg mixture of humus soil and sand (1:1). Soybean seedlings were watered with distilled water (50 mL/pot) as a control or with polyethylene glycol 6,000 (PEG-6000, Solarbio co., Beijing, China) at concentrations of 0%, 10%, 20%, and 30% concentrations (50 mL/pot). We applied PEG-6000 to induce osmotic stress. Each group consisted of three replications. The plants underwent numerous physiological and biochemical evaluations following the end of each treatment cycle (14 DAT, data is not shown). Finally, 20% polyethylene glycol (PEG) was finalized as the optimal concentration for further investigations.

In addition, the concentration of melatonin was selected before the final experiment. Plants in the control group were well watered with a ½ strength Hoagland nutrient solution, in the melatonin group plants were watered with a ½ strength Hoagland nutrient solution and foliar sprayed with 100 mL (100 μM concentration) melatonin solution and melatonin was applied every three alternate day (4 times and continue until 12 days of treatment). PEG treatment group were watered with a ½ strength Hoagland nutrient solution plus 20% PEG 6,000, and PEG + melatonin group were watered with a ½ strength Hoagland nutrient solution plus 20% PEG 6,000 plus 100 mL (100 μM concentration) melatonin solution. The designed parameters were recorded on the 14th day after the treatment, and samples were harvested for biochemical and gene expression analysis, followed by storage at -80 °C. At least three replicates were performed for each treatment and 32 plants were grown randomly for each treatment.

2.2 Determination of biomass and relative water content

At least five seedlings from each treatment group were washed with running water. Excess water was removed from the plant's surface by tissues before the stems and roots were separated. Following that, the fresh weight (FW) was calculated. The biomass and root-to-shoot ratio of soybean were determined. To determine the relative water content (RWC) of tissues (Jahan et al., 2019), leaves or roots with a specified FW were immersed in distilled water for 5 hours to saturation. The saturated weight (SW) was determined after the absorption of surface moisture. Then, they were dried in an oven at 72°C for 48 hours until their weight was constant (DW). The RWC was determined using the following formula: RWC = (FW-DW) / (SW-DW) × 100%. A similar procedure was used to calculate the RWC of the substrate. All previous measures were performed at least five times.

2.3 Gas exchange measurement

Gas exchange parameters were measured using a Licor-6400 portable photosynthesis system (Li-Cor, USA). Gas exchange traits of soybean leaves from the top in various treatments were determined between 9:00 and 11:00 am. The net photosynthetic rate (Pn), the stomatal conductance (Gs), the transpiration rate (Tr), and the intracellular CO_2 concentration (Ci) were recorded simultaneously at room temperature of 25°C, an intracellular CO_2 concentration of around 400 µmol mol^{-1}, an 800 µmol m^{-2} s^{-1} Photosynthetic photon flux density (Shen et al., 2019). The carboxylation efficiency (Pn/Ci) was calculated based on the values of Pn and Ci. The instantaneous water uses efficiency (WUEi) was calculated using the Pn/Tr formula.

2.4 Measurement of rapid chlorophyll fluorescence characteristics

Chlorophyll fluorescence is commonly employed to assess the effects of environmental stressors on the photosynthetic efficiency of plants. The fast chlorophyll fluorescence parameters of the middle blades of the 3rd to 5th mature leaves from the apex in various treatments were observed by a multifunctional plant efficiency analyzer (M-PEA, Hansatech, UK) and the variables were estimated according to Cai et al., (2020).

2.5 Measurement of antioxidant enzyme activity and reactive oxygen species

Tomato leaf and root samples (0.2 g) were homogenized with a mortar and pestle in 1.6 mL of 50 mM precooled sodium phosphate buffer (PBS, pH 7.8) on ice and centrifuged at 4°C for 20 min. The collected supernatant was used as a crude extract to detect antioxidant enzyme activities. The antioxidant enzyme namely superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) were determined following the method developed by Kazemi et al., (2010).

Malondialdehyde (MDA) concentration was assessed according to Altaf et al., (2021). Shortly, 0.5 g of leaf and root samples were homogenized in 5 mL of 5% (w/v) trichloroacetic acid (TCA) solution and then centrifuged at 4000 × g at 4° C for 10 min followed by supernatant collection. After that, 2 mL of TCA containing 0.67% thiobarbituric acid (TBA) solution was mixed to the collected supernatant followed by boiled in a water bath (100° C) for 30 minutes and later cooled on ice. The absorbance of aliquot was recorded at 450, 532, and 600 nm using a spectrophotometer. The MDA content unit was expressed as mircomoles per gram of FW.

Hydrogen peroxide content in the roots and leaves of soybean was examined by Wu et al., (2022). In brief, leaves (0.2 g) were sampled and homogenized in 1.6 mL of 0.1% precooled trichloroacetic acid (TCA) on ice and centrifuged at 4°C at 12000 g for 20 min. The supernatant was collected as the crude extract to determine the content of H_2O_2.

Superoxide anion production was estimated as indicated by Du et al., (2023) with minor modifications. In short, leaf and root tissues (0.2 g) were ground in 2 mL of 50 mM phosphate buffer (pH 7.8) followed by centrifugation at 12000 × g at 4°C for 20 min. Next, 0.5 mL of 50 mM phosphate buffer (pH 7.8) along with 0.1 mL of 10 mM hydroxylamine hydrochloride were incorporated in 0.5 mL supernatant and later incubation at room temperature for 30 min. Following after incubation, 1 mL of 17 mM sulfanilamide and 1 mL of 7 mM naphthylamine were added into the mixture solution and again incubated for 30 min. The absorbance reading was recorded at 530 nm.

2.6 Determination of ABA content

The endogenous ABA content of the soybean leaves was evaluated according to Ma et al., (2022). Briefly, 0.3 g leaf and root samples were homogenized in 3 mL precooled 50% chromatographic methanol (v/v), and the extracted was incubated at 4°C for 12 h, followed by centrifugation at 10000 rpm · min^{-1} for 10 min at 4°C, and the supernatant was stored at 4°C. 2 mL of precooled 80% methanol was added to the residue, extracted at 4°C for 12 h, and centrifuged at 10000 rpm · min^{-1} for 10 min at 4°C. After that, 2 mL of precooled 100% methanol was mixed to the residue and extracted for 12 h, and centrifuged as per the above conditions. Finally, all the extracts were collected and combined, and PVPP (crosslinked polyvinylpyrrolidone) was added into the extract at the rate of 0.2 g·FW^{-1} to adsorb phenols and pigments. After shaking at 4°C for 60 min, centrifuged the mixed solution like the above condition. The supernatant was passed slowly through the C18 column, collected in a centrifugal tube, and then kept in a freeze-drying machine. Thereafter, 2.5 mL of 50% methanol was added to the supernatant for dissolution, and passed through the 0.22 µm organic phase ultrafiltration membrane to determine ABA. The analyses were performed with a Hypersil ODS C18 column (250 mm ×4.0 mm, 5 µm) and a two-solvent system including methanol and ultrapure water (0.5% glacial acetic acid added). The quantitative value of ABA was calculated by an external standard calibration curve method.

2.7 Gene expression analysis by RT-PCR

To examine the effect of melatonin on the relative expression of particular genes in soybean leaves under drought stress, total RNA was extracted from soybean leaf tissues using the TotalRNA kit, and cDNA was synthesized after removing genome DNA using TransScript One-Step gDNA Removal and cDNA Synthesis Supermix (Transgen Biotech). After reversed transcription, the cDNA solution was diluted five times and utilized as a template. The specific primers used in this study are enlisted in Table S1. The SYBR ® Premix Ex TaqTM kit (Tli RNaseH Plus, TaKaRa, RR420A) was used for qRT-PCR as a master mix, and the reaction system was arranged in accordance with the guidelines. The relative gene expression was quantified following the formula developed by Livak and Schmittgen, (2001).

2.8 Statistical analysis

All data were statistically analyzed using IBM SPSS 24.0 (Statistical Package for the Social Sciences, SPSS Inc., Chicago, IL, USA). The least significant difference (LSD) test was used to examine the differences between treatments and at least three replications (n=3) were employed for each treatment Differences were considered significant at *P* <0.05. Origin 9.0 software was used to make figures.

3 Results

3.1 Melatonin improves soybean growth under osmotic stress

Osmotic stress severely inhibited plant growth, as evidenced by leaf desiccation, softening petioles, and plant wilting, while foliar application with melatonin resulted in a significant reduction in plant growth despite water loss and leaf wilting (Figure S1). Biomass measurements indicates that osmotic stress significantly reduced soybean plant growth, but melatonin largely counteracted this harsh effect. PEG treatment reduced the fresh weight of both shoots and roots to 77%, and 97%, respectively, than that of the control (Table 1). In contrast, while melatonin was incorporated, they were 88% and 126% of the controls and 39% and 40% greater than when PEG treatment alone was used. Similarly, the dry weights of the roots and shoots under PEG treatment were 53% and 44%, respectively, while the application of melatonin increased their dry weight by 66% and 21%, respectively, compared to their corresponding control. Based on dry weight, it was determined that PEG treatment had no discernible effect on the root/shoot ratio but that melatonin treatment resulted in 41% increase over PEG treatment alone (Table 1). These findings imply that melatonin has a significant ameliorating effect on the osmotic stress-induced growth inhibition of soybean.

