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Manganese Elicitation Enhances Flavanol Accumulation and Induces Somatic Embryogenesis in *Camellia sinensis* Callus Cultures

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ABSTRACT

The production of high-value flavanol metabolites in *Camellia sinensis* L. is often inconsistent due to environmental fluctuations, developmental variability, and limitations inherent in conventional field cultivation. In vitro culture offers a controlled and reproducible platform to optimize secondary metabolite biosynthesis while supporting rapid and uniform plant propagation. This study aimed to develop an integrated in vitro system capable of simultaneously enhancing flavanol production and inducing pro-embryo formation. Leaf tip explants were cultured on Murashige and Skoog (MS) medium supplemented with benzylaminopurine (BAP), 2,4-D, and manganese (Mn^{2+}) as an abiotic elicitor. Callus biomass was evaluated through wet and dry weight analysis, and flavanol content was quantified using high-performance liquid chromatography (HPLC). The results indicated that Mn^{2+} elicitation was associated with increased callus biomass, and HPLC profiling confirmed the presence of flavanol-related peaks by retention-time matching with a reference standard. Embryogenic calli were subsequently transferred to a benzylaminopurine-naphthalene acetic acid (BAP–NAA) liquid medium, where pro-embryonic structures were observed under microscopic examination. Because quantitative flavanol determination and embryogenesis efficiency metrics were not assessed in this study, the findings are presented as an integrated qualitative workflow linking elicitation, metabolite profiling, and early embryogenic initiation. Overall, this study provides the first integrated evidence that Mn^{2+} elicitation and hormone-regulated suspension culture can concurrently enhance metabolite production and somatic embryogenic initiation in *C. sinensis*. The optimized system represents a promising biotechnological strategy for sustainable industrial-scale flavanol production and the mass propagation of elite tea genotypes.

1. INTRODUCTION

Camellia sinensis L., the primary source of tea, is globally valued for its rich profile of secondary metabolites, especially flavonoids such as flavanols. These compounds, predominantly catechins including epigallocatechin gallate (EGCG), epicatechin (EC), and their derivatives, exhibit a wide range of pharmacological activities, including antioxidant, anti-inflammatory, anticancer, and cardioprotective effects [1]. Flavanols play critical roles in neutralizing reactive oxygen species (ROS), modulating redox homeostasis, and influencing key molecular pathways in human health [2]. In plants, flavanols contribute to adaptive responses against abiotic stresses, pathogen attack, and developmental regulation, underscoring their ecological and biological importance [3]. Given their rising commercial and therapeutic relevance, developing efficient and consistent strategies to enhance flavanol production in *C. sinensis* has become an urgent priority for both agricultural and biotechnological sectors.

Despite their importance, the accumulation of flavanols in field-grown tea plants is highly variable. Environmental fluctuations, including temperature extremes, drought, light intensity, and soil nutrient composition, substantially affect metabolite biosynthesis [4]. Younger leaves typically possess higher flavonoid levels than mature leaves or stems, yet their availability is limited by seasonality and growth rate [5]. Geographic and climatic factors, such as elevation and solar radiation, further modulate the flavonoid profile, frequently resulting in inconsistent yield and quality. Concurrently, conventional propagation of *C. sinensis* faces substantial constraints. Embryo development is particularly sensitive to environmental stressors; cold, freezing, heat waves, and drought trigger significant transcriptomic reprogramming, suppressing normal embryogenesis [6]. These biological and environmental limitations reduce the efficiency of large-scale metabolite harvesting and hinder the availability of uniform planting material.

In vitro culture has emerged as a promising alternative to address these limitations. Tissue and cell culture systems provide controlled environments independent of geographic constraints, enabling the manipulation of growth regulators, nutrient composition, and elicitors to stimulate secondary metabolite pathways [7]. Numerous studies have demonstrated that elicitors such as methyl jasmonate, salicylic acid, or hydrogen peroxide can enhance phenolic compound accumulation in medicinal plants [8]. Similarly, auxins and cytokinins orchestrate cell division, differentiation, and the acquisition of embryogenic competence. However, the integration of metabolite enhancement and embryo induction within a single in vitro workflow remains underexplored for *C. sinensis*. Existing literature often examines these processes separately, focusing either on flavonoid biosynthesis or on somatic embryogenesis, without elucidating the potential synergy between elicitor-driven stress responses and hormone-mediated developmental reprogramming [9].

