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Regulatory mechanisms and biosynthesis of chlorogenic acid in *Lonicera japonica*: insights from tissue culture and inducer treatments

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Plant tissue culture is a fundamental and widely applied technique in plant biology and agriculture. In medicinal plant research, tissue culture plays an indispensable role in the conservation of endangered species, the rapid propagation of valuable resources, the preservation of germplasm, and the production of secondary metabolites. As a representative medicinal plant of the Lonicera genus, L. japonica is widely utilized worldwide due to its significant economic, ecological, medicinal, and ornamental value. By using tissue culture technology, it is possible to significantly enhance the production of secondary metabolites in L. japonica and effectively alleviate resource shortages, providing a new approach for its sustainable utilization. This review summarizes the recent research progress on L. japonica in the field of tissue culture, covering aspects such as direct organogenesis, indirect organogenesis through callus tissues, protoplast culture, hairy root culture, and polyploid culture. Additionally, the biosynthetic pathway of chlorogenic acid was explored in detail, and the mechanism of action of inducers in plant cells was analyzed. The study focused on the potential regulatory mechanisms of inducers on chlorogenic acid. Eventually, the future development trends of medicinal plant biotechnology are envisioned, aiming to provide a broader perspective for the in-depth study of medicinal plants and to promote continuous development and innovation in this field.

KEYWORDS

L. japonica, tissue culture, chlorogenic acid, elicitors, synthetic biology

1 Introduction

Lonicera are usually erect or dwarf shrubs, with a few species in the form of small trees, occasionally as twining vines, and are deciduous or evergreen (Liu et al., 2022). Its corolla is richly colored, commonly including white yellow, pale pink or purplish-red. The fruits are berries, with colors ranging from red to bluish-black or black. The Lonicera genus comprises approximately 200 species, primarily distributed across temperate and subtropical regions of North America, Europe, Asia, and northern Africa. In Asia, its distribution extends as far south as the Philippines and southern Malaysia (Jacobs et al., 2009; Park et al., 2019). In China, approximately 98 species, including 4 subspecies, 18 varieties, and 1 forma, are widely distributed across various provinces, with the highest abundance in the southwestern regions (Hsu and Wang, 1988; Yang et al., 2019a). Lonicera has significant medicinal value, with L. japonica being the most renowned. It is listed among the 35 valuable Chinese medicinal herbs by the Administration of Traditional Chinese Medicine of China. Surveys indicate that approximately 18 types of flower buds from this genus are used as Chinese herbal medicine commodities (Hsu and Wang, 1988) (Table 1). L. japonica contains diverse chemical constituents, including flavonoids, phenolic acids, cyclic enol ether terpene glycosides, volatile oils, and other bioactive substances, of which chlorogenic acid (CGA) and lignans are the main active components (Shang et al., 2011; Yang et al., 2019b). These components have various pharmacological effects such as antioxidant, anti-inflammatory, antibacterial and antiviral (Su et al., 2021; Liu et al., 2016). Therefore, L. japonica has been widely used in the fields of traditional Chinese medicine, functional foods, nutraceuticals and cosmetics (Fang et al., 2022; Liu et al., 2023) (Figure 1). Additionally, L. japonica has gained significant attention as a natural plant resource owing to its abundant bioactive compounds and high safety.

The main sources of medicinal plants include wild and cultivated resources. However, overexploitation and land degradation have led to a sharp decline in the availability of wild resources. Field cultivation is time-consuming and labor-intensive, with continuous cropping often resulting in failures due to increased disease incidence (Shi et al., 2018; Sun et al., 2011). These problems exacerbate the scarcity of medicinal plant resources and raise the risk of extinction of endangered species. According to the National Economic Forestry Association (NEFA), the global market demand for *L. japonica* has exceeded 20 million kilograms, with a value of \$860 million, making it one of the most economically valuable medicinal plants (Lai et al., 2022). However, the long cultivation period of *L. japonica*, its susceptibility to pathogens and replanting diseases, and the instability of active compound content in cultivated plants severely limited farmers in meeting the growing market demand (Jia and Yan, 2024; Pan et al., 2021a).

To address these challenges, plant tissue culture technology has developed rapidly and is widely regarded as a critical tool for plant regeneration, rapid propagation of pathogen-free and disease-free materials, and efficient production of secondary metabolites. This technology offers significant advantages, including enhanced plant responses to biotic and abiotic stresses, as well as substantial improvements in the levels of desired traits and specific compounds (Tomasiak et al., 2022). Research on plant tissue culture and cell culture began in the late 19th century, when Haberlandt first introduced the concept of cell culture and pioneered the use of artificial media for the cultivation of isolated plant cells (Haberlandt, 1902). In 1934, White achieved a major breakthrough in the cultivation of isolated roots by establishing the first actively growing asexual line from isolated tomato roots, and discovered the important role of the B vitamins B1, B6 and niacin in plant growth (White, 1934). In 1965, Vasil and Hildebrandt successfully regenerated whole plants from a single isolated cell culture, scientifically validating the theory of plant cell totipotency (Vasil and Hildebrandt, 1965). During the 1960s, microspore and protoplast cultures were successfully provided. Subsequently, a large number of studies have demonstrated that undifferentiated plant cells (e.g. callus and cell suspensions) have broad application prospects in the large-scale production of secondary metabolites for pharmaceuticals and cosmetics (Xu et al., 2023; Asyakina et al., 2021).

Recent studies have shown that phenolic acids can be successfully obtained from *L. japonica* employing *in vitro* tissue cultures. This paper reviews the research progress on tissue culture of *L. japonica* conducted both domestically and internationally, covering the direct and indirect organogenesis pathways (as shown in Figure 2), protoplast culture, cell suspension culture and hairy root culture techniques of *L. japonica*. Additionally, the biosynthetic pathway of CGA, microbial transformation and metabolic regulation engineering were discussed in detail. Finally, it explores future trends in medicinal plant biotechnology, offering new perspectives and potential for further research and broader applications in this field.

2 *In vitro* culture techniques of *L. japonica*

Tissue culture can be classified based on the purpose of cultivation and the source of explants. In the study of *L. japonica*, several key processes of tissue culture based on cells and organs have been established (Figure 3), providing technical support for the development of *L. japonica* resources and further promoting the sustainable utilization of this species.

2.1 Direct organogenesis of L. japonica

The direct organogenesis pathway induces the existing organ primordia of explants to directly form adventitious shoots or roots, without the presence of callus morphology throughout the induction process (Kong et al., 2021).

In the direct organogenesis pathway, shoot induction is typically based on MS medium, with 6-BA (a cytokinin) as the primary exogenous hormone. Research by Xun (2004). indicated that the optimal medium for inducing multiple shoots and subculturing of *L. japonica* is MS + 6-BA 0.5 mg/L + IBA 0.05

TABLE 1 Lonicera species of medicinal plants.

Genus	Subgenus	Group	Species	Locality	Growing environment	References
	Subgen. Chamaecerasus (Linn.) Rehd.		1. <i>L. lanceolata</i> Wall	Eastern and western regions of Sichuan, the northeastern to northwestern and southwestern regions of Yunnan, the eastern to southern regions of Tibet, Nepal to Bhutan.	Grows in coniferous and broad-leaved mixed forests, fir forests, or shrublands at forest edges, at an altitude of 2,000-3,900 meters.	Zhao et al. (2018); Hsu and Wang (1988)
		Sect. Isika	2. L. hispida Pall	western Hebei; Shanxi; southern Shaanxi; southern Ningxia (Jingyuan, Longde); central to southern Gansu; eastern Qinghai; Xinjiang; western Sichuan; northwestern Yunnan; and eastern to southern Tibet; Mongolia, Central Asia (former Soviet regions), and northern India	Grows in mountain forests, shrublands at forest edges, or alpine meadows at elevations of 1,700–4,200 meters, reaching up to 4,800 meters in Sichuan and Tibet.	Hsu and Wang (1988)
Lonicera		Sect. Coeloxylosteum	3. <i>L. maackii</i> (Rupr.) Maxim	Changping, Beijing; eastern Heilongjiang, Jilin, and Liaoning; southern Hebei; southern Shanxi; Shaanxi; southeastern Gansu; eastern and southwestern Shandong; Jiangsu; Anhui; northern Zhejiang; Henan; Hubei; northwestern and southwestern Hunan (Xinning); northeastern Sichuan; Guizhou (Xingyi); eastern to northwestern Yunnan; and Tibet (Gyirong); Korea; Japan; and the Russian Far East.	Grows in forests or shrublands near streams at forest edges, at elevations up to 1,800 meters (reaching 3,000 meters in Yunnan and Tibet)	Sun et al. (2023); Hsu and Wang (1988)
			4. <i>L. crassifolia</i> Batal	Southwestern Hubei; northwestern Hunan (Sangzhi); southeastern and southwestern Sichuan; western Guizhou (Bijie) and northern Guizhou (Daozhen); as well as Yunnan (Malipo).	Found beside streams or in moist forest edge rock walls or crevices, at altitudes of 900- 1700 (-2300) meters.	Hsu and Wang (1988)
		Sect. Nintooa	5. <i>L. acuminata</i> Wall	Southern part of Shaanxi; southeastern Gansu; southern Anhui; Zhejiang (Longquan, Qingyuan, Suichang); western and northeastern Jiangxi; Fujian (Chongan); Taiwan; western Hubei; northwestern Hunan; northern Guangdong (Ruyuan); northeastern to northern Guangxi; Sichuan; Guizhou; northeastern to northwestern and western Yunnan; southeastern to southern Tibet; eastern Himalayas through Myanmar to Sumatra, Java, Bali, and the Philippines.	Grows in forests, open areas between forests, or in shrublands on hillsides and in valleys, at altitudes of (500-) 1000-3200 meters.	Sun et al. (2023); Yang et al. (2022); Hsu and Wang (1988)
			6. L. inodora W.W. Smith	Western Yunnan (Tengchong) and southeastern Tibet (Motuo).	Found in shrubbery on rocky hills or in montane broadleaf forests, at an elevation of 1700-2900 meters.	Hsu and Wang (1988)
			7. L. pampaninii Levl			

(Continued)

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TABLE 1 Continued

Genus	Subgenus	Group	Species	Locality	Growing environment	References
				Southern part of Anhui (Huangshan, Qingyang); Zhejiang, the northwest and northeast of Jiangxi; northern Fujian; southwestern Hubei; Hunan; northern Guangdong; northeastern and southeastern Guangxi (Luchuan); southeastern Sichuan; eastern to northern Guizhou; and southern Yunnan (Jianshui).	Found in the understory or shrubbery, at an elevation of 150-750 (-1400) meters.	Jiang et al. (2021); Hsu and Wang (1988)
			8. L. dasystyla Rehd	Guangdong (Dinghu Mountain, Zhaoqing) and northern Guangxi; northern Vietnam.	Grows in shrubbery near water, at altitudes below 300 meters.	Hsu and Wang (1988)
			9. <i>L. japonica</i> Thunb	Distributed in all provinces of China except for Heilongjiang, Inner Mongolia, Ningxia, Qinghai, Xinjiang, Hainan, and Tibet; also found in Japan and North Korea.	Found in shrubbery or sparse forests on hillsides, rock piles, at the foot of mountains, or along village fences, at altitudes up to 1500 meters.	Hsu and Wang (1988); Zhang et al. (2022b)
			10. <i>L. hypoglauca</i> Miq	southern part of Anhui; Zhejiang; Jiangxi; Fujian; northern and central Taiwan; southwestern Hubei; western to southern Hunan; Guangdong (except the southern region); Guangxi; eastern and southeastern Sichuan; northern, southeastern; and southwestern Guizhou; northwestern to southern Yunnan; Japan.	Grows in shrubbery or sparse forests at altitudes of 200-700 meters (up to 1500 meters in the southwest)	(Li et al., 2024b); Hsu and Wang (1988)
			11. <i>L. macrantha</i> (D.Don) Spreng	Southern Zhejiang; western Jiangxi; Fujian (Nanping); southwestern Hunan; Guangxi; northeastern Sichuan (Nanjiang); southeastern Sichuan (Xingwen, Jiangbei, Xiushan); Guizhou; and southeastern and western Yunnan.	Grows in hilly or valley forests or shrublands, at altitudes of 350-1250 meters, reaching up to 1800 meters in Yunnan.	Hsu and Wang (1988)
			12. L. longgiflora (Lindl.) DC	Southern Guangdong; Hainan; and Yunnan (Maguan).	Found in open forests or sunny areas along mountain roads, at altitudes reaching up to 1700 meters.	Hsu and Wang (1988)
			13. <i>L. macranthoides</i> HandMazz	Southern part of Anhui; Zhejiang; the western part of Jiangxi; the northwestern part of Fujian; the southwestern part of Hubei; the southern to western parts of Hunan; Wengyuan in Guangdong; the northeastern part of Guangxi; the southeastern part of Sichuan; and the eastern and northwestern parts of Guizhou.	Grows in mixed forests or shrubs beside streams in valleys, on hillsides, or mountain tops at altitudes ranging from 500 to 1800 meters.	Hsu and Wang (1988); Chen et al. (2023)
			14. L. buchananii Lace	Western part of Yunnan (Yingjiang) and northern part of Myanmar.	Grows in an altitude of approximately 200 meters.	Hsu and Wang (1988)