3.2 Melatonin enhances gas exchange characteristics of soybean leaves under osmotic stress

The gas exchange attributes, namely net photosynthetic rate (Pn), stomatal conductance (Gs), transpiration rate (Tr), respiration rate (Ci), and Pn/Ci were significantly influenced by osmotic stress, and the values of these gas exchange attributes were significantly reduced by 53%, 69%, 64%, 20%, and 42%, respectively, in soybean leaves exposed to PEG stress than in control plants (Figure 1). On the contrary, melatonin supplementation reversed these gas exchange characteristics except Ci and increased by 52%, 86%, 46%, and 33%, respectively, indicating that melatonin treatment potentially mitigates photosynthetic capacity under a stressful environment. In addition, melatonin treatment further augmented the WUEi value, which was higher with osmotic stress (Figure 1).

3.3 Melatonin modulates rapid chlorophyll fluorescence parameters of soybeans under osmotic stress

As displayed in Table 2, osmotic stress resulted in a notable reduction in the maximum photochemical efficiency (Fv/Fm) of photosystem II (PSII), which decreased by 19% from that of the untreated plants. However, the application of exogenous melatonin substantially elevated the Fv/Fm value, which increased by 13% in comparison with PEG-treated plants (Table 2). The results showed that the PSII parameters of soybean leaves was damaged under osmotic stress, while exogenous melatonin alleviated the inhibition of PSII caused by PEG-stress. In addition, osmotic stress differentially influenced rapid chlorophyll fluorescence parameters, reflecting as increased and/or decreased. The *W*k (the inhibition of oxygen evolving complex at the donor side of PSII reaction center), *M*o (the maximal rate of QA reduction at the receptor side of PSII reaction center), and φ_{Do} (the quantum yield of heat dissipation) values were increased in osmotically stressed soybean plants by 57%,174%, and 58%, respectively, compared with their corresponding control seedlings, and the ψ_o (the efficiency that a trapped exciton moves an electron into the electron transport chain beyond QA), φPo (he maximum quantum yield of PSIIreaction center), φRO (the quantum yield for reduction of end electron acceptors at the PSIacceptor side), φEo (the quantum yield of electron transport), *PI*ABS (photosynthetic capacity indexes which are based on absorption), and *PI*total (photosynthetic capacity indexes based on the total of PSI and PSII reaction centers) values were decreased by 40%, 35%, 63%, 40%, 76% and 69%, respectively in the same treatment conditions (Table 2). On the contrary, treatment with melatonin reversed their values under osmotic stress conditions. According to these findings, osmotic stress increases the quantum yield of heat dissipation while decreasing the electron transfer activities of the PSII reaction center, donor side and receptor side, and PSI reaction center. Treatment

TABLE 1 Interactive effect of melatonin on biomass production of soybean plants under osmotic stress.

Treatment	Fresh weight (g)		Dry weight (g)		Root/shoot ratio
	Shoot	Root	Shoot	Root	
Control	7.8 ± 0.15^b	2.5 ± 0.08^a	2.5 ± 0.06^a	0.45 ± 0.05^b	0.32± 0.01^a
Melatonin	8.5 ± 0.19^a	2.7 ± 0.07 ^{a}	2.6 ± 0.08^a	0.62 ± 0.07 ^{a}	0.32 ± 0.02^a
PEG	3.7 ± 0.12^d	1.0 ± 0.02^c	0.8 ± 0.02^c	0.12 ± 0.02^d	0.27 ± 0.01^b
Melatonin+ PEG	5.2 ± 0.18^c	1.5 ± 0.04^b	1.3 ± 0.04^b	0.18 ± 0.04^c	0.28 ± 0.01^b

Soybean seedlings cultivated on nutrient solution (Control), seedlings cultivated on nutrient solution and foliar sprayed with 100 µM melatonin for 14 days (Melatonin), seedlings treated with 20% PEG 6000 for 14 days (PEG), and seedlings treated with 100 µM melatonin and 20% PEG 6000 for 14 days (Melatonin+ PEG). At least three biological replicates were measured for each treatment. Difference letters indicate significant variations among the treatments at P<0.05.

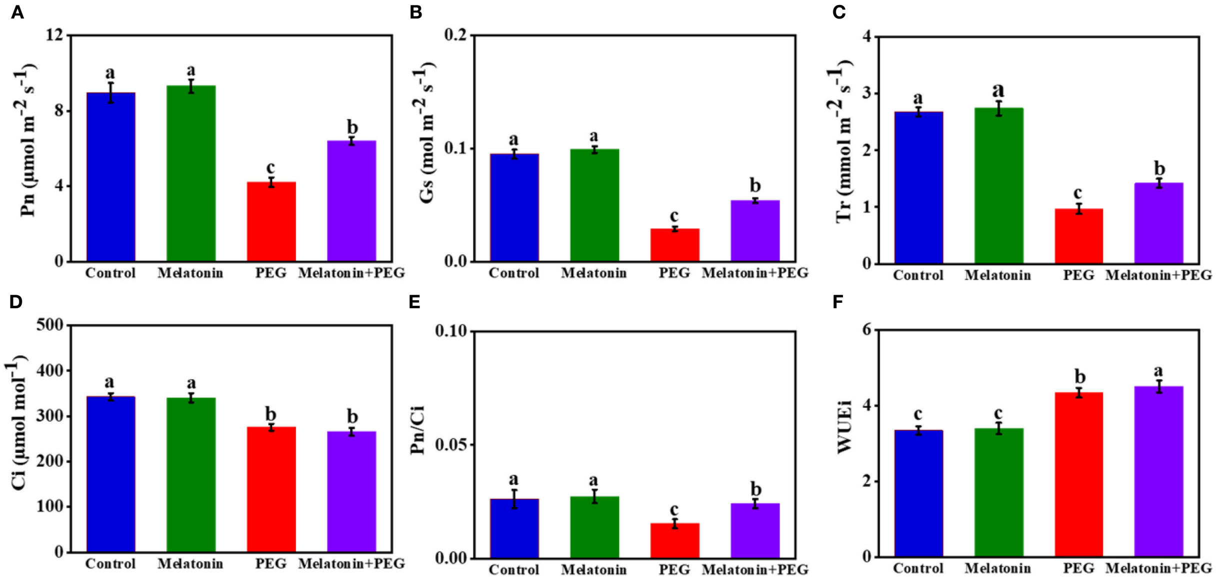


FIGURE 1
Interactive effect of melatonin on gas exchange parameters **(A)**, net photosynthetic rate (Pn); **(B)**, stomatal conductance (Gs); **(C)**, transpiration rate (Tr); **(D)**, intercellular carbon dioxide concentration (Ci); **(E)**, carboxylation efficiency (Pn/Ci)); **(F)**, water use efficiency (WUE)) of soybean leaf under osmotic stress. Soybean seedlings cultivated on nutrient solution (Control), seedlings cultivated on nutrient solution and foliar sprayed with 100 μM melatonin for 14 days (Melatonin), seedlings treated with 20% PEG 6000 for 14 days (PEG), and seedlings treated with 100 μM melatonin and 20% PEG 6000 for 14 days (Melatonin+ PEG). At least three biological replicates were measured for each treatment. Difference letters indicate significant variations among the treatments at P<0.05.

with melatonin prevents osmotic stress-induced down-regulation of photosynthetic electron transport in soybean leaves.

3.4 Melatonin triggers PSII reaction center protein gene expression in soybean leaves under osmotic stress

The relative transcript abundance of the core protein genes of the PSII reaction center were differentially expressed under osmotic stress conditions. The core genes *psbA* (encoding D1), *psbB* (encoding CP47), *psbD* (encoding D2), and *psaA* expression in soybean leaves under osmotic stress were significantly suppressed, and their expression were reversed after melatonin treatment (Figure 2). In addition, the relative gene expression of *CytB6F* was elevated in soybean leaves under PEG stress, and its expression was further up-regulated in melatonin-received plants under the same stress condition, indicating that melatonin has additive effects on core protein gene expression of PSII reaction center under osmotic stress environments (Figure 2).

TABLE 2 Interactive effect of melatonin on chlorophyll fluorescence attributes on soybean seedlings under osmotic stress.