Manganese (Mn^{2+}) ions present a compelling opportunity in this context. Beyond its role as an abiotic elicitor at elevated levels, Mn is an essential micronutrient required for normal plant physiology, including photosynthetic oxygen evolution and the activation of multiple enzymes. This dual nature makes Mn^{2+} a relevant candidate for designing in vitro systems that balance growth-supporting nutrition and controlled stress signaling. As an abiotic elicitor, Mn^{2+} can trigger oxidative stress pathways that upregulate key enzymes in the phenylpropanoid and flavonoid biosynthesis routes, including phenylalanine ammonia-lyase (PAL), chalcone isomerase (CHI), and flavonol synthase (FLS) [10, 11]. While Mn toxicity responses have been documented in several plant species, its targeted application to enhance flavanol accumulation in *C. sinensis* remains poorly characterized [12, 13]. Furthermore, it is unclear whether callus tissues exposed to Mn^{2+} retain their capacity to enter embryogenic pathways, an essential requirement for developing dual-function in vitro systems capable of supporting both metabolite production and propagation.

Accordingly, this study aimed to (1) evaluate the effects of Mn^{2+} supplementation on callus biomass accumulation; (2) profile flavanol-related peaks in callus extracts using High-performance liquid chromatography (HPLC) as a chromatographic identification approach; and (3) assess the early embryogenic response of Mn^{2+} treated calli after transfer to a benzylaminopurine-naphthalene acetic acid (BAP–NAA) liquid culture, thereby establishing a two-stage in vitro workflow linking elicitation, metabolite profiling, and pro-embryo initiation.

This research provides new insights into the dual modulation of secondary metabolism and early somatic embryogenesis in *C. sinensis*. The development of a unified system capable of improving flavanol production while simultaneously facilitating large-scale propagation represents a significant advancement in tea biotechnology. The outcomes have substantial implications for industrial metabolite production, sustainable tea cultivation, and the conservation of elite tea germplasm through reliable in vitro propagation technologies.

2. MATERIAL AND METHODS

2.1 Plant material and explant sterilization

Young leaf tips of *Camellia sinensis* L. were collected from healthy mother plants and prepared as explants for in vitro culture. Upon arrival in the laboratory, leaves were washed under running tap water for approximately ten minutes to remove dust and debris, followed by immersion in a mild detergent solution. All subsequent sterilization steps were conducted inside a laminar airflow cabinet. The leaves were first treated with 70% ethanol for 30 seconds and then immersed in 0.1% $HgCl_2$ for five minutes to eliminate surface microorganisms, after which they were rinsed three times using sterile distilled water. Sterile leaf tips of approximately two centimeters were excised aseptically and immediately inoculated onto culture media.

2.2 Callus induction on solid Murashige and Skoog medium

Callus induction was carried out by culturing sterilized leaf-tip explants of *Camellia sinensis* on solid Murashige and Skoog (MS) medium supplemented with 1.0 mg/L benzylaminopurine (BAP) and 1.0 mg/L 2,4-dichlorophenoxyacetic acid (2,4-D). To examine the elicitation effect on callus proliferation, manganese ions (Mn^{2+}) in the form of $MnSO_4 \cdot H_2O$ were incorporated into the medium at concentrations of 0, 80, 120, and 160 mg/L (approximately 0, 0.47, 0.71, and 0.95 mM, respectively). The medium also contained 30 g/L sucrose as a carbon source and was solidified with 8 g/L agar, with the pH adjusted to 5.8 before autoclaving at 121 °C for 15 minutes. Explants were positioned with the abaxial side in contact with the medium and incubated at 25 ± 2 °C under a 16/8-hour light–dark photoperiod. Throughout the culture period, explants were observed periodically to document physiological transitions, including initial wrinkling and swelling, followed by tissue disorganization and the eventual emergence of callus tissue. These morphological changes correspond directly to the developmental sequence illustrated in the results and were recorded regularly using a stereo microscope (Olympus CX-31) to ensure accurate characterization of callus formation under each Mn^{2+} treatment.

2.3 Callus biomass measurement

The growth response of callus tissue to varying concentrations of Mn^{2+} was evaluated through measurements of wet and dry biomass after 30 days of culture. Fresh calli from each treatment were carefully removed from the culture medium using sterile forceps and gently blotted with sterile filter paper to eliminate excess surface moisture before being weighed to obtain the wet weight values. Following this, the same callus samples were transferred to a drying oven set at 45 °C and dehydrated until a constant weight was achieved, which was confirmed by recording three consecutive identical measurements. This procedure ensured accuracy in determining the true dry biomass of the callus tissue. The resulting wet and dry weight data provided a quantitative assessment of callus proliferation under different Mn^{2+} elicitor concentrations, directly supporting the growth patterns presented in the results, where the highest biomass accumulation was observed in treatments supplemented with 160 mg/L Mn^{2+}.