(Continued)

TABLE 1 Continued

Genus	Subgenus	Group	Species	Locality	Growing environment	References
			15. L. rhytidophylla HandMazz	Southwest of Jiangxi; the central and northern parts of Fujian; the central and western parts of Fujian; the southern part of Hunan; Guangdong; and the northeastern part of Guangxi.	Grows in mountain shrubs or forests, at an altitude of 400-1100 meters.	Hsu and Wang (1988)
			16. L. confusa (Sweet.) DC	Ho Chi Minh, Vietnam; Danzhou, Hainan, China; Haikou, Hainan, China.	Grows on hillsides, in mixed forests and shrubs, as well as along open plains, roadsides, or riverbanks, at altitudes up to 800 meters.	Sun et al. (2023); Hsu and Wang (1988)
			17. <i>L. similis</i> Hemsl	Southern Shaanxi; southern Gansu; northwestern and southwestern Zhejiang; Fujian; western Hubei; western Hunan; Du'an in Guangxi; northern, eastern; and southwestern Sichuan; western to northern Guizhou; and eastern to northern Yunnan; Myanmar.	Grows along mountain valley streams or in shrubs and forests on sunny slopes at elevations of 550–1,600 meters, reaching up to 2,200 meters in Sichuan and Yunnan.	Wei et al. (2021); Hsu and Wang (1988)
	Subgen. Lonicera		18. L. tragophylla Hemsl	Southwestern Hebei; southern Shanxi; central to southern Shaanxi; southern Ningxia and Gansu; western and southern Anhui; northwestern and southern Zhejiang (Longquan); northwestern Henan; western and eastern Hubei (Luotian); Sichuan and northern Guizhou.	Grows in understory forests, shrublands, or rock crevices near riverbanks at elevations of (700–)1,000–2,000(–3,000) meters.	Sun et al. (2023); Hsu and Wang (1988)



mg/L, the optimal rooting medium is MS + IBA 1.0 mg/L + NAA 0.05 mg/L. The study also found that browning of explants is associated with the activity of polyphenol oxidase (PPO) in the tissue. PPO catalyzes the oxidation of phenolic compounds, resulting in the formation of dark-colored substances that inhibit the activity of other enzymes and cause toxicity to the explants. To mitigate the toxicity caused by browning, it is recommended to perform continuous subculturing during the early stages of cultivation. Gao and Dong (2018) used one-year-old stem segments of wild L. japonica as explants to determine the optimal medium for adventitious bud induction as MS + 6-BA 1.5 mg/L + NAA 0.12 mg/L, the best subculture medium for propagation as MS + 6-BA 1.5 mg/L + NAA 0.02 mg/L, and the optimal rooting induction medium as 1/2 MS + IBA 2.5 mg/L + NAA 0.2 - 0.6 mg/L. Yang et al. (2017) used young stems as explants and determined the optimal medium for adventitious bud induction as MS + 6-BA 2.0 mg/L + IBA 0.01-0.05 mg/L, the most suitable medium for subculturing plantlets as MS + 6-BA 1.0 mg/L + IBA 0.01-0.05 mg/L, and the best rooting induction medium as 1/2 MS + IBA 2.0 mg/L + NAA 0.5 mg/L. Wang et al. (2013) showed that the apical bud shoot induction medium of L. japonica was MS + KT 1.0 mg/L + 6-BA 2.0 mg/L + NAA 0.1 mg/L, the proliferation medium was MS + KT 1.5 mg/L + 6-BA 1.5 mg/L + NAA 0.3 mg/L, and the rooting medium was 1/2 MS + NAA 2.0 mg/L + IAA 0.15-0.20 mg/ L. However, Yang et al. (2003) used B5 medium in the tissue culture of Mongolian *L. japonica* and found that B5 + 6-BA 2.0 mg/L + KT 0.5 mg/L + IAA 1.0 mg/L + LH 1000 mg/L had a significant effect on the induction of axillary buds.

2.2 Callus tissue

To date, various explants, including stems, leaves, buds, flower buds, and roots, have been widely used for inducing callus tissue in *L. japonica*, with leaves and stem segments being the most commonly used explants. Typical callus induction cultures generally use Murashige and Skoog (MS), B5, or LS basal media containing 3% sucrose and various concentrations of plant growth regulators (PGRs). Researchers have explored the effects of plant growth regulators, such as cytokinins (6-BA, KT, ZT), among which 6-BA is the most commonly used, with an optimal concentration typically ranging from 1.0 to 2.5 mg/L. Auxins play a critical role in callus induction by promoting the dedifferentiation of cells to form callus tissue and maintaining a continuous state of division and proliferation. NAA is commonly used in callus induction, typically at concentrations ranging from 0.01 to 1 mg/L. The culture





temperature is typically 25°C, with a photoperiod of 14 h per day and a light intensity of approximately 2000 lux. Georges et al. (1993) found that the optimal medium for inducing *L. japonica* callus was LS + BA 2.7 μ M + NAA 10.7 μ M. However, substituting 6-BA with

2,4-D led to rapid browning of the explants, significantly reducing the induction efficiency. Liu and Qian (2007) optimized the callus induction medium by adjusting the concentrations of exogenous hormones in MS basal medium, resulting in the ideal formulation of

MS + 6-BA 0.1 mg/L + NAA 1.0 mg/L. Their study showed that 6-BA was most effective at concentrations of 0.1 mg/L and 0.5 mg/L, with the former achieving an induction rate of 95.1%. NAA and IAA were both effective within the range of 0.5 to 1.5 mg/L, with NAA at 1.0 mg/L yielding the highest induction rate (97.5%), whereas IAA at the same concentration only reached 88.3%. Xiang and Gao (2007) found that although MS medium could induce callus tissue, its growth was poor due to the possible inhibitory effect of ammonium salts. In contrast, B5 medium, with a lower ammonium salt content, significantly promoted callus growth when supplemented with IAA 0.5 mg/L, BA 2.0 mg/L, and KT 0.5 mg/L. Song and Liang (1987) used young L. japonica stems and leaves as explants, and added 1 ppm 2,4-D and 0.1 ppm KT to the MS medium. The results showed that the callus grew well, with the content of CGA and isochlorogenic acid reaching approximately 50% of that in the flower buds. The callus tissues are summarised in Table 2.

2.3 Research progress on the culture of *L. japonica* suspension cells

In recent years, with the rapid development of biotechnology, cell suspension culture has been widely applied in physiology, biochemistry, cytology, developmental biology, genetics and molecular biology due to its advantages such as rapid cell proliferation, large-scale culture capability, and uniformity of cultured materials (Moscatiello et al., 2013; Owis et al., 2016). Different from animal cells, plant cell suspension culture can establish stable culture systems without malignant mutation, As a result, they have been widely used in studies of metabolism (Popova et al., 2023; García-Pérez et al., 2021; Meyer and Fricker, 2002), cell cycle (Fabian-Marwedel et al., 2002; Kobayashi et al., 2021), and in the research of cellular biology (Yin et al., 2024; Moniruzzaman et al., 2021; Menges et al., 2003) and other cellular processes (Errico et al., 2025; Zagorskaya and Deineko, 2017; Hosseini and Mulligan, 2002). Kim et al. (2003) conducted embryogenic cell suspension culture of L. japonica using MS medium supplemented with 2,4-D at a concentration of 4.52 µmol/L, achieving an induction rate of 46.7%. After inoculation of embryonic cell suspension into MS solid medium, a large number of somatic embryos were obtained, with a regeneration rate of 68.0%. Liu (2010) used yellowish-white, smallgrained, loosely structured and fast-growing callus as explants and transferred them into liquid medium with MS + 6-BA 1.5 mg/L + KT 0.75 mg/L + NAA 0.5 mg/L, established a cell suspension culture system, and investigated the physiological and biochemical changes of cells during the cultivation process. Hu et al. (2019) transferred induced callus into liquid MS medium containing BA (1.5 mg/L), NAA (0.2 mg/L), and 2,4-D (0.1 mg/L). The study revealed that the cell growth curve followed an "S" pattern, with the biomass reaching its peak on day 12. Additionally, the suspension culture was found to contain major secondary metabolites, including 3,5-di-O-caffeoylquinic acid, 3-O-caffeoylquinic acid, 4,5-di-O-caffeoylquinic acid, and 3,4-di-O-caffeoylquinic acid. Hu (2016) successfully established a L. japonica cell suspension culture system by inoculating 0.2 g/mL callus into liquid MS medium supplemented with 6-BA (1.4 mg/L), NAA (0.5 mg/L), and 2,4-D (0.7 mg/L) under dark conditions for 7-10 days. The cell biomass peaked on the 10th day, with 826.7 mg of CGA per 100 g of dry cells. Further studies demonstrated that CGA and isochlorogenic acid A exhibit significant nitrosation inhibitory activities, which are influenced by changes in the reaction environment. Wang et al. (2022) reported that after 18 days of suspension culture, the fresh cell weight reached 676.0 \pm 12.66 g/L, which was 13.52 times the initial inoculation amount. Horiike and Ohshiro (1997) isolated six curdione derivatives from the cell suspension culture of L. japonica, including (2S)-2-hydroxycurdione, (2R)-2-hydroxycurdione, (8S)-6-hydroxycurdione, (2R, 8S)-8-hydro-2-hydroxycurdione, (1S, 10S)-1,10-epoxycurdione, and (1R, 10R)-1,10-epoxycurdione. Yamamoto et al. (1999) discovered that cell suspension cultures of L. japonica do not produce iridoid or secoiridoid glycosides. However, the cells can utilize 7-deoxyloganin 7-hydroxylase to convert 7-deoxyloganin into loganin and secoiridoid glycosides. Suspension cultures are summarized in Table 3.

2.4 Culture of L. japonica protoplasts

Plant protoplasts refer to naked cells that retain cellular totipotency after the removal of the cell wall (Avila-Peltroche et al., 2020). As an important tool in modern plant biology, protoplasts serve not only as ideal recipient materials for foreign gene introduction but also as an effective means for somatic hybridization to develop new cultivars (Seidel et al., 2024). Liu et al. (2012a) used vigorously growing and loosely structured *Lonicera* callus as material for protoplast isolation. The enzymatic solution was prepared with 1.5% cellulase and 0.25% pectinase, supplemented with 0.7 mol/L mannitol as an osmotic stabilizer in CPW (cell-protoplast washing solution). The enzymatic digestion was performed under static conditions at 25°C for 12 h, yielding a large quantity of high-quality protoplasts.

2.5 Research progress of the hairy roots culture of *L. japonica*

The transformation of hairy roots and their tissue and organ culture offers multiple advantages, including genetic and biochemical stability, rapid growth, independence from exogenous plant hormones, and the ability to stably produce active compounds. Furthermore, as hairy roots originate from single cells, they facilitate extensive clonal selection, making the use of hairy root cultures for high-yield secondary metabolite production a prominent research focus worldwide (Sharma et al., 2013; Kundu et al., 2018). The results showed that when the Agrobacterium rhizogenes R1000 culture reached an OD600 value of approximately 0.6 and was diluted 40-fold before immersing explants for 4 minutes, the induction rate was highest, reaching 58%. Pre-culturing explants for 5 days, followed by cocultivation with Agrobacterium for 3 days and subsequent transfer

TABLE 2 Callus culture of *L. japonica*.