Fluorescence	Control	Melatonin	PEG	Melatonin+ PEG
FV/FM	76.8 ± 0.95^{a}	77.5 ± 0.88^{a}	62.5 ± 0.75^{c}	72.2 ± 0.62^{b}
W_k	0.46 ± 0.03^{c}	0.44 ± 0.04^{c}	0.72 ± 0.05^{a}	0.53 ± 0.03^{b}
M_o	0.68 ± 0.04^{c}	0.63 ± 0.02^{d}	1.86 ± 0.14^{a}	0.95 ± 0.08^{b}
ψ_o	0.68 ± 0.02^{a}	0.73 ± 0.09^{a}	0.41 ± 0.02^{c}	0.45 ± 0.03^{b}
$_{\varphi}P_o$	0.86 ± 0.11^{a}	0.94 ± 0.13^{a}	0.56 ± 0.08^{c}	0.76 ± 0.10^{b}
$_{\varphi}E_o$	0.52 ± 0.08^{a}	0.53 ± 0.07^{a}	0.31 ± 0.11^{c}	0.49 ± 0.09^{b}
$_{\varphi}D_o$	0.26 ± 0.05^{bc}	0.25 ± 0.04^{c}	0.41 ± 0.13^{a}	0.28 ± 0.04^{b}
$_{\varphi}R_o$	0.19 ± 0.02^{a}	0.20 ± 0.03^{a}	0.07 ± 0.01^{b}	0.18 ± 0.03^{a}
PI_{ABS}	2.21 ± 0.18^{a}	2.28 ± 0.16^{a}	0.54 ± 0.08^{c}	2.11 ± 0.16^{b}
PI_{Total}	1.45 ± 0.19^{a}	1.54 ± 0.14^{a}	0.45 ± 0.07^{c}	1.26 ± 0.13^{b}

Soybean seedlings cultivated on nutrient solution (Control), seedlings cultivated on nutrient solution and foliar sprayed with 100 μM melatonin for 14 days (Melatonin), seedlings treated with 20% PEG 6000 for 14 days (PEG), and seedlings treated with 100 μM melatonin and 20% PEG 6000 for 14 days (Melatonin+ PEG). At least three biological replicates were measured for each treatment. Difference letters indicate significant variations among the treatments at P<0.05.

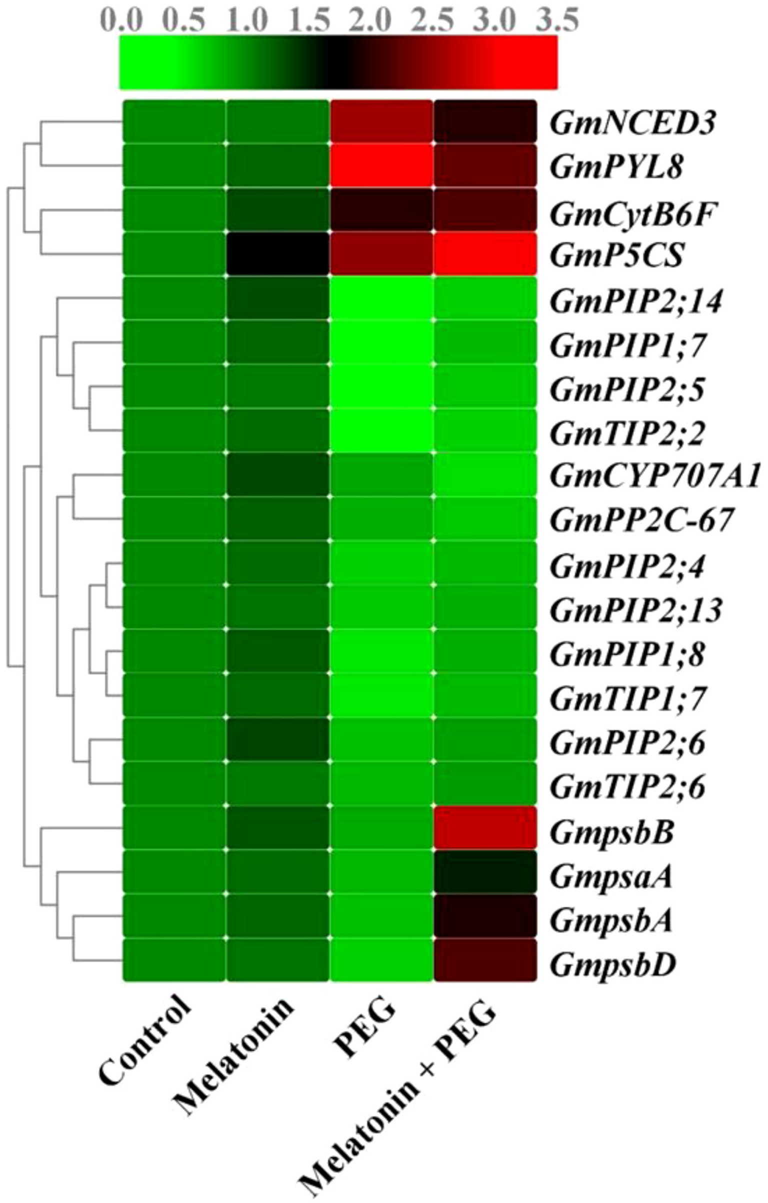


FIGURE 2
Interactive effect of melatonin on gene expression photosystem, aquaporins gene expression of soybean leaf under osmotic stress. Soybean seedlings cultivated on nutrient solution (Control), seedlings cultivated on nutrient solution and foliar sprayed with 100 μM melatonin for 14 days (Melatonin), seedlings treated with 20% PEG 6000 for 14 days (PEG), and seedlings treated with 100 μM melatonin and 20% PEG 6000 for 14 days (Melatonin+ PEG). At least three biological replicates were measured for each treatment. Difference letters indicate significant variations among the treatments at P<0.05.

3.5 Melatonin maintains relative water content of soybean seedlings and substrate under osmotic stress

After 14 days of osmotic stress, the relative water contents of roots and leaves of soybean seedlings decreased by 37% and 49%, respectively, compared with their corresponding control, whereas melatonin-received plants exposed to osmotic stress were uplifted their RWC content by 31% and 36%, respectively over only osmotic stress plants (Table 3), indicating that addition of melatonin significantly increases the water content in soybean under drought stress. In addition, we also determined the substrate water status. The relative water content of the matrix after PEG treatment was higher than that of the control (Table 3), while melatonin addition reduces water content in the substrate, indicating that PEG treatment blocks the water absorption so that much more water was left in the substrate, while melatonin treatment significantly improved water absorption of plants so that less water was left in the substrate than PEG alone.

TABLE 3 Interactive effect of melatonin on relative water content in soybean seedlings and substrate under osmotic stress.

Treatment	Root RWC (%)	Leaf RWC (%)	Substrate RWC (%)
Control	72.6 ± 2.54^a	81.2 ± 2.12^a	62.5 ± 0.98^b
Melatonin	73.3 ± 2.98^a	82.3 ± 2.32^a	61.5 ± 1.10^b
PEG	45.6 ± 1.52^c	41.5 ± 1.81^c	71.5 ± 1.28^a
Melatonin+ PEG	59.6 ± 1.85^b	56.3 ± 1.55^b	61.6 ± 0.84^b

Soybean seedlings cultivated on nutrient solution (Control), seedlings cultivated on nutrient solution and foliar sprayed with 100 µM melatonin for 14 days (Melatonin), seedlings treated with 20% PEG 6000 for 14 days (PEG), and seedlings treated with 100 µM melatonin and 20% PEG 6000 for 14 days (Melatonin+ PEG). At least three biological replicates were measured for each treatment. Difference letters indicate significant variations among the treatments at P<0.05.

3.6 Melatonin triggers free proline content and its gene expression of soybean seedlings under osmotic stress

Proline is a compatible osmotic solute in plants. Here, soybean plants exposed to osmotic stress increased proline contents in leaves and roots by 20% and 61%, respectively than control plants (Figure 3A). The exogenous addition of melatonin further elevated the proline content in both roots and leaves by 1.2- and 1.34 folds, respectively. Consistent with proline content, the core proline biosynthesis gene delta 1-pyrroline-5-carboxylate synthetase (*P5CS*) was highly expressed under drought stress, and its expression was further increased in melatonin-received soybean plants under osmotic stress (Figure 3B).

3.7 Melatonin balances antioxidant enzyme activity and ROS in soybean seedlings under osmotic stress

After 14 days of PEG treatment in soybean plants, SOD activities in leaves and roots decreased by 49% and 40%, respectively, compared with the control group (Figure 4A). On the contrary, the application of melatonin increased them by 39% and 14%, respectively. Similarly, under PEG treatment, CAT activities in the leaves and roots of the plants decreased by 65% and 56%, respectively, compared with control, and its activities increased in plants that received melatonin by 32% and 23%, respectively (Figure 4B). Consequently, treatment with PEG significantly decreased the POD activities in the leaves and roots of soybean plants by 45% and 50%, respectively, compared to control plants, and melatonin treatment increased their values by 42% and 40%, respectively (Figure 4C). These findings insight that melatonin treatment boosts antioxidant activities under osmotic stress.

PEG treatment significantly elevated the MDA content in the leaf and root of soybean plants by 1.75- and 1.85 times more over control treatment, and the co-addition of melatonin substantially reduced the leaf and root MDA content by 1.15- and 1.02 times more than control plants (Figure 4D). Under PEG stress, the rate of $O_2^{\cdot -}$ generation in soybean roots and leaves was 5.14- and 2.6 folds, respectively higher compared with control plants. Conversely, the production rates of $O_2^{\cdot -}$ in roots and leaves were lower in melatonin-applied plants when compared to only osmotic-treated plants (Figure 4E). These results denote that melatonin co-addition partially but significantly mitigates PEG-induced osmotic stress by lowering the production of MDA and $O_2^{\cdot -}$ in the leaf and root of soybean (Figure 4D-E). However, melatonin treatment significantly inhibits the H_2O_2 content in leaves and roots of soybean plants under osmotic stress in contrast to only PEG-treated plants (Figure 4F).

