**Supplementary Table 1: Bacterial genera commonly used as biostimulants**

|  |  |  |
| --- | --- | --- |
| **Bacterial genera** | **Gram Negative/Positive**  | **Mode of Existence**  |
| *Rhizobium* | Gram-negative | Anaerobic, symbiotic |
| *Azospirillum* | Gram-negative | Anaerobic, free-living |
| *Pseudomonas* | Gram-negative | Aerobic but showed facultative anaerobic, free living |
| *Burkholderia* | Gram-negative | Aerobic, free-living |
| *Bacillus* | Gram-positive | Anaerobic or aerobic, free living |

**Supplementary Table 2: Various commonly used biostimulants and their role in nutrient absorption**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Biostimulants** | **Source** | **Crop** | **Role**  | **References** |
| Humic acid  | Sludge of municipal waste  | *In vitro* assay  | * Inhibit urease enzyme activity in the soil
 | Liu et al. (2019) |
| Humic acid  | Vermicompost | Mangosteen | * Modulate charge balance of cytosol
* Increase membrane fluidity and permeability thereby increase the nutrient uptake
* Increase in the activity of the pyrophosphatase enzyme (H+ -PPase)
 | Gomes et al. (2019) |
| Humic acid | Coal  | Wheat | * Increase in micronutrient solubility by reducing pH of the soil
 | Khan et al. (2018) |
| Fulvic Acid  | VitaLink Fulvic (Commercial formulation) | Legume | * Enhanced N uptake by increasing the number of root nodules in legumes
 | Capstaff et al. (2020) |
| Humic acid | Leonardite  | Cucumber  | * Increase activity of plasma membrane H+-ATPase, upregulation of CsFRO1 and CsIRT1
* Increase activity of Fe (III) chelate-reductase
 | Aguirre et al. (2009) |
| Sea weed extract | *Ascophyllum nodosum* | Lettuce | * Enhanced the relative growth rate of lettuce in the low-K treatment
 | Chrysargyris et al. (2018) |
| Seaweed sap | *Kappaphycus alvarezii* and *Gracilaria edulis* | Maize | * Increased the uptake of macro and minor nutrients
 | Basavaraja et al. (2018) |
| Seaweed sap | *Ascophyllum nodosum and Durvillea potatorum* | Tomato  | * Mitigation of iron chlorosis
 | Carrasco-Gil et al. (2018) |
| Rosemary essential oil | Fresh rosemary foliage (steam-distillation) | Tomato | * Nutrient uptake and use efficiency (N, K, Mg, Fe and Zn)
 | Souri and Bakhtiarizade (2019) |
| Kelpak® and *Phomopsis columnaris* | *Ecklonia maxima* | *Noccaea goesingensis* | * Ni accumulation by 48%
 | Ważny et al. (2021) |

**Supplementary Table 3: Effect of salinity on plant growth and development**

|  |  |  |
| --- | --- | --- |
| **Growth stage/ Plant Structure** | **Action/Consequences** | **References** |
| Germination stage | Reduction in water imbibition by seed as a result of declined soil osmotic potential  | Javaid et al. (2022); Khan and Weber (2008) |
| Change in the metabolism of protein  | Rasheed (2009); Sarraf et al. (2022) |
| Significant decline in germination rate, plumule, root, and shoot length, and seed vigour | Khodarahmpour et al. (2012); Malik et al. (2022) |
| Plant physiology | Reduction in osmotic potential under high salinity  | Gama et al. (2007); Javaid et al. (2022); Kaymakanova and Stoeva (2008); Rodriguez et al. (1997);  |
| Decrease in plant water uptake, reduced turgidity in plant cells, declined cell division and regulation of stomata aperture, and consequently reduction in photosynthesis and ultimately death of plant tissues  | Ali et al. (2021); Marschner (1995); Munns et al. (2002) |
| Altered membrane permeability, destabilized membrane proteins  | Farouk et al. (2020); Grattan and Grieve (1992); Gupta et al.(2002) |
| Membrane interruption, nutrient imbalance, impaired ROS detoxification mechanisms, reduced photosynthesis, and reduced stomatal aperture | Rahnama et al. (2010); Shen et al. (2022) |
| Plant anatomy | Thicker leaves, epidermis, cell walls, and cuticles | Aslam et al. (2017) |
| Increased stomatal density on the lower side of leaves, amplified leaf thickness, palisade tissues, vascular bundle length, xylem rows, and reduced number of vessels  | Hussein et al. (2012); Raafat et al. (1991) |
| Thickening of endo-as well as exodermis, lignification of intercellular spaces in the exodermis | Degenhardt and Gimmler (2000); Gomes et al. (2011);  |
| Elevated suberin content in roots | Walker et al. (1985) |
| Plant morphology | Reduction in shoot growth, leaf development, and expansion, decreased internodal growth, and enhanced leaf abscission  | Ali et al. (2021); Zekri (1991)  |
| Dark green, thicker, and succulent leaves with declined leaf area and volume  | Aslam et al. (2017); Bray and Reid (2002) |
| Diminished biomass and leaf area | Ashraf and Bhatti (2000); Challabathula et al. (2022) |