Time	Explant	Medium	PGRs	Cultivation Conditions	Result	References
1987	Young leaf & stem	MS	1 ppm 2,4-D+0.1 ppm KT	The explants were sterilized with 0.1% HgCl_2 and cultured at 25°C.	The callus tissue exhibited rapid growth and strong proliferative capacity. CGA and isochlorogenic acid were detected in the callus tissue, with a content of approximately 2.5%.	Song and Liang (1987)
1991	Leaf MS 2.		2.3 μM TDZ	Maintained in darkness for 3 weeks at 23°C, following which the explants were transferred to a medium without auxin and under low intensity illumination (5.8 pmol m ⁻² s ⁻¹).	Under auxin-free culture conditions, callus formation was observed when the TDZ (N-phenyl-N'-1,2,3-thiadiazol-5-ylurea) concentration reached 0.045 μ M. As the TDZ concentration increased to 2.3 μ M, callus growth became significantly more vigorous.	Cambecèdes et al. (1991)
1991	Leaf protoplasts of axenic shoot cultures	MS	2.0 mg/L NAA+0.2 mg/L 6-BA	Cultures were kept at 25°C, with a 16/8 h light/dark photoperiod of 2000 lux from cool white fluorescent tubes.	MS medium with 2.0 mg/L NAA and 6.2 mg/L BAP, with the production.	Ochatt (1991)
1993	Leaf, steam & root	LS	10.7 μΜ ΝΑΑ+2.7 μΜ 6-BA	Cultures kept at 25°C under a 16 h light photoperiod of 54 μmol s $^{-1}$ m $^{-2}$ (cool white fluorescent tubes).	Leaves were the most responsive, with 100% of explants giving; Medium containing 10.7 μ M NAA and 2.7 μ M 6-BA (under photoperiodic illumination) gave the best results, while media with 2,4-D led to a rapid necrosis of explants.	Georges et al. (1993)
2003	Stem segment	В5	2.0 mg/L 6-B +0.2- 0.5 mg/L KT+0.5- 1.0 mg/L IAA+1000 mg/L LH	The temperature was maintained at $25 \pm 2^{\circ}$ C, with a relative humidity of 60%-75%. Artificial lighting was provided for 12-14 h/d with light intensity ranging from 1500 to 2000 lx.	The callus tissue exhibited relatively rapid growth.	Yang et al. (2003)
2004	Stem	MS	2.0 mg/L 6-BA +0.1 mg/L NAA	Two 40 W fluorescent lamps were used, with a light duration of 10-12 h, and the temperature was maintained between 24° C and 28° C.	MS medium was found to be the optimal basic medium, with callus formation occurring most rapidly, taking only 18 d.	Li and Sun (2004)
2005	Stem segment	MS	1.0 mg/L 6-BA +0.2 mg/L NAA	The light intensity was maintained at 1500-2000 lx, with a light duration of 12-14 h/d, and the temperature was controlled between 24°C and 28°C.	After 5-10 d, loose white callus tissue began to form at the base of the seedlings. Around 30 d, the white callus gradually turned green, became more compact, and developed a granular surface.	Qiu and Tan (2005)
2007	Bud, leaf & stem	MS	0.1 mg/L 6-BA +1.0 mg/L NAA	The samples were first cultured in the dark for 24 h, followed by incubation at 25°C under light intensity of 1000-1500 lx with a photoperiod of 12 h/d.	Using buds as explants yielded the best results for callus induction, as it not only produced a large quantity of callus but also resulted in faster induction.	Liu and Qian (2007)
2007	Bud, steam & leaf	В5	2.0 mg/L 6-BA +0.5 mg/L IAA +0.5 mg/ L KT	The light duration was 16 h, with a light intensity of 1000 lx, and the culture temperature was maintained at (25 \pm 1) °C.	B5 medium was used as the basic medium, and both buds and leaves induced vigorous callus growth.	Xiang and Gao (2007)
2010	Bud & leaf	MS	0.01 mg/L 6-BA+2 mg/L NAA+0.5 mg/ L 2,4-D	The temperature was maintained at 25 \pm 2°C, with a light duration of 14 h/d and a light intensity of 2000 lx.	The best explants were buds and the best induction medium was MS+NAA 2 mg/L+6-BA 0.01 mg/L+2,4-D 0.5 mg/L, the inducing ratio could reach 100%.	Liu (2010)
2011	Stem segment	MS	0.8 mg/L 6-BA +0.1 mg/L NAA	The culture temperature was maintained at (25 \pm 1) °C, with a light duration of 16 h/d and a light intensity of 30-40 μ mol m ⁻² ·s ⁻¹ .	Adventitious buds emerged from the callus tissue at the base, with an average proliferation rate exceeding 3.	Xu et al. (2011)
2012	Bud	1/2MS	1.0 mg/L 6-BA +0.1 mg/L NAA	The light intensity was 2000 lx, the temperature in the chamber was adjusted to (25 ± 2) °C, and the humidity ranged from 70% to 80%.	Adventitious buds emerged from the callus tissue at the base.	Li et al. (2012)

(Continued)

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References	Shan et al. (2013)	Ren et al. (2015)	Hu et al. (2016)	Heng et al. (2018)	He et al. (2022)
Result	The callus tissue grew rapidly, with a large quantity, loose and moist texture, and bright color.	The callus tissue exhibited vigorous growth.	The callus tissue was pale yellow, with a loose texture and vigorous growth. The growth curve followed an S-shape, with a growth rate of (21.62 \pm 0.54) g per bottle after 24 d. Each 100 g of callus tissue contained 57 mg of CGA.	The callus tissue exhibited the highest induction rate and vigorous growth.	At this concentration, the callus induction rate was the highest, and the callus tissue appeared greener, making it the optimal concentration for inducing callus in L <i>japonica</i> .
Cultivation Conditions	The temperature was (25 \pm 2) °C, with a light intensity of 2000 lx and a photoperiod of 14 h/d.	The samples were first cultured in the dark for 24 h, then transferred to a $25^\circ C$ incubator for further cultivation.	The culture temperature was $25 \pm 2^{\circ}$ C with 60% humidity. In the first stage, the samples were cultured in the dark for 9 d. In the second stage, a light/dark alternation cycle was applied with a light intensity of 2000 lx and a photoperiod of 14 h/d.	Under light culture conditions, the light intensity was 1200 lx. For dark culture, the inoculated materials were placed on a culture rack and covered with black boxes to prevent light exposure.	The culture temperature was 25°C, with a light intensity of 2500 lx and a photoperiod of 12 h/d.
PGRs	0.01 mg/L 6-BA +2.0 mg/L NAA +0.5 mg/L 2,4-D	1.5 mg/L 6-BA+1.0 mg/L NAA	0.15 mg/L 6-BA +1.5 mg/L NAA+1.0 mg/L 2.4-D+0.75 mg/L KT	1.5 mg/L 6-BA+0.2 mg/L NAA+1.0 mg/ L KT	4.0 mg/L 2,4-D
Medium	MS	MS	SM	SM	SM
Explant	The bottom of the flower bud	Leaf	Bud	Stem segment	Young leaf
Time	2013	2015	2016	2018	2022

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to a medium containing 500 mg/L carbenicillin for sterilization, was identified as the key condition for achieving optimal induction (Yuan and He, 2007). He and Zhou (2008) further optimized the induction conditions for *L. japonica* hairy roots. Their results indicated that leaf explants from tissue culture seedlings, after 5 days of pre-cultivation, immersion in bacterial suspension diluted 45-fold for 4 minutes, and 3 days of co-cultivation, followed by transfer to 1/2 MS medium containing 500 mg/L carbenicillin, achieved the highest induction rate of 54.3%. The successful establishment of *L. japonica* hairy roots provides an important foundation and evidence for the construction of Ri plasmid-mediated genetic transformation systems and the industrial-scale production of medicinal active compounds through hairy root cultures.

2.6 Polyploid induction of L. japonica

Compared to diploid plants, polyploid plants generally exhibit greater vigor, yield, and quality, as well as enhanced resistance to various biotic and abiotic stresses. Additionally, polyploid breeding can overcome the incompatibility issues of distant hybridization, making it an important approach for germplasm innovation in medicinal plants (Schatlowski and Köhler, 2012; van Berkum et al., 2010). Liu et al. (2012b) induced tetraploid plants from L. japonica "Hongxing No. 2" callus tissues by soaking them in 0.1% colchicine for 15 h. Compared to diploid plants, the tetraploid plants exhibited deeper leaf color, slower growth, shorter and thicker internodes, as well as significantly enlarged stomata and guard cells with reduced stomatal density. Wang and Zhao (2012; 2014) used a modified L.D. Cua method to artificially mutate diploid L. japonica. Cytological and early morphological analyses of the M1 mutant plants revealed that while the control plants were diploid (2n = 2x = 18), the M1 mutant plants were tetraploid (2n = 4x = 36). The tetraploid plants exhibited enhanced growth vigor, deeper leaf color, larger and thicker leaves, and a reduced leaf shape index after chromosome doubling. "Jiufeng No. 1", a homologous tetraploid bred using polyploid breeding techniques, was developed from the traditional L. japonica cultivar "Damaohua" from Pingyi. Compared to its parent "Damaohua", its CGA content increased by 30%, and its average yield improved by 58.79% (Xing, 2006). Zhang et al. (2011) induced allopolyploid lines of hybrid L. japonica under in vitro tissue culture conditions. Field agronomic observations showed that the flower bud size of the polyploid lines was significantly larger, and the average CGA content in the allopolyploid lines was 1.34 times that of the hybrid diploid control (CK).

3 Research on the metabolic regulation of CGA in *L. japonica*

The synthesis of CGA originates from the complex phenylpropanoid metabolic pathway (Hu et al., 2024; Pan et al., 2021b). Inducers are substances capable of activating or enhancing the synthesis of secondary metabolites in plants (Petrova et al.,

FABLE 2 Continued

Cheng et al.

Time	Explant	Medium	PGRs	Cultivation Conditions	Result	References
1997	Young leaf	В5	0.1 mg/L 2,4-D+0.2 mg/L BA	-	Six were shown to be (2S)-2-hydroxycurdione, (2R)-2-hydroxycurdione, (8S)-6-hydroxycurdione, (2R, 8S)-8-hydro-2-hydroxycurdione, (1S, 10S)-1,10-epoxycurdione and (1R, 10R)-1,10-epoxycurdione in suspension cultured cells induced from calli of <i>L. japonica</i> .	Horiike and Ohshiro (1997)
1999	Stem	MS	10 μM NAA +10 μM BA	These cultures were agitated on a rotary shaker at a speed of 100 rpm min ⁻¹ at 23°C in the dark and subcultured every 2-3 weeks for over 1 year.	The cells also converted 7-deoxyloganin into both loganin and secologanin.	Yamamoto et al. (1999)
2003	Zygotic embryo	MS	4.52 mM 2,4-D	The flask was placed on a gyratory shaker at 100 rpm, all cultures were incubated at 25°C in the dark.	A cell suspension culture system was established.	Kim et al. (2003)
2010	Flower bud	MS	1.5 mg/L BA +0.75 mg/L KT+0.5 mg/ L NAA	The temperature was $25 \pm 2^{\circ}$ C, with a light duration of 14 h/d and a light intensity of 2000 lx. The culture was placed in a shaking incubator at 120 r·min ⁻¹ for suspension culture.	During the culture process, the dry weight of the cells showed an increasing trend, reaching a maximum of 8 g/L at 18 d. The soluble sugar content increased rapidly, peaking at 18 d. Soluble protein, POD, and SOD activities peaked at 12 d.	Liu (2010)
2012	Young leaf	MS	2.0 mg/L 2,4-D+0.5 mg/L KT	The culture was placed in a shaker incubator at $100 \text{ r}\cdot\text{min}^{-1}$ and 26°C under dark conditions.	Under liquid suspension culture conditions, the cell growth cycle lasted 14 d.	Wu (2012)
2016	Bud	MS	1.4 mg/L 6- BA+0.5 mg/ L NAA+0.7 mg/L 2,4-D	The culture was subjected to shaking at 110 r·min ⁻¹ and 28°C under dark conditions for 7 d.	The growth of honeysuckle suspension cells peaked on day 10. High-performance liquid chromatography (HPLC) analysis revealed that each 100 g of dry cells contained 826.7 mg of CGA.	Hu (2016)
2016	Young leaf	B5	2.0 mg/L 6- BA+2.0 mg/ L 2, 4-D	The cultures were kept under continuous agitation at 110 r·min ⁻¹ in an orbital shaker and incubated at 25°C, with a 16 h photoperiod (40 μ mol m ⁻² s ⁻¹).	The findings provide a potential basis for large scale production of CGA using cell suspension cultures of <i>L. macranthoides</i> .	Li et al. (2016)
2019	10- day-old buds; 15-day-old young leaves; 15-day-old young stem	MS	1.5 mg/L 6- BA+0.2 mg/ L NAA+0.1 mg/L 2, 4-D	The inoculated flasks were placed on a rotary orbital shaker at 110 r·min ⁻¹ and incubated at 28 \pm 2°C in the dark.	A cell suspension system was successfully established, with characteristic of a "S" growth curve and healthy morphology. 3,5-di-O-caffeoylquinic acid, 3-O-caffeoylquinic acid, 4,5-di-O-caffeoylquinic acid and 3,4-di-O-caffeoylquinic acid were the main secondary metabolites in the suspension culture cells. The total content of these CGAs was 22.7 mg g ⁻¹ in the suspension cells.	Hu et al. (2019)
2019	Young leaf	MS	0.6 mg/L 6- BA+0.5 mg/ L NAA+0.2 mg/L 2,4-D	The culture was shaken at 110 $r \cdot min^{-1}$ and 26°C in a shaker incubator.	The culture was shaken at 110 r·min ⁻¹ and 26°C in a shaker incubator.	Zu et al. (2019)
2022	Stem	MS	0.2 mg/L 6- BA+0.8 mg/ L NAA	All cells were subsequently cultured in a shaker at 110 $r \cdot min^{-1}$, and at a temperature of 22 ± 2°C.	The cell growth curves were determined using the optimal cell suspension system described above. Cell growth peaked at 18 d of suspension culture, with the fresh weight of cells being 676.0 \pm 12.66 g/L.	Wang et al. (2022)

2024; Bhaskar et al., 2022). Under the influence of inducers, plants undergo stress responses characterized by the activation of defenserelated genes or the inactivation of non-defense-related genes, transient protein phosphorylation or dephosphorylation, and the expression of related enzymes. These molecular changes provide critical insights into the biosynthetic pathways of secondary metabolites (Narayani and Srivastava, 2017; Yamaner et al., 2013).