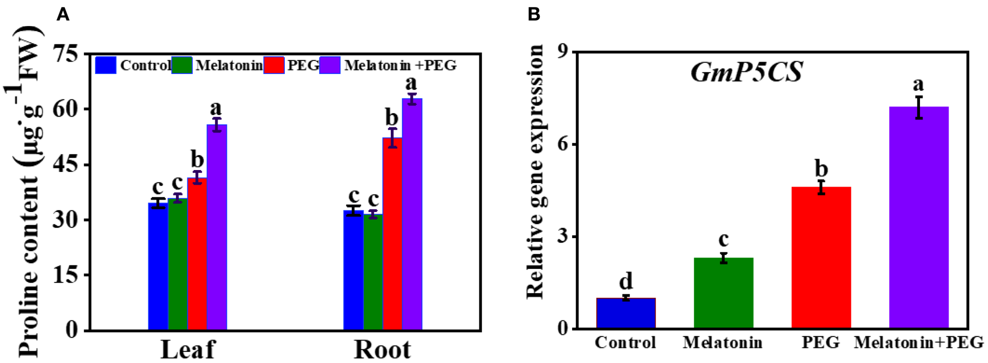


FIGURE 3
Interactive effect of melatonin on **(A)** proline content of soybean leaf and root and **(B)** *GmP5CS* expression of soybean leaf under osmotic stress. Soybean seedlings cultivated on nutrient solution (Control), seedlings cultivated on nutrient solution and foliar sprayed with 100 µM melatonin for 14 days (Melatonin), seedlings treated with 20% PEG 6000 for 14 days (PEG), and seedlings treated with 100 µM melatonin and 20% PEG 6000 for 14 days (Melatonin+ PEG). At least three biological replicates were measured for each treatment. Difference letters indicate significant variations among the treatments at P<0.05.

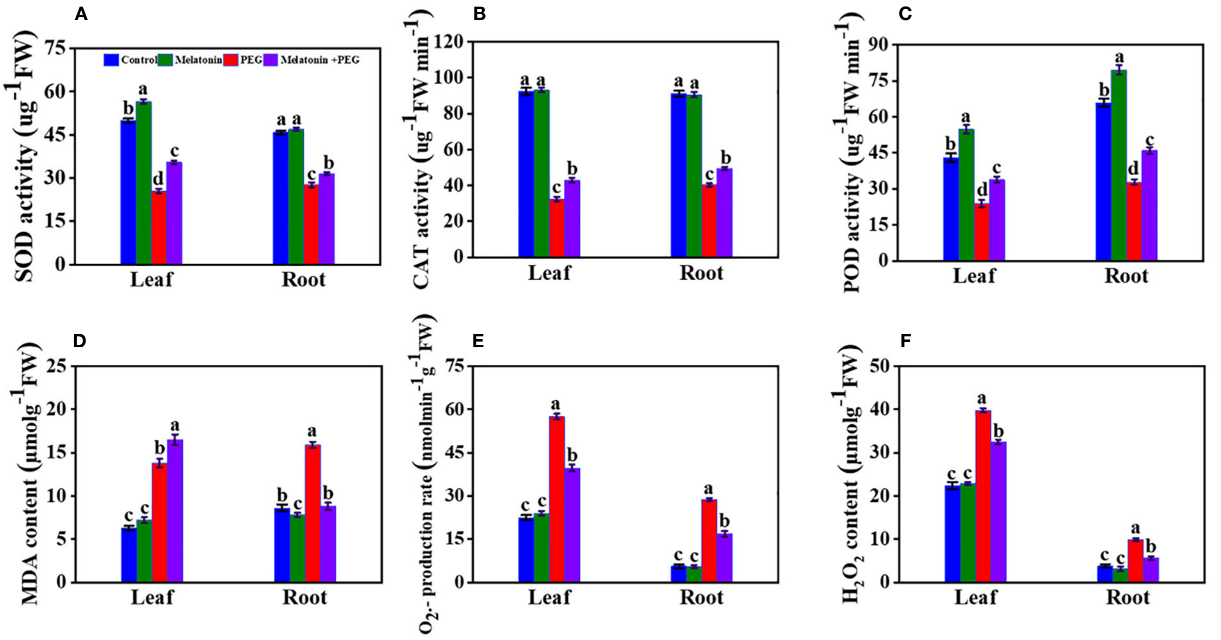


FIGURE 4
Interactive effect of melatonin on antioxidant enzyme activity of SOD **(A)**, CAT **(B)**, POD activity **(C)**, MDA content **(D)**, $O_2\bullet^-$ production rate **(E)** and H_2O_2 content **(F)** of soybean leaf and root under osmotic stress. Soybean seedlings cultivated on nutrient solution (Control), seedlings cultivated on nutrient solution and foliar sprayed with 100 µM melatonin for 14 days (Melatonin), seedlings treated with 20% PEG 6000 for 14 days (PEG), and seedlings treated with 100 µM melatonin and 20% PEG 6000 for 14 days (Melatonin+ PEG). At least three biological replicates were measured for each treatment. Difference letters indicate significant variations among the treatments at P<0.05.

3.8 Melatonin functions on endogenous ABA and related gene expressions in soybean seedlings under osmotic stress

ABA is a pivotal component that plays an essential role in the regulation of stress conditions in plants. After two weeks of osmotic stress, the endogenous ABA content was substantially increased in the roots and leaves of soybeans by 164% and 116%, respectively, compared with the control group (Figure 5A). Supplementation with melatonin significantly reduced the endogenous ABA content in the leaf but increased the root ABA content of soybean by 34% and 20%, respectively, compared with the only osmotic stressed plant. To understand molecular insight, we also assessed the expression of genes related to ABA metabolism. The relative gene expression of 9-cis-epoxycarotenoid dioxygenase 3 (*NCED3*) and *PYL8* were significantly up-regulated in osmotically stressed plants, and melatonin treatment inhibited their expression under the same stress conditions (Figure 5B). In contrast, PEG treatment significantly down-regulated the expression of *CYP707A1* and *PP2C-67* and their expression was further decreased with the supplementation of melatonin under the same stressful environment (Figure 5B and Figure 2).

3.9 Melatonin regulates aquaporin gene expressions in soybean seedlings under osmotic stress

In soybean plants, we sequenced the *PIP*-and *TIP*-related genes in accordance with the conserved sequences of the plant aquaporin genes, including *PIP* and *TIP*, which are confirmed by National Center for Biotechnology Information (NCBI). The relative transcript abundance of *PIP1;7*, *PIP1;8*, *PIP2;4*, *PIP2;5*, *PIP2;6, PIP2;13*, *PIP2;14*, *TIP1;7, TIP2;2*, and *PIP2;6* genes expression were down-regulated by 69%, 55%, 45%, 71%, 35%, 39%, 71%, 56%, 70%, 31%, respectively, compared to their corresponding control treatment (Figure 2); however, their expression was up-regulated with the supplementation with melatonin by 119%, 67%, 25%, 110%, 31%, 23%, 93%, 57%, 83%, and 28%, respectively, than the only osmotic stress plants (Figure 2). According to these findings, osmotic stress significantly inhibited the aquaporin gene expression in soybean plants, but melatonin treatment greatly reduced this suppression.

4 Discussion

Climate change poses significant threats to agricultural regions. Due to shifting climatic circumstances, the negative effects of water scarcity are not just limited to food security but could also result in restrictions in other areas of agricultural production. In this experiment, we investigated the putative role of melatonin on the drought stress tolerance of soybean seedlings. We observed that 20% PEG 6000 led to severe damage symptoms in soybean plants and slowed seedling growth, but the addition of melatonin greatly reduced this retardation effect (Figure S1 and Table 1). These findings support earlier studies indicating that melatonin increases the osmotic tolerance of soybean plants (Ahmad et al., 2019; Altaf et al., 2022). Furthermore, we found that no obvious

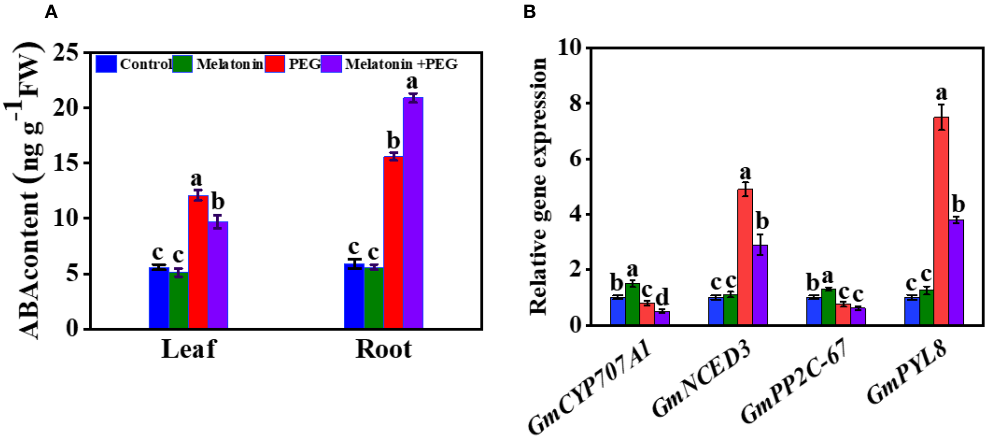


FIGURE 5
Interactive effect of melatonin on **(A)** ABA content of soybean leaf and root and **(B)** gene expression of ABA metabolism of soybean leaf under osmotic stress. Soybean seedlings cultivated on nutrient solution (Control), seedlings cultivated on nutrient solution and foliar sprayed with 100 μM melatonin for 14 days (Melatonin), seedlings treated with 20% PEG 6000 for 14 days (PEG), and seedlings treated with 100 μM melatonin and 20% PEG 6000 for 14 days (Melatonin+ PEG). At least three biological replicates were measured for each treatment. Difference letters indicate significant variations among the treatments at P<0.05.