2.4 Extraction of secondary metabolites

The extraction of secondary metabolites from the callus tissue was performed following the completion of the biomass measurement stage. Dried calli were first ground into a fine powder using a sterile mortar and pestle to increase extraction efficiency. The powdered samples were then subjected to a sequential solvent extraction process designed to isolate phenolic and flavanol compounds, beginning with distilled water to remove highly polar components, followed by partitioning with chloroform and ethyl acetate. The final and most critical extraction step utilized methanol, which served as the primary solvent for isolating phenolic compounds due to its high solubilizing efficiency for flavanols. The methanolic extracts were subsequently concentrated using a rotary evaporator set at 40 °C, allowing the solvent to evaporate without degrading heat-sensitive metabolites. The resulting crude extracts were filtered through 0.22 µm syringe filters to remove particulate impurities, yielding clear filtrates suitable for chromatographic analysis. This extraction procedure ensured the isolation of metabolite-rich fractions that were later analyzed using HPLC, forming the basis for identifying flavanol compounds as presented in the chromatographic results.

2.5 HPLC analysis for flavanol identification

HPLC was used for qualitative profiling of flavanol-related compounds. Samples were analyzed on a C18 reversed-phase column using an isocratic mobile phase of methanol: water (70:30, v/v) and UV detection at 280 nm. Peaks were assigned based on retention-time matching with the reference standard analyzed under identical conditions (target peak at approximately 7.2 min). Because an external calibration curve and method validation parameters (e.g., linearity, LOD, LOQ) were not established in this study, HPLC outputs were interpreted as qualitative evidence rather than absolute quantification.

2.6 Induction of pro-embryos in liquid MS medium

The induction of pro-embryos from callus tissue was carried out by transferring morphologically embryogenic calli into liquid MS medium enriched with specific growth regulators to stimulate somatic embryogenesis. Calli selected for this stage exhibited compact, friable, and pale-yellow tissue characteristics, indicating active meristematic potential. These calli were carefully transferred into liquid MS medium supplemented with 1.0 mg/L BAP and 0.5 mg/L naphthalene acetic acid (NAA), a hormonal combination known to promote cell division and early embryogenic differentiation. Cultures were maintained on a rotary shaker at 100 rpm to ensure continuous aeration and uniform nutrient distribution, which are essential for promoting the suspension culture environment required for embryogenic development. Observations were made every 3–5 days to monitor the formation of pro-embryonic structures, including early globular forms and organized cell clusters. Visual assessment under both macroscopic and microscopic examination revealed the gradual emergence of embryogenic protrusions and vascular-like tissues, confirming the successful transition of callus cells toward somatic embryogenesis. These developmental changes correspond directly to the morphological features documented in the results, demonstrating that the liquid MS medium enriched with BAP and NAA effectively supported the induction and early development of pro-embryos in *Camellia sinensis*.

2.7 Microscopic observation of embryogenic development and experimental design

The development of pro-embryonic structures in *Camellia sinensis* cultures was evaluated through both macroscopic and microscopic observations to confirm the progression of somatic embryogenesis and verify the effectiveness of the hormonal treatments used. Embryogenic calli grown in liquid MS medium supplemented with BAP and NAA were examined regularly for visible morphological changes, including the appearance of tissue protrusions, compact nodular formations, and early shoot-like structures. These macroscopic features were recorded using a digital camera to document the transition from undifferentiated callus to organized embryogenic tissue. For microscopic assessment, small sections of developing calli were mounted on slides and observed under an Olympus CX-31 stereo microscope to visualize meristematic cell clusters, dense cytoplasmic regions, and early vascular-like arrangements indicative of embryogenic competency. These observations provided detailed evidence supporting the developmental stages presented in the results section. The experimental setup followed a completely randomized design with three biological replicates for each Mn^{2+} concentration and embryogenic treatment. Given the descriptive and developmental nature of the study, data handling emphasized qualitative interpretation supported by biomass measurements and chromatographic confirmation, ensuring that all observed patterns accurately reflected the influence of elicitors and growth regulators on callus proliferation, metabolite accumulation, and embryogenic development.