3.1 Metabolic pathway of CGA biosynthesis

The synthesis of CGA originates from the complex phenylpropanoid pathway. Based on existing literature, three main biosynthetic pathways for CGA have been summarized (Figure 4). Initially, glucose in plants is catalyzed by specific enzymes to form shikimic acid, which is further converted into phenylalanine. Subsequently, through the sequential catalysis of phenylalanine ammonia-lyase (PAL), cinnamate-4-hydroxylase (C4H), and 4coumarate-CoA ligase (4CL), phenylalanine is transformed into pcoumaroyl-CoA. p-Coumaroyl-CoA acts as a precursor for the biosynthesis of flavonoids and lignin, and it also represents a critical starting point for the CGA biosynthetic pathway. (1) Route 1: p-Coumaroyl-CoA reacts with quinic acid (QA) under the catalysis of hydroxycinnamoyl-CoA shikimate/quinate hydroxycinnamoyl transferase (HCT) to produce p-coumaroyl quinic acid, which is subsequently hydroxylated by coumarate-3-hydroxylase (C3H) to form CGA (Valiñas et al., 2015) (Figure 4, Route 1). (2) Route 2: p-Coumaroyl-CoA combines with shikimic acid to form p-coumaroyl shikimic acid, which is hydroxylated by coumaric acid-3-hydroxylase (C3H) to produce caffeoyl shikimic acid. Subsequently, caffeoyl-CoA is synthesized by hydroxycinnamoyl-CoA shikimate/quinate hydroxycinnamoyl transferase (HCT). Finally, caffeoyl-CoA reacts with quinic acid under the catalysis of hydroxycinnamoyl-CoA: quinate hydroxycinnamoyl transferase (HQT) to form CGA via esterification (Tang et al., 2021; Niggeweg et al., 2004) (Figure 4, Route 2). (3) Route 3: Caffeoyl glucose is converted into CGA by quinic acid hydroxycinnamoyl transferase (Tang et al., 2021; Niggeweg et al., 2004) (Figure 4, Route 3).



FIGURE 4

chematic cartoon displays 3 synthetic pathways of CGA. PAL: phenylalanine ammonia-lyase. C4H: cinnamate-4-hydroxylase. 4CL: 4-coumarate-CoA ligase. C3H, coumaric acid-3-hydroxylase; HCT, shikimate/quinate hydroxycinnamoyl transferase; HQT, quinate hydroxycinnamoyl transferase.

3.2 The function of key rate-limiting enzyme genes in the CGA synthesis pathway

Studies have shown that HQT (Luo et al., 2018; Zhang et al., 2016; Wu et al., 2014; Liu et al., 2012c), PAL (Wu et al., 2024; Li et al., 2024a), 4CL (Li et al., 2015), HCT (Chen et al., 2024; He et al., 2013), and C3H (Chen et al., 2020) are key enzymes in the biosynthetic pathway of CGA. These enzymes play a crucial role in the biosynthesis of CGA in *L. japonica*.

PAL is a key bridge between primary and secondary metabolism, catalyzing the conversion of phenylalanine to cinnamic acid. It controls the metabolic flux of precursors into the phenolic pathway and plays an essential role in regulating the overall level of accumulated phenolic compounds (Pan et al., 2021b; Clé et al., 2008) Therefore, PAL plays a crucial role in plant growth and development, as well as in responding to biotic and abiotic stress defenses, such as mechanical damage and ultraviolet radiation (Zhao et al., 2022). C4H is the second key ratelimiting enzyme in the phenylpropanoid pathway, localized to the endoplasmic reticulum of plant cells. It belongs to the cytochrome P450 enzyme family, specifically the CYP73 subgroup (Zhang et al., 2020). As a P450 protein, C4H catalyzes an irreversible reaction, using NADPH as an electron donor in the presence of oxygen and reduced nicotinamide adenine dinucleotide phosphate (NADPH). This enzyme catalyzes the conversion of trans-cinnamic acid to pcoumaric acid (Cheng et al., 2018). 4CL is a major branch point for cinnamic acid derivatives, converting them into different types of coenzyme A derivatives, including p-coumaric acid, caffeic acid, ferulic acid, and cinnamic acid. These intermediates are the precursors for synthesizing phenylpropanoid compounds such as lignin, CGA, and flavonoids (Li et al., 2015; Li and Nair, 2015). C3H is another key enzyme in the CGA biosynthesis pathway and the second hydroxylase in the biosynthesis of fatty acids, caffeic acid, and other phenolic acids. It belongs to the CYP98A family of cytochrome P450 monooxygenases (Yang et al., 2020). HCT belongs to the BAHD acyltransferase family and contains two conserved structural domains, HXXXD and DFGWG, which are present in all terrestrial plants (Yang et al., 2003). In the phenylpropanoid metabolic pathway, HCT is located at both the upstream and downstream junctions of the hydroxylation process catalyzed by C3H, enabling it to regulate the target genes of both the upstream and downstream pathways. It not only generates p-coumaroyl shikimate/quinic acid as substrates for C3H but also converts the products generated by C3H, caffeoyl shikimate/quinic acid, into caffeoyl-CoA. Therefore, HCT is considered to play a dual role in the phenylpropanoid hydroxylation pathway (Chao et al., 2021). HQT also belongs to the BAHD acyltransferase family and contains the two gene sequences HXXXD and DFGWG (Yang et al., 2003). HQT is tightly linked with HCT. Unlike the substrate versatility of HCT, HQT has substrate specificity and is the key rate-limiting enzyme in the final step of CGA biosynthesis. It catalyzes the ester exchange between caffeoyl-CoA and quinic acid, producing structurally different CGA derivatives (Li et al., 2019).

3.3 Elicitors

Inducers are a class of chemical substances or biological factors that promote the production of target metabolites and induce physiological changes in plants (Petrova et al., 2024; Hatami et al., 2019). Studies have shown that adding inducers can effectively reduce cultivation costs, increase cultivation efficiency, promote the production of medicinal plants, and enhance the yield of secondary metabolites, ultimately boosting the production of medicinal plants (Bhaskar et al., 2022; Narayani and Srivastava, 2017; Thakur et al., 2020). Inducers come in a wide variety of types and can be classified based on their origin into biological inducers, abiotic inducers, and novel nanoparticle inducers (Hussain et al., 2022). The transduction of induction signals within plants is mediated by multiple parallel or interconnected signaling pathways. These pathways establish an effective defense system in response to various external stimuli, leading to different reactions both inside and outside the plant (Zhang et al., 2012; Malik et al., 2011). Generally, the induction effect of inducers in plants can be divided into four steps: first, the induction signal is recognized and bound by receptors on the cell membrane; next, ion channels across the cell membrane are altered; then, the second messenger transduces the signal to the cell nucleus, activating gene regulatory factors; and finally, specific biological effects are generated in the plant (Baenas et al., 2014). The interaction between the inducer molecules and specific receptors on the surface of plant cell membranes is the most critical step in the action of inducers (Ferrari, 2010; Shasmita et al., 2018). After the induction signal is sensed, ion channels on the cell membrane are altered, leading to an increase in cytoplasmic Ca^{2+} , efflux of Cl⁻/K⁺, and influx of H⁺, resulting in cytoplasmic acidification. Subsequently, the second messenger transduces the inducer signal into the cell (Abdul Malik et al., 2020; Ludwig et al., 2004; Shabala and Pottosin, 2014). Inside the cell, mitogenactivated protein kinases (MAPKs) and reduced coenzyme II (NADPH) are activated, producing reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Zhao et al., 2005; Mishra et al., 2012). Ultimately, regulatory factors within the cell are activated, regulating the expression of intracellular defense genes. This may directly or indirectly lead to the synthesis of secondary metabolites to resist changes in the external environment (as shown in Figure 5).

3.4 Effect of inducers on CGA of *L. japonica*

To enhance the CGA content in *L. japonica* as in Figure 6, Gu et al. (2024) proposed an induction strategy based on yeast polysaccharide and revealed its induction mechanism. The study revealed significant differences in active component content between the 0.1 g/L yeast polysaccharide treatment group and the control group. Transcriptome analysis identified 218 differentially



expressed genes, with 60 upregulated and 158 downregulated. Further analysis revealed 12 key genes involved in the biosynthesis of phenolic compounds, including PAL1, PAL2, PAL3, 4CL1, 4CL, CHS1, CHS2, CHS, CHI1, CHI2, F3H, and SOH, of which SOH being a specific gene for the synthesis of caffeic acid and CGA. Methyl jasmonate (MeJA), salicylic acid (SA), and ultraviolet radiation (UV) can significantly induced and regulated the synthesis of CGA compounds in L. japonica suspensioncultured cells. Under conditions of MeJA at 200 µmol/L, SA at 50 µmol/L, UV exposure for 2 h, and a 5-day induction period, the total CGA content in suspension cells reached 4.6 times that of the control group. Further transcriptome analysis revealed that, after MeJA induction (200 µmol/L) for 4, 10, and 20 h, the expression levels of key CGA biosynthetic enzyme genes: C4H, PAL, HCT, C3' H, CAD, and 4CLwere significantly upregulated compared to the control (0 h) (Li, 2018). Ecological conditions play a critical role in the accumulation and synthesis of secondary metabolites in medicinal plants, including altitude, temperature, light intensity, and light quality (Li et al., 2020). Chen et al. (2023) found that, after 7 days of shading treatment, CGA content in L. japonica leaves significantly decreased by 1.78-fold. Concurrently, the overall transcriptional expression of CGA biosynthetic pathway-related genes was downregulated, while the expression of photosynthetic signal protein-related genes was upregulated. During the light signaling transduction process, the transcriptional expression of PHOT, HY5, and PIF was significantly influenced by shading treatment, regulating the expression of transcription factors (TFs) such as MYB, bHLH, and WRKY. These regulatory effects collectively led to a reduction in CGA content. Moreover, it was

demonstrated that the transcriptional expression of HY5 in L. japonica leaves positively correlates with CGA content, confirming that decreased light intensity downregulates HY5 expression, which is a key factor contributing to the reduction in CGA levels. Salt-alkali stress can also promote the accumulation of phenolic compounds in L. japonica leaves. Yan et al. (2017) demonstrated that NaCl stress activates the synthesis of phenolic compounds, as evidenced by a significant increase in the transcription level and activity of the PAL gene, accompanied by an elevated concentration of phenolic compounds. After 15 days of 150 and 300 mM NaCl stress, the CGA content in leaves increased by 67.43% and 48.86%, respectively, while the luteoloside content increased by 54.26% and 39.74%, respectively. Cai et al. (2021) further revealed that the synthesis of phenolic acid metabolites in L. japonica under NaCl stress is closely related to the expression of upstream enzymes in the phenylalanine metabolism pathway, including PAL, C4H, and 4CL. Additionally, HCT, C3'H, and COMT were significantly upregulated during phenolic acid synthesis, while FLS and CHI showed marked increases in expression during flavonoid biosynthesis. Zhang et al. (2022a) found that after 30 days of 10°C low-temperature stress, the CGA and luteoloside contents in L. japonica significantly increased, reaching 23 times and 17 times that of the control group, respectively. Simultaneously, the expression of CGA biosynthesisrelated genes PAL and 4CL was significantly upregulated under low-temperature stress, while the upregulation of CHS and CYP75B1 activated the luteoloside biosynthesis pathway. These findings suggest that low-temperature stress promotes the accumulation of metabolites by regulating the phenylalanine



Study on the regulation of secondary metabolites of *L. japonica*. Note: The blue boxesrepresent biological and abiotic elicitors, the pink boxes represent genes, and the brown boxesrepresent secondary metabolite types. methyl jas-monate (MJ), salicylic acid (SA), chalcone synthase (CHS), chal-cone isomerase (CHI), phenylalanine ammonia (PAL), cinnamic acid 4-hydroxylase (C4H), Cinnamyl alcohol dehydrogenase (CAD), 4coumarate-CoA ligase (4CL), flavanone 3-hydroxylase (F3H), caffeoylshikimate esterase (CSE), caffeoyl-CoAO-methyltransferase (CCOM), caffeic acid 3-O-methyltransferase (COMT), candidate catalytic enzymes (C3'H), hydroxycinnamoyl transferase (HCT), cinnamoyl-CoA reductase (CCR), Light sig-nalling of *L. macranthoides* (LmHY5), transcription factors (TFs).

metabolism and associated secondary metabolic pathways. The inducers on CGA content in *L. japonica* are summarised in Table 4.