effect was observed on the root-to-shoot ratio of soybean plants under PEG treatment, whereas melatonin addition significantly increased the root-to-shoot ratio under osmotic stress (Table 1). This finding suggests that 20% PEG treatment is obviously enough to inhibit soybean root growth, whereas melatonin significantly lessens drought stress-induced root growth inhibition. The fact that melatonin improved fresh mass substantially more than dry mass (Table 1) indicates that melatonin enhances the water status of soybean seedlings exposed to osmotic stress, which can be corroborated by melatonin elevated RWC of soybean seedlings (Table 3). Moreover, melatonin also helps to reduce the water content of the growing substrate, indicating that it facilitates plant roots to absorb more water. A major improvement in higher RWC was observed in the melatonin-treated plants and results show the positive effect of melatonin on the water-retaining ability of the plants (Ali et al., 2021; Bhardwaj et al., 2022).

There are two basic explanations of the mechanism by which melatonin enhances drought tolerance in plants, one is increased photosynthesis. As a result, melatonin pretreatment increases leaf chlorophyll concentration and improves the gas exchange and photochemical efficiencies (Arnao and Hernández-Ruiz 2009; Arnao and Hernández-Ruiz, 2019). Melatonin can also augment the expression of genes related to the Calvin Cycle, including ribulose-1,5-bisphosphate carboxylase/oxygenase small subunit, triose-3-phosphate isomerase, fructose-1,6-bisphosphatase, fructose-1,6-bisphosphate aldolase, and transketolase under heat stress conditions (Jahan et al., 2021). The findings here demonstrated that melatonin lessened the osmotic stress-induced suppression of soybean leaf stomatal conductance and transpiration (Figure 1). Among the photosynthetic parameters, stomatal conductance is the rate liming component estimates the rate of gas exchange (i.e., carbon dioxide uptake) and transpiration (i.e., water loss) through the leaf stomata as determined by the degree of stomatal aperture and most vulnerable to osmotic stress. Plant growth is impaired by severe drought stress due to a decrease in stomatal opening, which limits CO_2 uptake and hence reduces photosynthetic activity (Chaves et al., 2009), and/or from changes in photosynthetic metabolism (Lawlor, 2002). Melatonin on the other hand helps to decrease stomatal closing and increase rate of stomatal opening by means of enhancing stomatal conducting activity (Jahan et al., 2021; Altaf et al., 2022) and thus helps to increase photosynthesis efficiency and biomass production under stress conditions. Melatonin supplementation increases drought tolerance in rapeseed through the enhancement of leaf stomatal conductance and water use efficiency (Dai et al., 2020). The second rationale is to strengthen the antioxidant system. Under drought conditions, tobacco seedlings supplemented with melatonin exhibited higher activities of SOD, POD, as well as other non-enzymatic antioxidants, which scavenged excess ROS and reduced lipid peroxidation production to prevent plant tissues from stress (Zuo et al., 2014; Chen et al., 2018; Iqbal et al., 2018). The findings of the current study supported the previous arguments. The carboxylation efficacy, Pn, WUEi, electron transport activities, and photosynthetic index values of soybean leaves under osmotic stress were all enhanced by applying melatonin (Figure 1 and Table 2). These findings are consistent with melatonin-enhanced leaf photosynthetic performance in other plant species (Wang et al., 2013; Shi et al., 2015; Ye et al., 2016), implying the stimulatory effects of melatonin on the photosynthesis processes under stress are similar in many respects.

The primary protein compound of the PSII reaction center is typically a heterodimer made of D1 and D2 proteins generated by psbA and psbD, respectively (Kiss et al., 2012). The inner light-harvesting complex comprises the CP43 and CP47 proteins, encoded by *psbB* and *psbC*, respectively (Bi et al., 2016). The major photochemical reactions are initiated by these protein complexes, all of which make up the PSII reaction center and control the absorption and transmission of the light energy received by the antenna pigments. The PSII reaction center is the central place of stress damage (Nishiyama and Murata, 2014). In the present investigation, we noticed that the expression of the *psbA*, *psbB*, *psbD*, and *psaA* genes was significantly suppressed by drought stress in soybean leaves, whereas the exogenous application of melatonin entirely reversed this negative effect (Figure 2), demonstrating that melatonin overturns the suppressive actions of drought stress on the central protein repair of the PSII reaction center (Jahan et al., 2021).

Our research also corroborated the notion that melatonin increases antioxidant enzyme activity. The result revealed that drought stress enhanced the SOD, POD, and CAT activities in soybean leaves and roots and melatonin supplementation fostered this improvement (Figure 4A–C). As a result, melatonin reduced O_2^- generation along with H_2O_2 and MDA contents (Figure 4D–F) and uplifted the activity of SOD, POD, and CAT. Accumulated evidence showed that melatonin increased antioxidant enzyme activity in different plant species under various stress conditions, including drought stress (Kaya and Doganlar, 2019; Dai et al., 2020; Hossain et al., 2020; Sharma et al., 2020), suggesting that melatonin enhances the antioxidant defenses of soybean leaves to neutralize ROS and inhibits membrane lipid peroxidation production in soybean plants, thereby enhancing plant stress tolerance (Figure 4D–F). The physiological implications of melatonin-induced reduction in H_2O_2 in soybean leaves under osmotic stress are also unclear. It is widely understood that an increase in H_2O_2 in guard cells causes stomatal closure, but melatonin can inhibit ABA-induced stomatal closure in apple leaves (Li et al., 2015) and Arabidopsis leaves (Zuo et al., 2014).

The outcome of water stress often increased endogenous ABA production as a result of both promoting synthesis and inhibiting catabolism (Li et al., 2015; Li et al., 2016). We also observed the accumulation of ABA in soybean plants under osmotic stress (Figure 5A). The increase in endogenous ABA content in leaf and root are likely the consequence of stimulation of synthesis and inhibition of catabolism because PEG treatment increased *NCED3* expression and decreased the expression of *CYP707A1* (Figure 5B and Figure 2) (Li et al., 2015). *PYL8* is an ABA receptor protein gene involved in ABA signaling (Antoni et al., 2013; Kundu and Gantait, 2017). *PYL8* expression was dramatically up-regulated in soybean leaves by osmotic stress (Figure 5B and Figure 2), showing that transduction of ABA signaling was facilitated during stress. In contrast, *PYL8* expression was down-regulated in leaves when treated with melatonin. This may prove that decrease ABA content in leaf is related with the down-regulation with *PYL8* expression. Kwak et al., (2003) demonstrated that ABA caused an increase in H_2O_2 by increasing the expression of the NADPH oxidase gene *Rboh* (respiratory burst oxidase homologue). Hence, the inhibition of ABA accumulation, which in turn increases stomatal conductance, maybe the responsible for melatonin-induced reduction of H_2O_2 in soybean leaves during osmotic stress.

Osmotic stress substantially suppressed the transcription of plasma membrane *PIPs* and tonoplast *TIP* in soybean leaves (Figure 2), demonstrating that osmotic stress can readily impede water transduction in soybean. Conversely, supplementation with melatonin substantially reduced the suppressive activities of osmotic stress on the transcription of *PIPs* and *TIPs*, revealing that melatonin enhances water absorption and transportation in soybeans, explaining the mechanism underlying melatonin-improved RWC content in plant tissues. These findings help to partially explain why melatonin not only stimulates stomatal opening but also enhances the water balance of plants in drought-stressed environments. The present findings were also confirmed by previous investigations, which concluded that melatonin application triggered the water absorption capacity of the root through the mRNA regulation of *PIP* aquaporins in maize and elevated the *TIP* aquaporins expression in barley under drought stress conditions (Kurowska et al., 2019; Qiao et al., 2020). Plant aquaporin gene expression is stimulated by H_2O_2 under stress conditions (Prado and Maurel, 2013; Cai et al., 2020). Moreover, ABA plays a significant regulatory role in promoting rice *PIPs* gene expression (Lian et al., 2006; Ding et al., 2016). In this study, PEG treatment increased ABA and H_2O_2 levels in soybean plants, but the plants still exhibited severe damage symptoms and aquaporin gene expression was repressed. As a result, we cannot fully validate the association between ABA and aquaporin gene expression, and it requires further investigation. On the contrary, the positive effect of melatonin is evident. In leaves, melatonin treatment reduced ABA production under osmotic stress, improved stomatal conductance, and enabled CO_2 entry, which is advantageous for photosynthesis. These effects cause plants to continue absorbing and distributing surface water, ultimately conferring plant drought tolerance.