3. RESULT AND DISCUSSION

3.1 Callus induction and early morphogenic responses

Leaf explants of *Camellia sinensis* showed a progressive morphogenic transition beginning with surface wrinkling and swelling, followed by tissue softening and the formation of disorganized parenchymatous masses. These developmental changes, documented in Figure 1, demonstrated that the combination of 2,4-D and BAP successfully initiated callus formation. The explants displayed visible dedifferentiation as early as the second week, and by the fourth week, compact calli with uniform pale-yellow coloration had formed, indicating active cell proliferation.

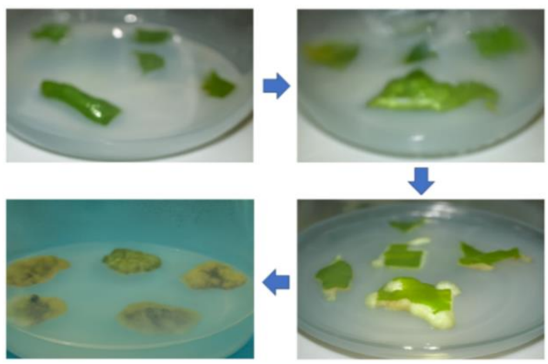


Figure 1. Progression of callus development from swollen tissue to compact callus masses

The strong callogenetic response observed aligns with previous evidence that 2,4-D is the most effective auxin for inducing dedifferentiation and cellular totipotency, particularly in woody or recalcitrant species. According to a study [14], 2,4-D stimulates reactivation of auxin-responsive genes, destabilizes tissue polarity, and drives cells into proliferative states. Meanwhile, the presence of BAP enhanced mitotic activity and encouraged organized cell division, processes commonly associated with cytokinin-mediated reprogramming [15, 16]. The synergy between auxin and cytokinin is a long-established requirement for effective callus induction, where auxin drives dedifferentiation, while BAP promotes sustained proliferative capacity. The results, therefore, confirm that the hormonal formulation used in this study is suitable for initiating stable callus cultures in *C. sinensis*.

3.2 Effects of Mn^{2+} elicitation on callus biomass

Mn^{2+} supplementation was associated with higher callus wet and dry biomass, with the highest values observed at 160 mg L^{-1} (Figure 2). Because inferential statistics (e.g., ANOVA) were not performed, the biomass trends are described descriptively. Increasing Mn^{2+} levels produced progressively larger callus masses, indicating that Mn^{2+} served as a positive abiotic elicitor within the concentration range tested. The enhanced biomass accumulation is consistent with elicitation mechanisms, which demonstrates that elicitors stimulate the production of ROS functioning as intracellular secondary messengers [17]. These ROS activate transcription factors regulating growth, metabolic energy allocation, and cell division pathways, ultimately increasing proliferative activity. Mn^{2+} may have acted similarly, triggering mild oxidative stress that upregulated PAL activity, an enzyme central to the phenylpropanoid pathway and biomass expansion. Comparable responses have been reported in rice cell suspensions treated with salicylic acid, yeast extract, or chitosan, where elicitation enhanced biomass and metabolite yields simultaneously [17-19]. The results indicate that Mn^{2+}, at optimal concentrations, promotes both cellular growth and metabolic activation, supporting its role as an effective abiotic elicitor for tea callus cultures.

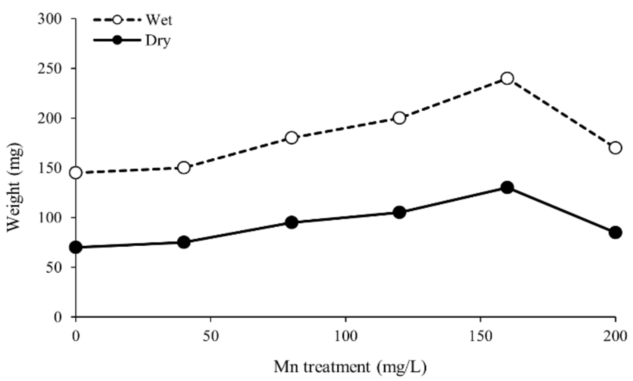


Figure 2. Wet and dry biomass of callus

3.3 Flavanol profiling using high-performance liquid chromatography

HPLC profiling of methanolic extracts consistently showed a peak at approximately 7.2 min that matched the retention time of the reference standard (Figure 3). This finding supports the presence of flavanol-related compounds in callus extracts under the tested culture conditions. However, because peak areas were not converted into concentrations using an external standard curve, the HPLC output is discussed qualitatively rather than as comparative quantification across Mn^{2+} treatments. As explained, elicitor-induced ROS play a central role in activating the phenylpropanoid pathway by stimulating enzymes such as PAL, CHS, CHI, and FLS [17, 20, 21]. The similarity between these findings and the present study suggests that Mn^{2+} elicitation activated comparable biosynthetic cascades in *C. sinensis*.