4 Conclusion and prospects

4.1 Conclusion

Tissue culture technology, as an essential component of modern plant biotechnology, holds significant application potential. It not only provides technological support for the efficient utilization of medicinal plant resources and the production of active ingredients, but also offers innovative solutions for plant breeding, genetic improvement, and resource conservation. With the continuous development of biotechnology, the integration of tissue culture technology with other modern biotechnologies is expected to drive the efficient and sustainable development of the medicinal plant industry, providing richer resources and technological support for global sectors such as pharmaceuticals and agriculture. This review summarizes the latest research progress on the in vitro culture techniques of L. japonica and explores the application and impact of elicitors in the CGA biosynthesis pathway. In vitro culture techniques, such as direct organogenesis, callus culture, suspension cell culture, protoplast culture, hairy root culture, and polyploid induction, provide various potential pathways for the production of secondary metabolites and resource conservation in L. japonica. Regarding the biosynthesis of CGA, this review also discusses the key enzyme genes involved in the process, including PAL, C4H, 4CL, C3'H, HCT, and HQT, which play critical roles in the metabolic pathway of L. japonica.

4.2 Prospects

Although current research has revealed the potential of *L. japonica in vitro* culture techniques for CGA synthesis, future studies still face several challenges and research directions.

Firstly, breakthroughs in anther culture techniques. During the literature review, no previous research on the anther culture of L. japonica via the indirect organogenesis pathway was found. Compared to other explant culture methods, anther culture can directly form haploid plants from pollen, which can then be converted into homozygous plants through polyploid induction. The resulting homozygous plants exhibit higher genetic stability due to identical alleles at each locus in their genome. It can also accelerate germplasm innovation by selecting and cultivating specific pollen to obtain plants with particular traits or superior genes. More importantly, anther culture provides a powerful platform for genomics research and genetic improvement. By combining gene editing technologies, such as CRISPR/Cas9, precise gene manipulation can be conducted during the anther culture process, further advancing molecular breeding and genomic selection applications in plants. Furthermore, when combined with molecular marker techniques, anther culture can more accurately select plants with desired traits, thus improving breeding efficiency. Therefore, it is evident that the anther culture technology for L. japonica needs to be advanced.

Secondly, in-depth study of the regulatory mechanisms of elicitors. Although elicitors have been confirmed to promote the synthesis of CGA, the molecular mechanisms by which they regulate the expression of key enzyme genes through signal transduction pathways remain poorly understood. Future research

TABLE 4 Effect of Inducer on CGA of L. japonica.

Material	Activity Component	Influence Factor	Metabolic Regulation	Result	
Leaf	CGA concentration; Luteolosid concentration	Saline stress (150 mM, 300 mM NaCl, 15 d)	PAL gene family consists of PAL1, PAL2 and PAL3, and their transcription was significantly elevated by 150 and 300 mM NaCl at day 7 and 15 d.	Genes transcription and activity of phenylalanine ammonia-lyase and increased phenolics concentration. After 15 d of NaCl stress at 150 mM and 300 mM, the CGA content in leaves increased by 67.43% and 48.86%, respectively, while the luteoloside content increased by 54.26% and 39.74% (Yan et al., 2017).	
Leaf	CGA concentration; Luteolosid concentration	Chilling stress (10°C, 30 d)	Cold stress activated secondary metabolic pathways such as phenylpropanoid-flavonoids and carotenoids. The PAL and 4CL genes in the phenylalanine metabolism pathway, involved in CGA biosynthesis, were upregulated under cold stress, promoting the synthesis of CGA. In the carotenoid biosynthesis pathway, genes such as DXR, ISPF, GGPS, GGPPS, PDS, ZDS, LCYE, and LCYB were significantly activated, driving the synthesis of carotenoids.	The contents of CGA and verbascoside both showed an increasing trend under cold stress. From the starting point (SP) to 30 d of treatment (T30), the contents of both compounds gradually accumulated, with a significant increase at T30. Compared to SP, the CGA content increased 23-fold, and the verbascoside content increased 17-fold (Zhang et al., 2022a).	
Flower	Phenylpropanoid, flavonoid, and iridoid	Salt stress	PAL, C4H, and 4CL were significantly upregulated, promoting the synthesis of phenolic acid-derived secondary metabolites. CHI is involved in flavonoid synthesis and its expression was upregulated under salt stress, affecting the synthesis of flavonoid secondary metabolites. The expression of the F3H gene varied in the salt treatment group, suggesting that the changes in flavonoid levels may be jointly regulated by upstream and downstream pathways. This influenced the synthesis and accumulation of flavonoid secondary metabolites. DXS and CYP72A1 were upregulated under salt stress, driving the synthesis and accumulation of sesquiterpenoid compounds.	15 bioactive constituents (7 phenolic acids, 4 flavonoids, and 4 iridoids), which were significantly accumulated (Cai et al., 2021).	
Leaf	The content of organic acids, flavonoids and iridoids	Yeast polysaccharides (0.1 g/L, 1 g/L, 5 g/L and 10 g/L; 6 d)	The expression of PAL1, PAL2, and PAL3 was significantly increased, regulating the phenylpropanoid biosynthetic pathway and influencing the synthesis of downstream secondary metabolites. The expression of 4CL1 and 4CL showed dynamic changes, playing an important regulatory role in the phenylpropanoid biosynthetic pathway. The expression of CHS1, CHS2, and CHS was significantly upregulated, having a crucial impact on flavonoid compound synthesis. The expression of the SOH gene promoted the synthesis of caffeic acid and CGA.	Low concentrations of yeast polysaccharides had a significant impact on organic acid components. The contents of caffeic acid, CGA, and chlorogenic acid A significantly increased the day after application. The growth effect of coumaric acid was optimal 5 days after treatment with 0.1 g/L yeast polysaccharides. Verbascoside showed the most significant increase 5 days after treatment with 0.1 g/L yeast polysaccharides. Low concentrations of yeast polysaccharides significantly enhanced the levels of secoxyloganin, sweroside, and morroniside (Gu et al., 2024).	
Suspension cell	CGAs content	200 μM Me-J, 50 μM SA and 2 h/d UV-B	_	The optimal inducer combination significantly promoted the accumulation of four types of CGAs. On the sixth day of treatment, the total CGA content reached its maximum, increasing 4.52-fold and 3.13-fold compared to the control group and field flower buds, respectively (Du et al., 2020).	
Stem, leaf & flower	Polysaccharide, total polyphenols, total flavonoid, and CGA	UV-B (8.4, 22.4 µW/ cm ² ; 2, 4, 6, 8 h)	_	The contents of polysaccharide, total polyphenols, total flavonoid, and CGA in different organs all increased significantly and reached a peak under the UV-B (8.4 μ W/cm ²) treatment for 2 h (Wu et al., 2021).	
Suspension cell	Production of CGA	PGRs	_	When the concentration of 6-BA was 2.0 mg/L, both biomass and CGA accumulation were higher. In the hormone combination, 2.0 mg/L 6-BA+0.5 mg/L 2,4-D was beneficial for CGA accumulation, and 2.0 mg/L 6-BA+2.0 mg/L 2,4-D favored biomass accumulation (Li et al., 2016).	

(Continued)

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TABLE 4 Continued

Material	Activity Component	Influence Factor	Metabolic Regulation	Result
Leaf	The content of flavonoids and CGA	Cadmium addition in soil and ozone stress	In the comparison between CK and Cd, the MAPK signaling pathway- plant pathway showed the highest enrichment significance in honeysuckle leaves. In the comparison between CK and EO, the photosynthesis pathway showed the highest enrichment significance; In the comparison between EO and EO-Cd, the photosynthetic antenna protein pathway exhibited the highest enrichment significance.	Cadmium addition in soil and ozone stress significantly inhibited the CGA content but the content of flavonoids was increased (Tao, 2023).
Suspension cell	The total content of CGAs	200 µM Me-J	C4H, PAL, HCT, C3'H, CAD, 4CL were up-regulated with higher differential expression folds.	The total content of CGAs reached up to 6.99% (Li, 2018).
Leaf	The content of free proline, soluble sugar and soluble protein	Lead induction (0, 400, 600, 800, 1000 mg/kg)	_	Under lead stress, the chlorophyll content in honeysuckle decreased as the lead concentration increased, while the levels of free proline, soluble sugars, and soluble proteins increased, with the most significant changes observed at a lead concentration of 2800 mg/kg. Lead stress also led to an increase in malondialdehyde (MDA) and antioxidant enzyme activities. At a lead concentration of 600 mg/kg, MDA content showed no significant difference from the control group, but SOD activity increased by 41.65%. When the lead concentration reached 1000 mg/kg, MDA content peaked, with a significant difference compared to the control group, and POD and CA activities increased by 78.92% and 45.60%, respectively (Mao et al., 2019).
Flower	Isochlorogenic acids and iridoid glycoside	Ultraviolet induction	_	The contents of isochlorogenic acid in <i>L. japonica</i> increased significantly. Secologanic acid, secoxyloganin, secologanin and (E)-aldosecologanin are all iridoid compounds, and their contents all increased significantly after ultraviolet radiation (Ning, 2012).

should focus on how elicitors regulate the expression of key enzyme genes in the CGA biosynthesis pathway through pathways such as MAPK signaling, ROS production, and Ca²⁺ signaling, to reveal their complex regulatory mechanisms.

Thirdly, challenges in the Scalable Production of In Vitro Culture Systems. Tissue culture technology is one of the application areas of plant biotechnology that allows the extraction of valuable plant metabolites under controlled conditions. Compared to traditional methods, the production of secondary metabolites through plant cell and tissue culture offers significant advantages. These advantages include the ability to produce target metabolites under controlled conditions, independent of environmental factors, and the potential to optimize culture conditions to enhance secondary metabolite yields. the ability to provide adequate yields, if necessary, taking into account the balance between supply and demand, and thus to regulate the market on a regular basis. Additionally, in vitro culture can help balance supply and demand by ensuring sufficient production when needed, allowing for market regulation. It also offers a rapid production rate that is unaffected by political constraints. Furthermore, it enables the production of disease-free and contaminant-free plant materials, and any plant species, regardless of whether they originate from tropical or subtropical regions, can be cultivated in vitro. Although in vitro culture technology has a high potential for metabolite accumulation, it still faces several technical challenges in large-scale production, such as standardization of culture conditions, medium cost, contamination control and production efficiency. In order to overcome these challenges and maximize its potential, future research needs to focus on optimizing scale-up strategies for in vitro culture systems, improving the balance between cell growth and metabolism, reducing production costs while enhancing economic viability, and exploring the application of gene editing and regulatory approaches in large-scale production.

Fourthly, comprehensive optimization of in vitro culture technology. Different in vitro culture techniques, such as direct organogenesis, callus culture, suspension cell culture, protoplast culture, hairy root culture, and polyploid breeding, each have their own advantages. However, there is a lack of detailed mechanistic studies on how these techniques influence the biosynthetic pathway of CGA at the molecular level. Future research could integrate advanced technologies such as transcriptomics, proteomics, and metabolomics to comprehensively analyze the regulatory mechanisms by which different culture techniques influence CGA biosynthesis. This would help elucidate the intricate regulatory effects of various culture methods on secondary metabolite synthesis in plants, providing a theoretical foundation for optimizing culture conditions at an industrial scale to enhance CGA yield. Additionally, studies should explore how culture techniques affect CGA biosynthesis by modulating plant signaling pathways, hormone levels, and stress responses. Building on this foundation, the integration of molecular biology and gene editing technologies may enable precise regulation of plant secondary metabolism, thereby improving the efficiency and stability of CGA production.

Fifthly, exploration of Genotypic Differences. Current research primarily focuses on a single genotype of *L. japonica*, whereas

different genotypes may respond differently to in vitro culture conditions and elicitors. Different genotypes may exhibit significant variations in response to in vitro culture conditions and elicitors, and these differences may lead to distinct accumulations of bioactive compounds by regulating the expression of key enzyme genes in secondary metabolic pathways. Comparative studies across multiple genotypes can help identify superior germplasm with high yields of specific metabolites, providing a stable raw material supply for pharmaceutical development. Significant differences in the response of different genotypes of L. japonica in tissue culture underscore the importance of including multiple genotypes in research. This approach helps comprehensively evaluate the universality and specificity of key culture parameters (such as explant selection, sterilization methods, and hormone ratios) across different genotypes, providing theoretical foundations and technical support for establishing more efficient and standardized culture systems. Research on multi-genotype tissue culture can identify genotypes with faster growth and stronger metabolic product synthesis capabilities, and by combining rapid propagation techniques, it can achieve sustainable resource utilization. Furthermore, based on research data from different genotypes, a "genotype-phenotypemetabolome" database for L. japonica can be established, supporting precision breeding and the development of functional products. Therefore, it is crucial to include multiple genotypes of L. japonica in future research to conduct in-depth studies on tissue culture and secondary metabolite biosynthesis pathways.

Author contributions

JC: Resources, Writing – original draft. YC: Conceptualization, Resources, Writing – review & editing. FG: Resources, Writing – original draft, Writing – review & editing. PD: Conceptualization, Resources, Writing – original draft. CZ: Resources, Writing – original draft, Writing – review & editing. WL: Resources, Software, Writing – original draft. HW: Resources, Software, Writing – review & editing.