**Supplementary Table 4: Role of biostimulants in mitigating salt stress in crop plants**

|  |  |  |  |
| --- | --- | --- | --- |
| **Types of BS**  | **Crop** | **Mechanisms** | **References** |
| Algal extract yield  | Soybean(*Glycine max*) | Enhanced seed yield | Islam et al. (2021) |
| Humic acid extracts  | Common bean (*Phaseolus vulgaris*), Rice (*Oryza sativa*)  | Activation of anti-oxidative enzymatic function, enhanced ROS scavenging enzymes, detoxifying harmful free oxygen radicals synthesized, augmented endogenous proline levels and minimised membrane leakage in plants under drought and saline stress | Aydin et al. (2012); García et al. (2012) |
| Arbuscular mycorrhizal fungi (AMF) | Tomato (*Lycopersicon esculentum*)  | Negating the salinity-induced oxidative stress | Abdel Latef and Chaoxing (2011) |
| Plant growth-promoting rhizobacteria | Wheat (*Triticum aestivum*)  | Improved growth and yield | Upadhyay and Singh (2015) |
| Retrosal® (Valagro S.p.A) | Lettuce (*Lactuca sativa*) | Maintain the salinity level (NaCl) of water exposed, more accumulation of proline and less ABA content | Bulgari et al. (2019) |

**Supplementary Table 5: Physio-biochemical modulations of the plant by biostimulants**

|  |  |  |
| --- | --- | --- |
| **Particular** | **Role/Action**  | **References** |
| **Ionic compartmentalization and homeostasis** |
| Proline | Higher accumulation of proline in antioxidant defense mechanisms improves salt tolerance | Hoque et al. (2007); Meena et al., (2019) |
| Na+/Cl- ratio | A high concentration of Na+ is toxic for the plant cell causing a low metabolic rate for the plant, destabilization of the membrane, and cell division becoming slow finally changing the homeostasis of the mineral nutrients | Munns and Tester, (2008) |
| Regular control of Na+ entry in the cytoplasm of the cell through Na+/H+ antiporter *via* vacuolar-type H+-ATPase (V-ATPase) and the vacuolar pyrophosphatase (V-PPase) and its compartmentalization into the vacuole maintain the ionic homeostasis in plants | Mansour (2022) |
| Na+ and Cl- ion concentration within the cell, maintains the growth of the plant  | Assaha et al. (2017); Hasegawa et al., (2000) |
| **Osmotic adjustment** |
| Compatible solute  | More production of polyols, glycine betaine, and soluble sugar maintain the required osmotic potential inside the cell | Kerepesi and Galiba (2000) |
| Compatible solute maintains the cell's pH, detoxified harmful chemicals, and free radicles produced during the oxidation process are clean and  | Mansour et al. (2000); Slama et al. (2015) |
| Reduction of osmotic potential due to excess accumulation of compatible salt  | Hasegawa et al. (2000) |
| Proline | During the stress condition proline serves as a reserve organic nitrogen to recover the stress,pyrroline carboxylic acid synthetase and pyrroline carboxylic acid reductase play a vital role to produce proline from the primary precursor glutamate in the biosynthetic pathway  | Qamar et al. (2015) |
| Increasing the enzyme activity responsible for the production of proline in antioxidant defense mechanisms improves salt tolerance | Hoque et al. (2007);Meena et al. (2019) |
| **Antioxidants** |
| Glutathione | ROS scavenging, stress-responsive gene expression, detoxification of xenobiotics | Massange-Sánchez et al. (2021); Mullineaux and Rausch (2005); Noctor et al. (2012)  |
| Tocopherol | Reduced lipid peroxidation, cell signaling, regulation of growth regulation phytohormones | Krieger-Liszkay and Trebst (2006); Maeda et al. (2006); Munné-Bosch and Alegre (2002) |
| Carotenoids | Heat dissipation, protection of photosynthetic apparatus, regulation of hormonal biosynthesis | Jin et al. (2015); Ruiz-Sola and Rodríguez-Concepción (2012)  |
| Flavonoids | Inhibition of ROS-producing enzymes; antioxidant barriers | Agati et al., (2012); Mierziak (2014) |
| Ascorbic acid | ROS scavenging, Heat dissipation, synthesis of growth regulating phytohormones, induction of cell signaling, and cell division | Gallie (2013); Massange-Sánchez et al. (2021); Smirnoff (2000) |
| **Other compounds** |
| Silicon | Si improves plant growth either directly by blocking the transport of Na+ ions into the plant, or indirectly by activating different physiological processes to ameliorate the effect of salinity stress | Dhiman et al. (2021) |
| Helicase proteins (DESD-box helicase and OsSUV3 dual helicase) | Maintains or improves photosynthesis and antioxidant enzyme machinery | Tuteja et al. (2013) |