Proline is an important osmolytes triggers plant adaptability under osmotic stress. Surprisingly, melatonin application can promote higher proline accumulation both root and leaf of soybean seedling even if the intensity of water stress in plants is not as severe as with PEG treatment alone and the core proline biosynthesis gene delta 1-pyrroline-5-carboxylate synthetase expression also increased under osmotic stress (Figure 3). Our finding indicates that melatonin-induced proline content in soybean plants is not the product of stress-related damage but rather an active accumulation of osmotic solutes, which may have significant implications for increasing tolerance to drought. These findings, consistent with the earlier study, noted that melatonin-mediated proline activation promotes drought tolerance *via* cell membrane stability and reduced ROS generation (Wang et al., 2019). Collectively, osmotic stress inhibits growth of soybean seedlings, whereas melatonin application mitigates osmotic stress induced growth inhibition by means of increasing photosynthesis efficiency and regulating ABA and aquaporin gene expression.

5 Conclusion

In conclusion, our findings show that exogenous melatonin application significantly increased plant biomass specifically root biomass, which might be contribute to improves root water uptake and conductivity, increases photosynthetic activity amid drought conditions, and exerts osmotic stress to soybean plants. Under osmotic stress, the co-addition of melatonin significantly inhibited excess ROS production and reduced MDA levels, thus-facilitates reduction of cellular damage. Osmotic stress greatly decreased antioxidant defense system and melatonin helped to elevated antioxidant enzyme activities. In addition, melatonin prevents ABA synthesis in leaves and increase endogenous ABA content in roots, thereby boosting ABA signaling and generating less H_2O_2 accumulation in plant under osmotic stress, and the latter stimulates the up-regulation of aquaporin genes (*PIPs* and *TIPs*) expression, thus increasing the stomatal aperture and transpiration pull. This is advantageous to uptake water, enabling CO_2 entrance into mesophyll cells and retaining the photosynthetic efficiency of leaves. These studies shed light on the underlying mechanisms of melatonin-mediated improvement of plant drought tolerance. Thus more studies like field trials can be conducted to unravel the efficacy of melatonin in reducing drought-induced growth inhibition under water-limited soil conditions and high-throughput molecular analysis may be the best alternative to get more insightful mechanisms.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Materials, further inquiries can be directed to the corresponding author/s.

Author contributions

XZ: Conceptualization, design of experiment, Methodology, hunting fund; MJ: Conceptualization, performed experiment, Data curation, and Original draft preparation; CJ; Resources, LS: Software, XL: Review and editing. DJ and JN: Revise and editing. All authors contributed to the article and consented to the submitted version.

Funding

The authors declare that no financial support was received for the research, authorship, and/or publication of this article.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2023.1193666/full#supplementary-material

References

Ahmad, P., Jamsheed, S., Hameed, A., Rasool, S., Sharma, I., Azooz, M., et al. (2014). "Drought stress induced oxidative damage and antioxidants in plants," in *Oxidative damage to plants* (Amsterdam, Paris: Elsevier), 345–367. doi: 10.1016/B978-0-12-799963-0.00011-3

Ahmad, S., Kamran, M., Ding, R., Meng, X., Wang, H., Ahmad, I., et al. (2019). Exogenous melatonin confers drought stress by promoting plant growth, photosynthetic capacity and antioxidant defense system of maize seedlings. *PeerJ* 7, e7793. doi: 10.7717/peerj.7793

Alam, H., Zamin, M., Adnan, M., Shah, A. N., Alharby, H. F., Bamagoos, A. A., et al. (2022). Exploring suitability of salsola imbricata (Fetid saltwort) for salinity and drought conditions: a step toward sustainable landscaping under changing climate. *Front. Plant Sci.* 13. doi: 10.3389/fpls.2022.900210

Alexandersson, E., Danielson, JÅ, Råde, J., Moparthi, V. K., Fontes, M., Kjellbom, P., et al. (2010). Transcriptional regulation of aquaporins in accessions of arabidopsis in response to drought stress. *Plant J.* 61, 650–660. doi: 10.1111/j.1365-313X.2009.04087.x

Alharby, H. F., and Fahad, S. (2020). Melatonin application enhances biochar efficiency for drought tolerance in maize varieties: modifications in physio-biochemical machinery. *Agron. J.* 112, 2826–2847. doi: 10.1002/agj2.20263

Ali, M., Kamran, M., Abbasi, G. H., Saleem, M. H., Ahmad, S., Parveen, A., et al. (2021). Melatonin-induced salinity tolerance by ameliorating osmotic and oxidative stress in the seedlings of two tomato (*Solanum lycopersicum* l.) cultivars. *J. Plant Growth Regul.* 40, 2236–2248. doi: 10.1007/s00344-020-10273-3

Altaf, M. A., Shahid, R., Ren, M. X., Altaf, M. M., Khan, L. U., Shahid, S., et al. (2021). Melatonin alleviates salt damage in tomato seedling: a root architecture system, photosynthetic capacity, ion homeostasis, and antioxidant enzymes analysis. *Scientia Hortic.* 285, 110145. doi: 10.1016/j.scienta.2021.110145

Altaf, M. A., Shahid, R., Ren, M. X., Naz, S., Altaf, M. M., Khan, L. U., et al. (2022). Melatonin improves drought stress tolerance of tomato by modulating plant growth, root architecture, photosynthesis, and antioxidant defense system. *Antioxidants* 11, 309. doi: 10.3390/antiox11020309

Antoni, R., Gonzalez-Guzman, M., Rodriguez, L., Peirats-Llobet, M., Pizzio, G. A., Fernandez, M. A., et al. (2013). Pyrabactin Resistance1-Like8 plays an important role for the regulation of abscisic acid signaling in root. *Plant Physiol.* 161, 931–941. doi: 10.1104/pp.112.208678

Arnao, M., and Hernández-Ruiz, J. (2009). Protective effect of melatonin against chlorophyll degradation during the senescence of barley leaves. *J. Pineal Res.* 46, 58–63. doi: 10.1111/j.1600-079X.2008.00625.x

Arnao, M. B., and Hernández-Ruiz, J. (2019). Melatonin: a new plant hormone and/or a plant master regulator? *Trends Plant Sci.* 24, 38–48. doi: 10.1016/j.tplants.2018.10.010

Bhardwaj, K., Raina, M., Sanfratello, G. M., Pandey, P., Singh, A., Rajwanshi, R., et al. (2022). Exogenous melatonin counteracts salinity and cadmium stress *via* photosynthetic machinery and antioxidant modulation in *Solanum lycopersicum* l. *J. Plant Growth Regul.*, 1–17. doi: 10.1007/s00344-022-10843-7

Bi, A., Fan, J., Hu, Z., Wang, G., Amombo, E., Fu, J., et al. (2016). Differential acclimation of enzymatic antioxidant metabolism and photosystem II photochemistry in tall fescue under drought and heat and the combined stresses. *Front. Plant Sci.* 7, 453. doi: 10.3389/fpls.2016.00453

Bienert, G. P., Møller, A. L., Kristiansen, K. A., Schulz, A., Møller, I. M., Schjoerring, J. K., et al. (2007). Specific aquaporins facilitate the diffusion of hydrogen peroxide across membranes. *J. Biol. Chem.* 282, 1183–1192. doi: 10.1074/jbc.M603761200

Bortoluzzi, M. P., Heldwein, A. B., Trentin, R., Maldaner, I. C., and da Silva, J. R. (2020). Risk of occurrence of water deficit in soybean cultivated in lowland soils. *Earth Interact.* 24, 1–9. doi: 10.1175/EI-D-19-0029.1

Cai, C., He, S., An, Y., and Wang, L. (2020). Exogenous 5-aminolevulinic acid improves strawberry tolerance to osmotic stress and its possible mechanisms. *Physiologia Plantarum* 168 (4), 948–962. doi: 10.1111/ppl.13038

Carvalho, Md (2008). Drought stress and reactive oxygen species. *Plant Signaling Behav.* 3, 156–165. doi: 10.4161/psb.3.3.5536

Chai, Q., Gan, Y., Turner, N. C., Zhang, R. Z., Yang, C., Niu, Y., et al. (2014). Water-saving innovations in Chinese agriculture. *Adv. Agron.* 126, 149–201. doi: 10.1016/B978-0-12-800132-5.00002-X

Chaves, M. M., Flexas, J., and Pinheiro, C. (2009). Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell. *Ann. Bot.* 103, 551–560. doi: 10.1093/aob/mcn125

Chen, Y. E., Mao, J. J., Sun, L. Q., Huang, B., Ding, C. B., Gu, Y., et al. (2018). Exogenous melatonin enhances salt stress tolerance in maize seedlings by improving antioxidant and photosynthetic capacity. *Physiologia Plantarum* 164, 349–363. doi: 10.1111/ppl.12737

Cohen, I., Zandalinas, S. I., Fritschi, F. B., Sengupta, S., Fichman, Y., Azad, R. K., et al. (2021). The impact of water deficit and heat stress combination on the molecular response, physiology, and seed production of soybean. *Physiologia Plantarum* 172, 41–52. doi: 10.1111/ppl.13269

Cotrim, M. F., Gava, R., Campos, C. N. S., de David, C. H. O., IdA, R., Teodoro, L. P. R., et al. (2021). Physiological performance of soybean genotypes grown under irrigated and rainfed conditions. *J. Agron. Crop Sci.* 207, 34–43. doi: 10.1111/jac.12448