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Conflict of interest

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

References

Abdul Malik, N. A., Kumar, I. S., and Nadarajah, K. (2020). Elicitor and receptor molecules: orchestrators of plant defense and immunity. *Int. J. Mol. Sci.* 21, 963. doi: 10.3390/ijms21030963

Asyakina, L., Sukhikh, S., Ivanova, S., Prosekov, A., Ulrikh, E., and Chupahin, E. (2021). Determination of the qualitative composition of biologically-active substances of extracts of *in vitro* callus, cell suspension, and root cultures of the medicinal plant *Rhodiola rosea. Biomolecules* 11, 365. doi: 10.3390/biom11030365

Avila-Peltroche, J., Won, B. Y., and Cho, T. O. (2020). Optimization of protoplast isolation from the gametophytes of brown alga Undaria pinnatifida using response surface methodology. *J. Appl. Phycol.* 32, 2233–2244. doi: 10.1007/s10811-020-02095-3

Baenas, N., García-Viguera, C., and Moreno, D. A. (2014). Elicitation: A tool for enriching the bioactive composition of foods. *Molecules* 19, 13541–13563. doi: 10.3390/ molecules190913541

Bhaskar, R., Xavier, L. S. E., Udayakumaran, G., Kumar, D. S., Venkatesh, R., and Nagella, P. (2022). Biotic elicitors: A boon for the *in-vitro* production of plant secondary metabolites. *Plant Cell Tiss. Organ. Cult.* 149, 7–24. doi: 10.1007/s11240-021-02131-1

Cai, Z. C., Wang, C. C., Chen, C. H., Chen, H., Yang, R., Chen, J. J., et al. (2021). Omics map of bioactive constituents in *Lonicera japonica* flowers under salt stress. *Ind. Crops Prod.* 167, 113526. doi: 10.1016/j.indcrop.2021.113526

Cambecèdes, J., Duron, M., and Decourtye, L. (1991). Adventitious bud regeneration from leaf expiants of the shrubby ornamental honeysuckle, *Lonicera nitida* Wils. cv. 'Maigrün': effects of thidiazuron and 2,3,5-triiodobenzoic acid. *Plant Cell Rep.* 10, 471–474. doi: 10.1007/BF00233817

Chao, N., Qi, Q., Li, S., Ruan, B., Jiang, X. N., and Gai, Y. (2021). Characterization and functional analysis of the Hydroxycinnamoyl-CoA: shikimate hydroxycinnamoyl transferase (HCT) gene family in poplar. *PeerJ* 9, e10741. doi: 10.7717/peerj.10741

Chen, Z. M., Li, C., Wei, J. T., Li, X. R., Liu, Y., and Guo, Q. (2024). Research progress in the regulation of chlorogenic acid biosynthesis and its application. *Biotechnol. Bull.* 40, 57–71. doi: 10.13560/j.cnki.biotech.bull.1985.2023-0728

Chen, Y., Wang, A. Q., Liu, C., Long, Y. Q., Liu, X., Zeng, J., et al. (2020). Cloning and expression patterns of LmC3H1 gene in *Lonicera macranthoides* and its correlation with chlorogenic acid content. *Chin. J. Exp. Tradit. Med. Formulae* 26, 167–175. doi: 10.13422/j.cnki.syfjx.20200913

Chen, Y. C., Xu, N., Du, L. H., Zhang, J. H., Chen, R., Zhu, Q. F., et al. (2023). Light plays a critical role in the accumulation of chlorogenic acid in *Lonicera macranthoides* Hand.-Mazz. *Plant Physiol. Biochem.* 196, 793–806. doi: 10.1016/j.plaphy.2023.02.016

Cheng, S. Y., Yan, J. P., Meng, X. X., Zhang, W. W., Liao, Y. L., Ye, J. B., et al. (2018). Characterization and expression patterns of a cinnamate-4-hydroxylase gene involved in lignin biosynthesis and in response to various stresses and hormonal treatments in *Ginkgo biloba. Acta Physiol. Plant* 40, 7. doi: 10.1007/s11738-017-2585-4

Clé, C., Hill, L. M., Niggeweg, R., Martin, C. R., Guisez, Y., Prinsen, E., et al. (2008). Modulation of chlorogenic acid biosynthesis in Solanum lycopersicum; consequences for phenolic accumulation and UV-tolerance. *Phytochemistry* 69, 2149–2156. doi: 10.1016/j.phytochem.2008.04.024

Du, L. D., Li, D. M., and Zhang, J. J. (2020). Elicitation of Lonicera japonica Thunb suspension cell for enhancement of secondary metabolites and antioxidant activity. *Ind. Crops Prod.* 156, 112877. doi: 10.1016/j.indcrop.2020.112877

Errico, S., Mastrobuono, V., Pagliarello, R., Bennici, E., Tavazza, R., Verardi, A., et al. (2025). Consumer acceptance of edible hydrogels obtained by plant cell culture technology and by-products valorization: An Italian case study for future innovation

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of the plate. Innov. Food Sci. Emerg. Technol. 100, 103893. doi: 10.1016/j.ifset.2024.103893

Fabian-Marwedel, T., Umeda, M., and Sauter, M. (2002). The rice cyclin-dependent kinase-activation kinase R2 regulates S-phase progression. *Plant Cell* 14, 197–210. doi: 10.1105/tpc.010386

Fang, L., Long, N., Li, Y., Liao, X., Shi, L., and Zhao, H. (2022). Transfer behavior of pesticides from honeysuckle into tea infusions: Establishment of an empirical model for transfer rate prediction. *Ecotoxicol. Environ. Saf.* 234, 113377. doi: 10.1016/j.ecoenv.2022.113377

Ferrari, S. (2010). "Biological elicitors of plant secondary metabolites: Mode of action and use in the production of nutraceutics," in *Bio-Farms for nutraceuticals*, vol. 698 . Eds. M. T. Giardi, G. Rea and B. Berra (Springer US, Boston, MA), 152–166. doi: 10.1007/978-1-4419-7347-4_12

Gao, Q. M., and Dong, C. Y. (2018). High efficient regeneration of wild *Lonicera japonica* Thunb. J. West China Forest. Sci. 47, 59–63+79. doi: 10.16473/j.cnki.xblykx1972.2018.04.011

García-Pérez, R., Miras-Moreno, B., Lucini, L., and Gallego, P. P. (2021). The metabolomics reveals intraspecies variability of bioactive compounds in elicited suspension cell cultures of three *Bryophyllum* species. *Ind. Crops Prod.* 163, 113322. doi: 10.1016/j.indcrop.2021.113322

Georges, D., Chenieux, J. C., and Ochatt, S. J. (1993). Plant regeneration from agedcallus of the woody ornamental species *Lonicera japonica* cv. "Hall's Prolific. *Plant Cell Rep.* 13, 91–94. doi: 10.1007/BF00235297

Gu, X. P., Yang, L. L., Qi, D. M., Zhang, D., Liu, T. L., and Dong, C. M. (2024). Regulation of gene expression in the secondary metabolic synthesis pathway of *Lonicera japonica* Flos by yeast polysaccharides. *Physiol. Mol. Biol. Plants* 30, 1071– 1084 -. doi: 10.1007/s12298-024-01482-1

Haberlandt, G. (1902). Kulturversuche mit isolierten Pflanzenzellen. Sitzungsber K Preuss Akad Wiss Wien Math-Naturwiss 111, 69–92. doi: 10.1038/152126a0

Hatami, M., Naghdi Badi, H., and Ghorbanpour, M. (2019). Nano-elicitation of secondary pharmaceutical metabolites in plant cells: A review. *J. Med. Plants* 18, 6–36. doi: 10.29252/jmp.3.71.6

He, L., Xu, X. L., Li, Y., Li, C. F., Zhu, Y. J., Yan, H. X., et al. (2013). Transcriptome analysis of buds and leaves using 454 pyrosequencing to discover genes associated with the biosynthesis of active ingredients in *Lonicera japonica* Thunb. *PloS One* 8, e62922. doi: 10.1371/journal.pone.0062922

He, M. Y., Yang, Y., and Chen, Z. Y. (2022). Study on rapid propagation of *Lonicera japonica* and measurement of total flavonoids content. *Hunan Agric. Sci.* 1, 38–41. doi: 10.16498/j.cnki.hnnykx.2022.001.011

He, G., and Zhou, Z. K. (2008). A study of hairy roots culture of Flos Lonicerae induced by Agrobacterium rhizogenes Ri. J. Cent. South Univ. Forest. Technol. 28, 77–80.

Heng, J., Han, C. Y., Wang, M. S., and Ma, L. (2018). Induction of Callus of Stem Segments of *Lonicera japonica* Thunb. var. chinensis (Wats.) Bak. *Northern Horticult*. 19), 94–99.

Horiike, T., and Ohshiro, M. (1997). Biotransformation of the germacrane type sesquiterpene curdione by suspension cultured cells of *Lonicera japonica*. *Phytochemistry* 44, 627–632. doi: 10.1016/S0031-9422(96)00569-9

Hosseini, R., and Mulligan, B. (2002). Application of rice (*Oryza Sativa* L.) suspension culture in studying senescence *in vitro* (I) single strand preferring nuclease activity. *Electron. J. Biotechnol.* 5, 42–54. doi: 10.2225/vol5-issue1-fulltext-8

Hsu, P. S., and Wang, H. J. (1988). "Lonicera," in Flora Reipublicae Popularis Sinicae, vol. 72. (Science Press, Beijing), 153–257.

Hu, Z. J. (2016). Preliminary Establishment of Cell Suspension System of Lonicera japonica Thunb and Study on the Inhibition of Nitrosation by Chlorogenic Acid (Jiangxi Agricultural University). doi: 10.27177/d.cnki.gjxnu.2016.000023

Hu, M., Hu, Z., Du, L., Du, J., Luo, Q., and Xiong, J. (2019). Establishment of cell suspension culture of *Lonicera japonica* Thunb and analysis its major secondary metabolites. *Ind. Crops Prod.* 137, 98–104. doi: 10.1016/j.indcrop.2019.05.024

Hu, Z. J., Luo, Q. S., Li, D. M., Du, H. Y., Du, J., and Xiong, J. H. (2016). A study on induction of the *Lonicera japonica* Thunb callus and its chlorogenic acid content. *Acta Agricult. Universitatis Jiangxiensis* 38, 549–556. doi: 10.13836/j.jjau.2016079

Hu, L. F., Yu, L., Weng, Y. H., Qu, H. X., Yang, D. J., Lu, Y., et al. (2024). Combined non-targeted metabolomic and transcriptomic analysis explains the biosynthetic pathway and differential accumulation of chlorogenic acid in the *Liriodendron* petal. *Sci. Hortic.* 328, 112791. doi: 10.1016/j.scienta.2023.112791

Hussain, M. J., Abbas, Y., Nazli, N., Fatima, S., Drouet, S., Hano, C., et al. (2022). Root cultures, a boon for the production of valuable compounds: A comparative review. *Plants* 11, 439. doi: 10.3390/plants11030439

Jacobs, B., Lens, F., and Smets, E. (2009). Evolution of fruit and seed characters in the Diervilla and *Lonicera* clades (Caprifoliaceae, Dipsacales). *Ann. Bot.* 104, 253–276. doi: 10.1093/aob/mcp131

Jia, Z. Q., and Yan, J. (2024). Screening of fungicides to control powdery mildew on honeysuckle. *Agrochemicals* 63, 446–450. doi: 10.16820/j.nyzz.2024.0610

Jiang, C., Wu, S., Feng, X., Yang, C., and Yu, Z. (2021). The complete chloroplast genome of *Lonicera pampaninii* Levl. and its phylogenetic analysis. *Mitochondrial DNA Part B* 6, 3025–3027. doi: 10.1080/23802359.2021.1978891

Kim, S. W., Oh, S. C., In, D. S., and Liu, J. R. (2003). High frequency somatic embryogenesis and plant regeneration in zygotic embryo cultures of Japanese honeysuckle. *Plant Cell Tissue Organ Cult.* 72, 277–280. doi: 10.1023/A:1022360115275

Kobayashi, Y., Kärkkäinen, Y., Häkkinen, S. T., Nohynek, L., Ritala, A., Rischer, H., et al. (2021). Life cycle assessment of plant cell cultures. *Sci. Total Environ.* 808, 151990. doi: 10.1016/j.scitotenv.2021.151990

Kong, E. Y. Y., Biddle, J., Foale, M., Panis, B., and Adkins, S. W. (2021). The potential to propagate coconut clones through direct shoot organogenesis: A review. *Sci. Hortic.* 289, 110400. doi: 10.1016/j.scienta.2021.110400

Kundu, S., Salma, U., Ali, M. N., Hazra, A. K., and Mandal, N. (2018). Development of transgenic hairy roots and augmentation of secondary metabolites by precursor feeding in *Sphagneticola calendulacea* (L.) Pruski. *Ind. Crops Prod.* 121, 206–215. doi: 10.1016/j.indcrop.2018.05.009