The successful detection of flavanols through HPLC reaffirms that Mn^{2+} acts not only as a growth elicitor but also as a metabolic enhancer, improving the accumulation of high-value phytochemicals in cultured tea tissues.

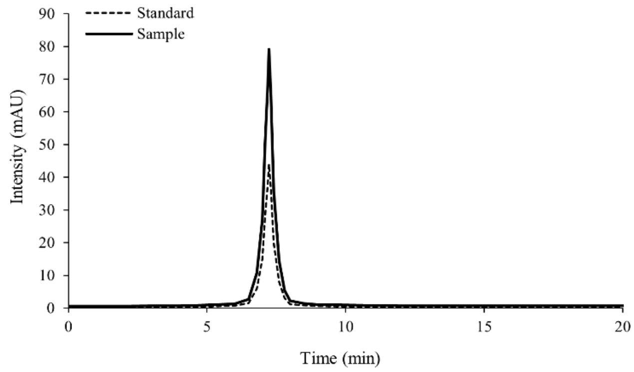


Figure 3. HPLC chromatogram showing flavanol peak matching the reference standard

3.4 Induction of pro-embryos in liquid MS medium

Pro-embryogenic structures were observed after transferring selected calli to BAP–NAA liquid medium (Figure 4). While embryogenic features were noted in calli derived from Mn^{2+}-supplemented media, the present work did not quantify embryogenesis efficiency (e.g., number of pro-embryos per gram callus or percentage of responding explants) nor formally compare responses against an untreated control in a statistical framework.

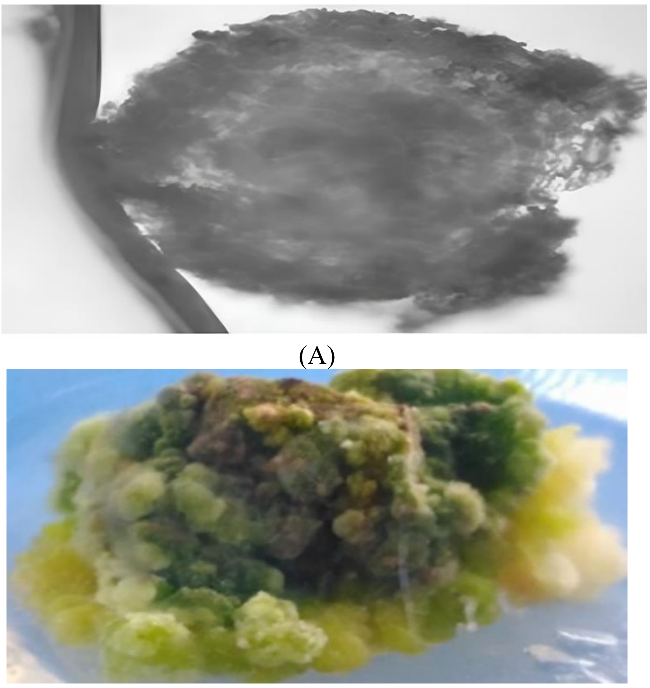
Figure 4. Pro-embryo induction in liquid MS medium showing embryogenic nodules

The embryogenic response reflects the established role of cytokinins and auxins in embryo initiation. BAP enhances cell division and activates meristematic gene networks, while NAA reinforces auxin gradients that determine embryo polarity and tissue fate. Studies in orchids and rice confirm that the BAP–NAA combination effectively stimulates embryogenic transition, particularly when tissues are physiologically primed [11, 22]. Elicited calli, enriched with secondary metabolites and responding to ROS-mediated signaling, are often more competent for embryogenesis because elicitors modify cellular redox states and may influence epigenetic activation of embryogenic pathways [23-25]. The results demonstrate that the callus lines generated in this study not only biosynthesize flavanols but also maintain high embryogenic plasticity, a dual property with significant biotechnological value.