**Supplementary Table 6: Different biostimulants used in mitigating temperature stress**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Biostimulants**  | **Crop**  | **Mode of application**  | **Role**  | **References** |
| **High temperature stress**  |
| CycoFlow (sugar cane molasses with yeast extract) | Tomato | Fertigation  | Increased ascorbic acid, pollen viability, and antioxidant activity  | Francesca et al. (2020) |
| Boosten and Megafol derived from seaweed,Sabion derived from animal collagen | Tomato | Foliar application | CO2 assimilation by stabilizing chlorophyll pigments Improved root growth aid in nutrient uptake  | Niu et al. (2022) |
| Seaweed extract  | Wheat | Seed pre-treatment and fertigation  | Increased antioxidant capacity and chlorophyll stability  | Jócsák et al. (2022) |
| KIEM® (2% organic nitrogen, 2% molybdenum, and 21% organic carbon) | Soybean | Seed priming  | Increased germination percentage, upregulation genes involved in DNA repair, protection from enzymatic degradation and methyl transferases. Reduced oxidative stress  | Campobenedetto et al. (2020) |
| Salicylic acid (SA)Jasmonic acid (JA)Nitric oxide (NO) as sodium nitroprusside (SNP) | Kimchi cabbage (*Brassica rapa* L. *ssp. pekinensis*) | Foliar application | Reduced the transpiration up to 50% compared to only heat-treated plants, biostumilant able to maintain higher rate of photosynthesis and yield under heat stress | Lee et al. (2019) |
| Protein hydrolysate (CycoFlow) | Tomato  | Soil application  | Reduced H2O2 accumulationand MDA content and increased antioxidant content | Francesca et al. (2022) |
| Humic acid along with *Bacillus cereus* | Tomato  | Soil inoculation | Reduced ABA and increased SA on contrary to non-stressed plants, Enhanced the stability of PSII under heat stress | Khan et al. (2020a) |
| **Low temperature Stress** |
| Smoke-water and KAR1 | *Ceratotheca triloba* | Seed priming  | Improved germination by mitigating detrimental effects of low temperature  | Masondo et al., (2018) |
| Asahi SL (synthetic biostimulant)Goëmar Goteo (extract of *Ascophyllum nodosum*) | *Coriandrum sativum* | Foliar application | Increased rate of transpiration and stomatal conductance maximum, quantum yield of PSI | Pokluda et al., (2016) |
| Ruter AA, Terra Sorb and Razormin | Rapeseed and Wheat | Foliar spray | More accumulation of free amino acids, (most probably proline and glutamine) | Gaveliene et al. (2018) |

**Table 7: Study cases using modern methods of analysis of biostimulants on different plants under stress**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **S. No.**  | **Study** | **Methods used** | **Stress condition** | **Inference** | **Reference** |
| 1 | EnNuVi® ALPAN® on tomato; foliar application | Transcriptome studies (RNA-seq and qPCR); qualitative analyses | Drought | Upregulation of drought-responsive genes; stabilization of photosynthetic pigment levels | Hamedeh et al. (2022) |
| 2 | Seaweeds extract on maize; foliar application | Metabolomics and molecular networking; LC-MS and computational tools; Physiological studies | Drought | Alteration in primary and secondary metabolic pathways | Tinte et al., (2022) |
| 3 | Microbial consortia on maize; soil application | Metabolomics and molecular networking; LC-MS and bioinformatics tools; Biochemical analysis of metabolites | Drought | Alteration in amino acids profile, TCA intermediates, phenolics, and hormones involved in various pathways | Othibeng et al. (2022) |
| 4 |  Phylgreen (*Ascophyllum nodosum* extracts) and Delfan Plus (animal L-α amino acids) on *Arabidopsis thaliana* plants; foliar application | Transcriptome studies, physiological, and biochemical analyses |  | Upregulation of heat stress response genes and antioxidant systems, reduction of oxidative damage in leaf morphology | Cocetta et al. (2022) |
| 5 | Calcium based biostimulant on tomato; foliar application | Transcriptomics and physiological profiling, RT-qPCR (real-time quantitative reverse transcription PCR) | Water-deficient | Expression of 9 genes involved in nutrient metabolism and osmotic stress were validated; increased photosynthetic rate and chlorophyll content | Rico-Chávez et al. (2022) |
| 6 | Protein hydrolysates on *Arabidopsis thaliana*; seed priming | High throughput screening and untargeted metabolomics; physiological analyses | High salt | Reduced content of stress-related molecules, Increased photosynthetic rates | Sorrentino et al. (2021) |
| 7 | *Ascophyllum nodosum* extracts | Transcriptomics, physical, biochemical analyses | Heat  | Expression of heat shock proteins, increased flower development, fruit production, soluble sugars accumulation | Carmody et al. (2020) |

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