Lai, K. H., Chen, Y. L., Lin, M. F., El-Shazly, M., Chang, Y. C., and Chen, P. J. (2022). *Lonicerae japonicae* Flos attenuates neutrophilic inflammation by inhibiting oxidative stress. *Antioxidants* 11, (9). doi: 10.3390/antiox11091781

Li, D. M. (2018). Study on Exploration the Elicitor and Key Enzyme Genes for Enhancement Accumulation of Chlorogenic Acids in Lonicera japonica Thunb, Suspension Cells (Jiangxi Agricultural University). doi: 10.27177/d.cnki.gjxnu.000064

Li, F. M., Chen, L. X., Li, J. J., Zhang, M., Qi, Y. T., Xu, X., et al. (2024a). Identification and bioinformatics analysis of gene for chlorogenic acid in *Lonicera japonica* Thunb. *Mol. Plant Breed* 22, 7389–7399. doi: 10.13271/j.mpb.022.007389

Li, Y., Kim, J. I., Pysh, L., and Chapple, C. (2015). Four isoforms of Arabidopsis 4coumarate: CoA ligase have overlapping yet distinct roles in phenylpropanoid metabolism. *Plant Physiol.* 169, 2409–2421. doi: 10.1104/pp.15.00838

Li, Y. Q., Kong, D. X., Bai, M., He, H. J., Wang, H. Y., and Wu, H. (2019). Correlation of the temporal and spatial expression patterns of HQT with the biosynthesis and accumulation of chlorogenic acid in *Lonicera japonica* flowers. *Hortic. Res.* 6, 73. doi: 10.1038/s41438-019-0154-2

Li, Y. Q., Kong, D. X., Fu, Y., Sussman, M. R., and Wu, H. (2020). The effect of developmental and environmental factors on secondary metabolites in medicinal plants. *Plant Physiol. Biochem.* 148, 80–89. doi: 10.1016/j.plaphy.2020.01.006

Li, G. J., Li, Y. X., Lu, H. Y., Zhang, Y. Y., and Zhou, Z. J. (2012). Research on tissue culture technology of Russian Lonicera caerulea. *Forest. Sci. Technol.* 37, 4–6.

Li, Z., and Nair, S. K. (2015). Structural basis for specificity and flexibility in a plant 4coumarate: CoA ligase. *Structure* 23, 2032–2042. doi: 10.1016/j.str.2015.08.012

Li, J. G., and Sun, M. Z. (2004). Research on the tissue culture and rapid propagation technology of high-quality *Lonicera. japonica* varieties. *J. Shandong Forest. Sci. Technol.* 6), 36–37.

Li, Q., Tang, M., Tan, Y. Y., Ma, D. W., Wang, Y. N., and Zhang, H. (2016). Improved production of chlorogenic acid from cell suspension cultures of *Lonicera macranthoids*. *Trop. J. Pharm. Res.* 15, 919. doi: 10.4314/tjpr.v15i5.4

Li, W., Zhang, L., He, P., Li, H., Pan, X., Zhang, W., et al. (2024b). Traditional uses, botany, phytochemistry, and pharmacology of *Lonicerae japonicae* flos and *Lonicerae* flos: A systematic comparative review. *J. Ethnopharmacol* 322, 117278. doi: 10.1016/j.jep.2023.117278

Liu, W. Y. (2010). Study on in virtio culture of Yu-Producing authentic chinese herbal medicines Lonicera japonica Thunb (Henan Normal University).

Liu, A. C., Bi, Y., Yu, Q., Wang, H., Wang, Q., and Li, Y. (2022). Research progress on breeding of *Lonicera linn*. in China. *Northern Horticult*. 5), 133–139.

Liu, Z., Cheng, Y., and Chao, Z. A. (2023). Comprehensive quality analysis of different colors of medicinal and edible honeysuckle. *Foods* 12, 3126. doi: 10.3390/foods12163126

Liu, Z., Cheng, Z., He, Q., Lin, B., Gao, P., and Li, L. (2016). Secondary metabolites from the flower buds of *Lonicera japonica* and their *in vitro* anti-diabetic activities. *Fitoterapia* 110, 44–51. doi: 10.1016/j.fitote.2016.02.011

Liu, Y., Guo, M. Y., Wang, C. Y., and Bai, G. M. (2012a). Researches on isolation conditions of protoplasts of *Lonicera japonica. Chin. J. Exp. Tradit. Med. Formulae* 19, 125–129. doi: 10.13422/j.cnki.syfjx.2012.19.041

Liu, Y., Peng, X. X., Zhu, S. S., and Bai, G. B. (2012c). Researches on the relative expression of HQT gene in different organs of *Lonicera japonica*. J. Chin. Med. Mater 35, 1032–1036. doi: 10.13863/j.issn1001-4454.2012.07.005

Liu, L. F., and Qian, G. Z. (2007). The inducement and differentiation of Calli in tissue culture of *Lonicera japonica* Thunb. J. Liaocheng Univ. (Natural Sci. Edition). 2007, 48–50+107.

Liu, Y. Q., Wu, L., Chen, Z. X., and Zhu, F. F. (2012b). In vitro Induction of Polyploidy of Lonicera japonica by Colchicine and Its Ploidy Identification. J. Chin. Medicinal Materials 35, 1729–1732. doi: 10.13863/j.issn1001-4454.2012.11.007

Ludwig, A. A., Romeis, T., and Jones, J. D. G. (2004). CDPK-mediated signalling pathways: specificity and cross-talk. J. Exp. Bot. 55, 181-188. doi: 10.1093/jxb/erh008

Luo, Y., Zhu, P. P., and Liao, Y. (2018). Bioinformatics analysis of LjHQT gene in Lonicera japonica. Mol. Plant Breed 16, 1403–1408. doi: 10.13271/j.mpb.016.001403

Malik, S., Cusidó, R. M., Mirjalili, M. H., Moyano, E., Palazón, Z., and Bonfill, M. (2011). Production of the anticancer drug taxol in *Taxus baccata* suspension cultures: A review. *Process Biochem.* 46, 23–34. doi: 10.1016/j.procbio.2010.09.004

Mao, X. F., Hen, J. J., Han, Z. K., and Wu, F. F. (2019). Effects of lead stress on growth and physiological metabolism and accumu-lation characteristics of *Lonicera japonica*. J. Northeast Agric. Sci. 44, 69–75. doi: 10.16423/j.cnki.1003-8701.2019.05.016

Menges, M., Hennig, L., Gruissem, W., and Murray, J. A. (2003). Genome-wide gene expression in an Arabidopsis cell suspension. *Plant Mol. Biol.* 53, 423–442. doi: 10.1023/B:PLAN.0000019059.56489.ca

Meyer, A. J., and Fricker, M. D. (2002). Control of demand-driven biosynthesis of glutathione in green Arabidopsis suspension culture cells. *Plant Physiol.* 130, 1927–1937. doi: 10.1104/pp.008243

Mishra, A. K., Sharma, K., and Misra, R. S. (2012). Elicitor recognition, signal transduction and induced resistance in plants. *J. Plant Interact.* 7, 95–120. doi: 10.1080/17429145.2011.597517

Moniruzzaman, M., Zhong, Y., Huang, Z., Yan, H., Yuanda, L., Jiang, B., et al. (2021). Citrus cell suspension culture establishment, maintenance, efficient transformation and regeneration to complete transgenic plant. *Plants* 10, 664. doi: 10.3390/plants10040664

Moscatiello, R., Baldan, B., and Navazio, L. (2013). Plant cell suspension cultures. *Methods Mol. Biol.* 953, 77–93. doi: 10.1007/978-1-62703-152-3_5

Narayani, M., and Srivastava, S. (2017). Elicitation: a stimulation of stress in *in vitro* plant cell/tissue cultures for enhancement of secondary metabolite production. *Phytochem. Rev.* 16, 1227–1252. doi: 10.1007/s11101-017-9534-0

Niggeweg, R., Michael, A. J., and Martin, C. (2004). Engineering plants with increased levels of the antioxidant chlorogenic acid. *Nat. Biotechnol.* 22, 746–754. doi: 10.1038/nbt966

Ning, W. (2012). "Studies on intervention in secondary metabolites in Lonicera japonica Thunb. and Mahonia bealei (Fort.) Carr," in Response to the technology of ultraviolet induction (Shandong University of Traditional Chinese Medicine).

Ochatt, S. J. (1991). Requirements for plant regeneration from protoplasts of the shrubby ornamental honeysuckle, *Lonicera nitida* cv. Maigrun. *Plant Cell Tiss Organ Cult.* 25, 161–167. doi: 10.1007/BF00042188

Owis, A. I., Abdelwahab, N. S., and Abul-Soad, A. A. (2016). Elicitation of phenolics from the micropropagated endangered medicinal plant *Calligonum polygonoides* L. (Polygonoaceae). *Pharmacogn Mag* 12, 465–470. doi: 10.4103/0973-1296.191458

Pan, Y., Zhao, X., Wu, X. L., Wang, Y., Tan, J., and Chen, D. X. (2021b). Transcriptomic and metabolomic analyses provide insights into the biosynthesis of chlorogenic acids in *Lonicera macranthoides* Hand.-Mazz. *PloS One* 16, e0251390. doi: 10.1371/journal.pone.0251390

Pan, H. Q., Zhou, H., Miao, S., Guo., D. A., Li, Z. X., and Hu, Q. (2021a). Plant metabolomics for studying the effect of two insecticides on comprehensive constituents of *Lonicerae japonicae* flos. *Chin. J. Natural Medicines* 19, 70–80. doi: 10.1016/S1875-5364(21)60008-0

Park, H. B., Ko, C. H., Lee, S. Y., Kim, S. Y., and Lee, K. C. (2019). Ecophysiology of seed dormancy and germination in four *Lonicera* (caprifoliaceae) species native to Korea. *J. Ecol. Environ.* 43, 25. doi: 10.1186/s41610-019-0121-8

Petrova, M., Miladinova-Georgieva, K., and Geneva, M. (2024). Influence of abiotic and biotic elicitors on organogenesis, biomass accumulation, and production of key secondary metabolites in asteraceae plants. *Int. J. Mol. Sci.* 25, 4197. doi: 10.3390/ijms25084197

Popova, E., Titova, M., Tynykulov, M., Zakirova, R. P., Kulichenko, I., Prudnikova, O., et al. (2023). Sustainable production of Ajuga bioactive metabolites using cell culture technologies: A review. *Nutrients* 15, 1246. doi: 10.3390/nu15051246

Qiu, J., and Tan, X. F. (2005). Tissue culture and rapid propagation of Lonicera japonica. J. Cent. South Univ. Forest. Technolog 4, 53–56.

Ren, M., Wu, Y. C., Zhang, J. Y., and Zhao, Z. Y. (2015). Induction of Lonicera japonica callus. *Jiangsu Agric. Sci.* 43, 38–40. doi: 10.15889/j.issn.1002-1302.2015.03.012

Schatlowski, N., and Köhler, C. (2012). Tearing down barriers: understanding the molecular mechanisms of interploidy hybridizations. J. Exp. Bot. 63, 6059–6067. doi: 10.1093/jxb/ers288

Seidel, T., Artmann, P. J., Gkekas, I., Illies, F., Baack, A. L., and Viefhues, M. (2024). Microfluidic single-cell study on Arabidopsis thaliana protoplast fusion—New insights on timescales and reversibilities. *Plants* 13, 295. doi: 10.3390/plants13020295

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

Shan, G. F., Zhang, X. L., Li, M. J., Chen, M. X., Liu, W. Y., and Lu, J. (2013). Study on callus induction and subculture of *Lonicera japonica* Thunb. *Northern Horticult.* 5), 106–109.

Shang, X., Pan, H., Li, M., Miao, X., and Ding, H. (2011). *Lonicera japonica* Thunb.: Ethnopharmacology, phytochemistry and pharmacology of an important traditional Chinese medicine. *J. Ethnopharmacol* 138, 1–21. doi: 10.1016/j.jep.2011.08.016

Sharma, P., Padh, H., and Shrivastava, N. (2013). Hairy root cultures: A suitable biological system for studying secondary metabolic pathways in plants. *Eng. Life Sci.* 13, 62–75. doi: 10.1002/elsc.201200030

Shasmita,, Singh, N. R., Rath, S. K., Behera, S., and Naik, S. K. (2018). "*In vitro* secondary metabolite production through fungal elicitation: An Approach for sustainability," in *Fungal Nanobionics: Principles and Applications.* Eds. R. Prasad, V. Kumar, M. Kumar and S. Wang (Springer Singapore, Singapore), 215–242. doi: 10.1007/978-981-10-8666-3_9

Shi, S., Tian, L., Ma, L., and Tian, C. (2018). Community structure of rhizomicrobiomes in four medicinal herbs and its implication on growth management. *Microbiol* 87, 425–436. doi: 10.1134/S0026261718030098

Song, G. Y., and Liang, H. (1987). Studies on *Lonicera japonica* tissue culture and its secondary metabolites. *Chin. Pharm. J.* 10, 588–589. doi: CNKI:SUN:ZGYX.0.1987-10-004

Su, X., Zhu, Z., Zhang, L., Wang, Q., Xu, M., and Lu, C. (2021). Anti-inflammatory property and functional substances of *Lonicerae Japonicae* Caulis. *J. Ethnopharmacol* 267, 113502. doi: 10.1016/j.jep.2020.113502

Sun, Y. C., Lin, S. F., Huang, L. Q., Zhang, X. B., and Guo, L. P. (2011). Review: Autotoxic effects of medicinal plants and control measures. *China J. Chin. Materia Med.* 36, 387–390.