Dai, L., Li, J., Harmens, H., Zheng, X., and Zhang, C. (2020). Melatonin enhances drought resistance by regulating leaf stomatal behaviour, root growth and catalase activity in two contrasting rapeseed (*Brassica napus* l.) genotypes. *Plant Physiol. Biochem.* 149, 86–95. doi: 10.1016/j.plaphy.2020.01.039

Dalal, M., Sahu, S., Tiwari, S., Rao, A. R., and Gaikwad, K. (2018). Transcriptome analysis reveals interplay between hormones, ROS metabolism and cell wall biosynthesis for drought-induced root growth in wheat. *Plant Physiol. Biochem.* 130, 482–492. doi: 10.1016/j.plaphy.2018.07.035

Deshmukh, R. K., Nguyen, H. T., and Belanger, R. R. (2017). Aquaporins: dynamic role and regulation. *Front. Plant Sci.* 8. doi: 10.3389/fpls.2017.01420

Ding, L., Li, Y., Wang, Y., Gao, L., Wang, M., Chaumont, F., et al. (2016). Root ABA accumulation enhances rice seedling drought tolerance under ammonium supply: interaction with aquaporins. *Front. Plant Sci.* 7, 1206. doi: 10.3389/fpls.2016.01206

Dong, S., Jiang, Y., Dong, Y., Wang, L., Wang, W., Ma, Z., et al. (2019). A study on soybean responses to drought stress and rehydration. *Saudi J. Biol. Sci.* 26, 2006–2017. doi: 10.1016/j.sjbs.2019.08.005

Du, N., Xue, L., Xue, D., Dong, X., Yang, Q., Jahan, M. S., et al. (2023). The transcription factor SlNAP1 increases salt tolerance by modulating ion homeostasis and ROS metabolism in *Solanum lycopersicum*. *Gene* 849, 146906. doi: 10.1016/j.gene.2022.146906

Dubey, A., Kumar, A., Abd_Allah, E. F., Hashem, A., and Khan, M. L. (2018). Growing more with less: breeding and developing drought resilient soybean to improve food security. *Ecol Indic* 105, 425–437. doi: 10.1016/j.ecolind.2018.03.003

Dutta, T., Neelapu, N. R. R., Wani, S. H., and Surekha, C. (2019). Role and regulation of osmolytes as signaling molecules to abiotic stress tolerance. *Plant Signaling Molecules*, 459–477. doi: 10.1016/B978-0-12-816451-8.00029-0

FAO (2021) *Land & water*. Available at: http://www.fao.org/land-water/databases-and-software/crop-information/soybean/en/ (Accessed 25, December 2022).

FAOSTAT (2019) *Crops and livestock products*. Available at: http://www.fao.org/faostat/en/#data/QCL (Accessed 25, December 2022).

Hossain, M. M., Lam, H.-M., and Zhang, J. (2015). Responses in gas exchange and water status between drought-tolerant and-susceptible soybean genotypes with ABA application. *Crop J.* 3, 500–506. doi: 10.1016/j.cj.2015.09.001

Hossain, M. S., Li, J., Sikdar, A., Hasanuzzaman, M., Uzizerimana, F., Muhammad, I., et al. (2020). Exogenous melatonin modulates the physiological and biochemical mechanisms of drought tolerance in tartary buckwheat (*Fagopyrum tataricum* (L.) gaertn). *Molecules* 25, 2828. doi: 10.3390/molecules25122828

Iqbal, H., Yaning, C., Waqas, M., Shareef, M., and Raza, S. T. (2018). Differential response of quinoa genotypes to drought and foliage-applied H_2O_2 in relation to oxidative damage, osmotic adjustment and antioxidant capacity. *Ecotoxicology Environ. Saf.* 164, 344–354. doi: 10.1016/j.ecoenv.2018.08.004

Jahan, M. S., Guo, S., Sun, J., Shu, S., Wang, Y., Abou El-Yazied, A., et al. (2021). Melatonin-mediated photosynthetic performance of tomato seedlings under high-temperature stress. *Plant Physiol. Biochem.* 167, 309–320. doi: 10.1016/j.plaphy.2021.08.002

Jahan, M. S., Wang, Y., Shu, S., Zhong, M., Chen, Z., Wu, J., et al. (2019). Exogenous salicylic acid increases the heat tolerance in tomato (*Solanum lycopersicum* l) by enhancing photosynthesis efficiency and improving antioxidant defense system through scavenging of reactive oxygen species. *Scientia Hortic.* 247, 421–429. doi: 10.1016/j.scienta.2018.12.047

Kaya, A., and Doganlar, Z. B. (2019). Melatonin improves the multiple stress tolerance in pepper (*Capsicum annuum*). *Scientia Hortic.* 256, 108509. doi: 10.1016/j.scienta.2019.05.036

Kazemi, N., Khavari-Nejad, R. A., Fahimi, H., Saadatmand, S., and Nejad-Sattari, T. (2010). Effects of exogenous salicylic acid and nitric oxide on lipid peroxidation and antioxidant enzyme activities in leaves of *Brassica napus* l. under nickel stress. *Scientia Hortic.* 126, 402–407. doi: 10.1016/j.scienta.2010.07.037

Khan, M. N., Zhang, J., Luo, T., Liu, J., Rizwan, M., Fahad, S., et al. (2019). Seed priming with melatonin coping drought stress in rapeseed by regulating reactive oxygen species detoxification: antioxidant defense system, osmotic adjustment, stomatal traits and chloroplast ultrastructure perseveration. *Ind. Crops Products* 140, 111597. doi: 10.1016/j.indcrop.2019.111597

Kiss, É, Kós, P. B., Chen, M., and Vass, I. (2012). A unique regulation of the expression of the *psbA*, *psbD*, and *psbE* genes, encoding the D1, D2 and cytochrome b559 subunits of the photosystem II complex in the chlorophyll d containing cyanobacterium acaryochloris marina. *Biochim. Biophys. Acta (BBA)-Bioenergetics* 1817, 1083–1094. doi: 10.1016/j.bbabio.2012.04.010

Kundu, S., and Gantait, S. (2017). Abscisic acid signal crosstalk during abiotic stress response. *Plant Gene* 11, 61–69. doi: 10.1016/j.plgene.2017.04.007

Kurowska, M. M., Wiecha, K., Gajek, K., and Szarejko, I. (2019). Drought stress and re-watering affect the abundance of TIP aquaporin transcripts in barley. *PloS One* 14 (12), e0226423. doi: 10.1371/journal.pone.0226423

Kwak, J. M., Mori, I. C., Pei, Z. M., Leonhardt, N., Torres, M. A., Dangl, J. L., et al. (2003). NADPH oxidase *AtrbohD* and *AtrbohF* genes function in ROS-dependent ABA signaling in arabidopsis. *EMBO J.* 22, 2623–2633. doi: 10.1093/emboj/cdg277

Lawlor, D. W. (2002). Limitation to photosynthesis in water-stressed leaves: stomata vs. metabolism and the role of ATP. *Ann. Bot.* 89, 871–885. doi: 10.1093/aob/mcf110

Li, X., Tan, D. X., Jiang, D., and Liu, F. (2016). Melatonin enhances cold tolerance in drought-primed wild-type and abscisic acid-deficient mutant barley. *J. Pineal Res.* 61, 328–339. doi: 10.1111/jpi.12350

Li, C., Tan, D. X., Liang, D., Chang, C., Jia, D., and Ma, F. (2015). Melatonin mediates the regulation of ABA metabolism, free-radical scavenging, and stomatal behaviour in two malus species under drought stress. *J. Exp. Bot.* 66, 669–680. doi: 10.1093/jxb/eru476

Lian, H. L., Yu, X., Lane, D., Sun, W. N., Tang, Z. C., and Su, W. A. (2006). Upland rice and lowland rice exhibited different *PIP* expression under water deficit and ABA treatment. *Cell Res.* 16, 651–660. doi: 10.1038/sj.cr.7310068

Liu, J., Wang, W., Wang, L., and Sun, Y. (2015). Exogenous melatonin improves seedling health index and drought tolerance in tomato. *Plant Growth Regul.* 77, 317–326. doi: 10.1007/s10725-015-0066-6

Livak, K. J., and Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods* 25, 402–408. doi: 10.1006/meth.2001.1262

Ma, S., Zhou, X., Jahan, M. S., Guo, S., Tian, M., Zhou, R., et al. (2022). Putrescine regulates stomatal opening of cucumber leaves under salt stress *via* the H_2O_2-mediated signaling pathway. *Plant Physiol. Biochem.* 170, 87–97. doi: 10.1016/j.plaphy.2021.11.028

Mutava, R. N., Prince, S. J. K., Syed, N. H., Song, L., Valliyodan, B., Chen, W., et al. (2015). Understanding abiotic stress tolerance mechanisms in soybean: a comparative evaluation of soybean response to drought and flooding stress. *Plant Physiol. Biochem.* 86, 109–120. doi: 10.1016/j.plaphy.2014.11.010