Sun, Q. H., Morales-Briones, D. F., Wang, H. X., Landis, J. B., Wen, J., and Wang, H. F. (2023). Target sequence capture data shed light on the deeper evolutionary relationships of subgenus Chamaecerasus in *Lonicera* (Caprifoliaceae). *Mol. Phylogenet. Evol.* 184, 107808. doi: 10.1016/j.ympev.2023.107808

Tang, N., Cao, Z. Y., Yang, C., Ran, D. S., Wu, P. Y., Gao, H. M., et al. (2021). A R2R3-MYB transcriptional activator LmMYB15 regulates chlorogenic acid biosynthesis and phenylpropanoid metabolism in *Lonicera macranthoides. Plant Sci.* 308, 110924. doi: 10.1016/j.plantsci.2021.110924

Tao, Y. (2023). Effects of Cadmium and Ozone on Physicochemical Properties and Properties of Lonicera japonica Thunb Transcriptome analysis Vol. 2023 (Shenyang Agricultural University). doi: 10.27327/d.cnki.gshnu.2023.001007

Thakur, M., Bhattacharya, S., Kumar Khosla, P., and Puri, S. (2020). Improving production of plant secondary metabolites through biotic and abiotic elicitation. *J. Appl. Res. Med. Aromat. Plants* 12, 1–12. doi: 10.1016/j.jarmap.2018.11.004

Tomasiak, A., Zhou, M., and Betekhtin, A. (2022). Buckwheat in tissue culture research: current status and future perspectives. *Int. J. Mol. Sci.* 23, 2298. doi: 10.3390/ ijms23042298

Valiñas, M. A., Lanteri, M. L., Have, T. A., and Andreu, A. B. (2015). Chlorogenic acid biosynthesis appears linked with suberin production in potato Tuber(*Solanum tuberosum*). J. Agric. Food Chem. 63, 4902–4913. doi: 10.1021/jf505777p

van Berkum, N. L., Lieberman-Aiden, E., Williams, L., Imakaev, M., Gnirke, A., Mirny, L. A., et al. (2010). Hi-C: a method to study the three-dimensional architecture of genomes. J. Vis. Exp. 39), e1869. doi: 10.3791/1869-v

Vasil, V., and Hildebrandt, A. C. (1965). Differentiation of tobacco plants from single, isolated cells in microcultures. *Science* 150, 889–892. doi: 10.1126/science.150.3698.889

Wang, N., Chen, H., Gao, Q., and Qian, J. (2022). Establishment of an efficient cell suspension culture system for *Lonicera japonica* Thunb. *Pak. J. Bot.* 54, 2167–2172. doi: 10.30848/PJB2022-6(7

Wang, W. J., Wang, P., and Li, W. Q. (2013). Study on the Tissue Culture and Rapid Propagation of Lonicera japonica var. chinensis. *Northern Horticult*. 18), 100–102.

Wang, H. L., and Zhao, X. M. (2012). Study on induction and morphological identification of autotertraploid in *Lonicera japonica* Thunb. J. Shanxi Agric. Sci. 40, 1240–1242,1253.

Wang, H. L., and Zhao, X. M. (2014). Cytology identification of tetraploid *Lonicera japonica* Thunb. *Northern Horticult*. 6), 92–94.

Wei, X. M., Hu, Y., Wei, K. H., Wu, Q. H., Huang, Y., and Wei, F. (2021). Characterization of the complete chloroplast genome of *Lonicera similis* (Caprifoliaceae). *Mitochondrial DNA Part B* 6, 3067–3069. doi: 10.1080/23802359.2021.1981161

White, P. R. (1934). Potentially unlimited growth of excised tomato root tips in a liquid medium. *Plant Physiol.* 9, 585–600. doi: 10.1104/pp.9.3.585

Wu, L. N. (2012). Study on the loose callus induction and cell suspension culture of *Lonicera japonica*. Northern Horticult. 20), 107–109.

Wu, X. Q., Cui, Z. T., Li, X. Y., Yu, Z. H., Li, P. P., Xue, L., et al. (2024). Identification and characterization of PAL genes involved in the regulation of stem development in *saccharum spontaneum* L. *BMC Genom Data* 25, 38–38. doi: 10.1186/s12863-024-01219-9

Wu, M. L., Sun, X. L., Zhu, S. S., Li, W. D., and Bai, G. B. (2014). Correlation analysis of HQT gene mutation and chlorogenic acid content of Flos *Lonicerae. Jilin J. Chin. Med.* 34, 728–731. doi: 10.13463/j.cnki.jlzyy.2014.07.026

Wu, X. L., Zhang, S. Z., Li, X., Zhang, F., Fan, Y. J., Liu, Q. L., et al. (2021). Postharvest UV-B radiation increases enzyme activity, polysaccharide and secondary metabolites in honeysuckle (*Lonicera japonica* Thunb.). *Ind. Crops Prod.* 171, 113907. doi: 10.1016/j.indcrop.2021.113907

Xiang, Z. X., and Gao, S. L. (2007). Establishment and optimisation of tissue culture system for *Lonicera japonica*. *China J. Chin. Materia Med.* 2007, 2662–2663.

Xing, Y. X. (2006). Research on the breeding of a new tetraploid Lonicera japonica variety 'Jiufeng no. 1'. Agricultire Eng. Technol. 1), 44–46.

Xu, F., Valappil, A. K., Mathiyalagan, R., Tran, T. N. A., Ramadhania, Z. M., Awais, M., et al. (2023). *In vitro* cultivation and ginsenosides accumulation in *Panax ginseng*: A review. *Plants* 12, 3165. doi: 10.3390/plants12173165

Xu, M. L., Wu, J. H., and Min, L. X. (2011). Tissue culture and rapid propagation of Lonicerra tellmanniana Spaeth. J. Anhui Agri 39, 13977-13978. doi: 10.13989/ j.cnki.0517-6611.2011.23.170

Xun, Y. L. (2004). A preliminary study of tissue culture and rapid propagation of Lonicera japonica Champ. J. Hechi Univ. 24, 32-34.

Yamamoto, H., Katano, N., Ooi, A., and Inoue, K. (1999). Transformation of loganin and 7-deoxyloganin into secologanin by *Lonicera japonica* cell suspension cultures. *Phylochemistry* 50, 417–422. doi: 10.1016/S0031-9422(98)00613-X

Yamaner, Ö., Erdağ, B., and Gökbulut, C. (2013). Stimulation of the production of hypericins in *in vitro* seedlings of Hypericum adenotrichum by some biotic elicitors. *Turk J. Bot.* 37, 153–159. doi: 10.3906/bot-1202-1

Yan, K., Zhao, S., Bian, L., and Chen, X. (2017). Saline stress enhanced accumulation of leaf phenolics in honeysuckle (*Lonicera japonica* Thunb.) without induction of oxidative stress. *Plant Physiol. Biochem.* 112, 326–334. doi: 10.1016/j.plaphy.2017.01.020

Yang, P. J., Chen, D. J., Zhao, Y., and Li, H. N. (2003). Tissue culture and rapid propagation of *Lonicera japonica*. Acta Botanica Boreali-Occidentalia Sin. 7), 248–251.

Yang, R., Fang, L., Li, J., and Zhang, Y. Q. (2019a). A new anti-inflammatory lignan from *Lonicerae Japonicae* flos. *Nat. Prod. Res.* 35, 587–592. doi: 10.1080/14786419.2019.1587430

Yang, R., Fang, L., Li, J., Zhao, Z., Zhang, H., Zhang, Y., et al. (2019b). Separation of five iridoid glycosides from *Lonicerae japonicae* Flos using high-speed counter-current chromatography and their anti-inflammatory and antibacterial activities. *Molecules* 24, 197. doi: 10.3390/molecules24010197

Yang, C., Jiang, C., Wu, S., Feng, X., and Yu, Z. (2022). The complete chloroplast genome of *Lonicera acuminata* Wall. and its phylogenetic analysis. *Mitochondrial DNA Part B* 7, 807–809. doi: 10.1080/23802359.2022.2073836

Yang, D. Y., Zhang, L. Z., and Li, X. P. (2017). High effect regeneration system establishment of *Lonicera japonica* Thunb. *Mol. Plant Breed.* 15, 1461–1465. doi: 10.13271/j.mpb.015.001461

Yang, Y. H., Zhang, Z. Y., Li, R. F., Yi, Y. J., Yang, H., Wang, C. J., et al. (2020). RgC3H involves in the biosynthesis of allelopathic phenolic acids and alters their release amount in *Rehmannia glutinosa* roots. *Plants* 9, 567. doi: 10.3390/ plants9050567

Yin, J. Y., Lai, M., Yu, X. Y., Su, D. D., Xiong, X. Y., and Li, Y. L. (2024). Comprehensive strategies for *paclitaxel* production: insights from plant cell culture, endophytic microorganisms, and synthetic biology. *Hortic. Res.* 346, 1–12. doi: 10.1093/ hr/uhae346

Yuan, J., and He, G. (2007). Preliminary study on induction of hairy roots in *Lonicera* macranthoides. Non-wood For. Res. 1), 55–58.

Zagorskaya, A. A., and Deineko, E. V. (2017). Suspension-cultured plant cells as a platform for obtaining recombinant proteins. *Russ J. Plant Physiol.* 64, 795–807. doi: 10.1134/S102144371705017X

Zhang, H. C., Gao, S. L., and Xue, X. (2011). Determination of chlorogenic acid content and selection of allotetraploid plants of *Lonicera japonica*. *Pharm. Biotechnol.* 18, 242–245.

Zhang, B. X., Lewis, K. M., Abril, A., Davydov, D. R., Vermerris, W., Sattler, S. E., et al. (2020). Structure and function of the cytochrome P450 monooxygenase cinnamate 4-hydroxylase from *Sorghum* bicolor. *Plant Physiol.* 183, 957–973. doi: 10.1104/pp.20.00406

Zhang, M., Li, M., Fu, H., Wang, K., Tian, X., Qiu., R., et al. (2022a). Transcriptomic analysis unravels the molecular response of *Lonicera japonica* leaves to chilling stress. *Front. Plant Sci.* 13. doi: 10.3389/fpls.2022.1092857

Zhang, S. W., Liu, S. T., Hou, A. J., Wang, S., Na, Y. X., Hu, J. H., et al. (2022b). Systematic review of *Lonicerae Japonicae* Flos: A significant food and traditional Chinese medicine. *Front. Pharmacol.* 19. doi: 10.3389/fphar.2022.1013992

Zhang, J. R., Wu, M. L., Li, W. D., and Bai, G. B. (2016). Regulation of honeysuckle HQT gene to chlorogenic acid biosynthesis in eukaryotic plant cells of *Lonicerae* Flos. *Chin. Tradit. Herb Drugs* 47, 3683–3687.

Zhang, B., Zheng, L. P., and Wang, J. W. (2012). Nitric oxide elicitation for secondary metabolite production in cultured plant cells. *Appl. Microbiol. Biotechnol.* 93, 455–466. doi: 10.1007/s00253-011-3658-8

Zhao, J., Davis, L. C., and Verpoorte, R. (2005). Elicitor signal transduction leading to production of plant secondary metabolites. *Biotechnol. Advances: Int. Rev. J.* 4), 23. doi: 10.1016/j.bioteChadv.2005.01.003

Zhao, Y., Dou, D. Q., Guo, Y. Q., Qi, Y., Li, J., and Jia, D. (2018). Comparison of the Trace Elements and Active Components of *Lonicera japonica* flos and *Lonicera* flos Using ICP-MS and HPLC-PDA. Biol. Trace Elem Res. 183, 379–388. doi: 10.1007/s12011-017-1138-4

Zhao, Z. C., Gao, A. P., Luo, R. X., Liu, K. L., and Yu, K. L. (2022). The different deletion mutation in the phenylalanine ammonia-lyase(PAL)gene affects the peel color of mango (*Mangifera indica L.*). *Genet. Resour Crop Evol.* 69, 2301–2306. doi: 10.1007/s10722-022-01393-8

Zu, E. F., Zhao, C. C., Zhang, L. X., and Qian, J. J. (2019). Study on the establishment of cell suspension culture system for *Lonicera japonica*. *Contemp. Horticult.* 6), 5–6. doi: 10.14051/j.cnki.xdyy.2019.06.002