Nishiyama, Y., and Murata, N. (2014). Revised scheme for the mechanism of photoinhibition and its application to enhance the abiotic stress tolerance of the photosynthetic machinery. *Appl. Microbiol. Biotechnol.* 98, 8777–8796. doi: 10.1007/s00253-014-6020-0

Parent, B., Hachez, C., Redondo, E., Simonneau, T., Chaumont, F., and Tardieu, F. (2009). Drought and abscisic acid effects on aquaporin content translate into changes in hydraulic conductivity and leaf growth rate: a trans-scale approach. *Plant Physiol.* 149, 2000–2012. doi: 10.1104/pp.108.130682

Prado, K., and Maurel, C. (2013). Regulation of leaf hydraulics: from molecular to whole plant levels. *Front. Plant Sci.* 4, 255. doi: 10.3389/fpls.2013.00255

Qiao, Y., Ren, J., Yin, L., Liu, Y., Deng, X., Liu, P., et al. (2020). Exogenous melatonin alleviates PEG-induced short-term water deficiency in maize by increasing hydraulic conductance. *BMC Plant Biol.* 20, 1–14. doi: 10.1186/s12870-020-02432-1

Raja, V., Majeed, U., Kang, H., Andrabi, K. I., and John, R. (2017). Abiotic stress: interplay between ROS, hormones and MAPKs. *Environ. Exp. Bot.* 137, 142–157. doi: 10.1016/j.envexpbot.2017.02.010

Rajora, N., Vats, S., Raturi, G., Thakral, V., Kaur, S., Rachappanavar, V., et al. (2022). Seed priming with melatonin: a promising approach to combat abiotic stress in plants. *Plant Stress* 4, 100071. doi: 10.1016/j.stress.2022.100071

Shahzad, A., Gul, H., Ahsan, M., Wang, D., and Fahad, S. (2023). Comparative genetic evaluation of maize inbred lines at seedling and maturity stages under drought stress. *J. Plant Growth Regul.* 42, 989–1005. doi: 10.1007/s00344-022-10608-2

Sharma, A., Wang, J., Xu, D., Tao, S., Chong, S., Yan, D., et al. (2020). Melatonin regulates the functional components of photosynthesis, antioxidant system, gene expression, and metabolic pathways to induce drought resistance in grafted *Carya cathayensis* plants. *Sci. Total Environ.* 713, 136675. doi: 10.1016/j.scitotenv.2020.136675

Sharmin, R. A., Bhuiyan, M. R., Lv, W., Yu, Z., Chang, F., Kong, J., et al. (2020). RNA-Seq based transcriptomic analysis revealed genes associated with seed-flooding tolerance in wild soybean (*Glycine soja* sieb. & zucc.). *Environ. Exp. Bot.* 171, 103906. doi: 10.1016/j.envexpbot.2019.103906

Shen, Jl, Wang, Y., Shu, S., Jahan, M. S., Zhong, M., Wu, J. Q., et al. (2019). Exogenous putrescine regulates leaf starch overaccumulation in cucumber under salt stress. *Scientia Hortic.* 253, 99–110. doi: 10.1016/j.scienta.2019.04.010

Shi, H., Jiang, C., Ye, T., Tan, D. X., Reiter, R. J., Zhang, H., et al. (2015). Comparative physiological, metabolomic, and transcriptomic analyses reveal mechanisms of improved abiotic stress resistance in bermudagrass [*Cynodon dactylon* (L). pers.] by exogenous melatonin. *J. Exp. Bot.* 66, 681–694. doi: 10.1093/jxb/eru373

Shivaraj, S., Sharma, Y., Chaudhary, J., Rajora, N., Sharma, S., Thakral, V., et al. (2021). Dynamic role of aquaporin transport system under drought stress in plants. *Environ. Exp. Bot.* 184, 104367. doi: 10.1016/j.envexpbot.2020.104367

Soltani, N., Dille, J. A., Burke, I. C., Everman, W. J., VanGessel, M. J., Davis, V. M., et al. (2017). Perspectives on potential soybean yield losses from weeds in north America. *Weed Technol.* 31, 148–154. doi: 10.1017/wet.2016.2

Sudhakaran, S., Thakral, V., Padalkar, G., Rajora, N., Dhiman, P., Raturi, G., et al. (2021). Significance of solute specificity, expression, and gating mechanism of tonoplast intrinsic protein during development and stress response in plants. *Physiologia Plantarum* 172, 258–274. doi: 10.1111/ppl.13386

Šurbanovski, N., Sargent, D. J., Else, M. A., Simpson, D. W., Zhang, H., and Grant, O. M. (2013). Expression of *Fragaria vesca PIP* aquaporins in response to drought stress: *PIP* down-regulation correlates with the decline in substrate moisture content. *PloS One* 8, e74945. doi: 10.1371/journal.pone.0074945

Tiwari, R. K., Lal, M. K., Kumar, R., Chourasia, K. N., Naga, K. C., Kumar, D., et al. (2021). Mechanistic insights on melatonin-mediated drought stress mitigation in plants. *Physiologia Plantarum* 172, 1212–1226. doi: 10.1111/ppl.13307

Wang, J., Chen, J., Sharma, A., Tao, S., Zheng, B., Landi, M., et al. (2019). Melatonin stimulates activities and expression level of antioxidant enzymes and preserves functionality of photosynthetic apparatus in hickory plants (*Carya cathayensis* sarg.) under PEG-promoted drought. *Agronomy* 9, 702. doi: 10.3390/agronomy9110702

Wang, L., Liu, L., Ma, Y., Li, S., Dong, S., and Zu, W. (2018). Transcriptome profilling analysis characterized the gene expression patterns responded to combined drought and heat stresses in soybean. *Comput. Biol. Chem.* 77, 413–429. doi: 10.1016/j.compbiolchem.2018.09.012

Wang, P., Sun, X., Li, C., Wei, Z., Liang, D., and Ma, F. (2013). Long-term exogenous application of melatonin delays drought-induced leaf senescence in apple. *J. Pineal Res.* 54, 292–302. doi: 10.1111/jpi.12017

Wu, J., Zhu, M., Liu, W., Jahan, M. S., Gu, Q., Shu, S., et al. (2022). *CsPAO2* improves salt tolerance of cucumber through the interaction with CsPSA3 by affecting photosynthesis and polyamine conversion. *Int. J. Mol. Sci.* 23, 12413. doi: 10.3390/ijms232012413

Ye, J., Wang, S., Deng, X., Yin, L., Xiong, B., and Wang, X. (2016). Melatonin increased maize (*Zea mays* l.) seedling drought tolerance by alleviating drought-induced photosynthetic inhibition and oxidative damage. *Acta Physiol. Plant.* 38, 48. doi: 10.1007/s11738-015-2045-y

Zhang, N., Zhang, H. J., Zhao, B., Sun, Q. Q., Cao, Y. Y., Li, R., et al. (2014). The RNA-seq approach to discriminate gene expression profiles in response to melatonin on cucumber lateral root formation. *J. Pineal Res.* 56, 39–50. doi: 10.1111/jpi.12095

Zuo, B., Zheng, X., He, P., Wang, L., Lei, Q., Feng, C., et al. (2014). Overexpression of *MzASMT* improves melatonin production and enhances drought tolerance in transgenic arabidopsis thaliana plants. *J. Pineal Res.* 57, 408–417. doi: 10.1111/jpi.12180

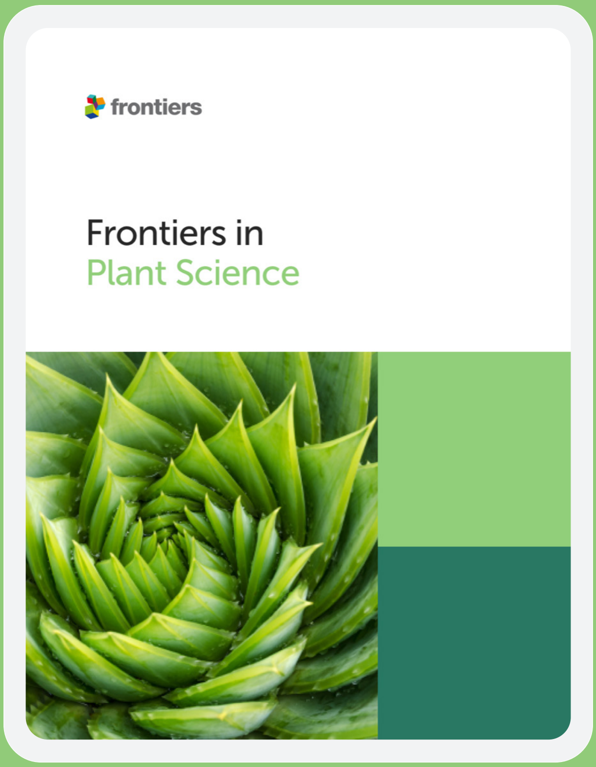
Frontiers in
Plant Science

Cultivates the science of plant biology and its applications

The most cited plant science journal, which advances our understanding of plant biology for sustainable food security, functional ecosystems and human health.

Discover the latest Research Topics

See more →



frontiers

Frontiers in
Plant Science

Frontiers

Avenue du Tribunal-Fédéral 34
1005 Lausanne, Switzerland
frontiersin.org

Contact us

+41 (0)21 510 17 00
frontiersin.org/about/contact