Mechanistically, the apparent coupling between biomass enhancement, flavanol-related peak detection, and embryogenic initiation may be explained by shared regulatory nodes rather than fully independent pathways. Mn^{2+}-associated oxidative signaling can modulate cellular redox status and phenylpropanoid activation, which in turn may influence hormonal sensitivity and developmental reprogramming. In tissue culture systems, ROS–hormone crosstalk is frequently discussed as a trigger that shifts cells toward competence states, while cytokinins and auxins subsequently drive organized division and polarity establishment. Although the present study does not directly test these molecular nodes, framing Mn^{2+} as a modulator of redox–hormone balance provides a coherent rationale for integrating elicitation and embryo initiation within a two-stage workflow.

3.5 Microscopic and macroscopic characterization of embryogenic development

Microscopic observations revealed dense meristematic cell clusters, early vascular-like tissues, and tightly arranged cytoplasmic regions indicative of somatic embryo formation (Figure 5(A)). Macroscopic evaluation (Figure 5(B)) further supported these findings, showing the emergence of nodular protrusions and early shoot-like structures on the callus surface.



(A)

(B)

Figure 5. Microscopic (A) and macroscopic (B) features of somatic embryo initiation in *C. sinensis* callus

These characteristics align with classical descriptions of early-stage somatic embryogenesis in tea and other woody perennials. Studies have shown that elicited tissues often show enhanced embryogenic traits because stress-induced signaling (e.g., ROS accumulation, phenolic enrichment) activates embryogenic genes and reprograms cell identity [17, 26, 27]. This phenomenon suggests that Mn^{2+} elicitation may have preconditioned the callus toward embryogenic transition, enhancing developmental responsiveness in BAP–NAA liquid medium. Thus, the microscopic and macroscopic features observed confirm successful induction of somatic embryogenesis, illustrating a clear developmental progression supported by both hormonal cues and elicitor-modifiable physiology.

4. CONCLUSIONS

This study successfully demonstrated that the integration of Mn^{2+} elicitation with an optimized hormonal regime can simultaneously enhance secondary metabolite production and somatic embryogenic competence in *Camellia sinensis* callus cultures. The combination of 2,4-D and BAP effectively induced vigorous callus formation, while the addition of Mn^{2+} at concentrations up to 160 mg L^{-1} significantly increased callus biomass and activated flavanol biosynthesis, as confirmed by HPLC analysis. Furthermore, calli derived from elicited treatments exhibited strong embryogenic responses when transferred to liquid MS medium containing BAP and NAA, leading to the formation of globular and pre-heart stage pro-embryos. Microscopic and macroscopic characterizations validated the presence of meristematic cell clusters and organized embryogenic structures, indicating successful initiation of somatic embryogenesis. Overall, these findings highlight the dual functional benefits of Mn^{2+} as an abiotic elicitor capable of promoting both metabolic activation and developmental reprogramming. The developed system offers a promising biotechnological platform for the large-scale production of flavanols and for efficient propagation of elite tea genotypes through somatic embryo-based regeneration.

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REFERENCES

[1] Baranwal, A., Aggarwal, P., Rai, A., Kumar, N. (2022). Pharmacological actions and underlying mechanisms of catechin: A review. Mini Reviews in Medicinal Chemistry, 22(5): 821-833. https://doi.org/10.2174/1389557521666210902162120

[2] Sarkar, C., Chaudhary, P., Jamaddar, S., Janmeda, P., Mondal, M., Mubarak, M.S., Islam, M.T. (2022). Redox activity of flavonoids: Impact on human health, therapeutics, and chemical safety. Chemical Research in Toxicology, 35(2): 140-162. https://doi.org/10.1021/acs.chemrestox.1c00348

[3] Patil, J.R., Mhatre, K.J., Yadav, K., Yadav, L.S., Srivastava, S., Nikalje, G.C. (2024). Flavonoids in plant-environment interactions and stress responses. Discover Plants, 1(1): 68. https://doi.org/10.1007/s44372-024-00063-6

[4] Jangpangi, D., Patni, B., Chandola, V., Chandra, S. (2025). Medicinal plants in a changing climate: understanding the links between environmental stress and secondary metabolite synthesis. Frontiers in Plant Science, 16: 1587337. https://doi.org/10.3389/fpls.2025.1587337

[5] Vilkickyte, G., Raudone, L. (2021). Phenological and geographical effects on phenolic and triterpenoid content in Vaccinium vitis-idaea L. leaves. Plants, 10(10): 1986. https://doi.org/10.3390/plants10101986

[6] Sciorio, R., Cantatore, C., D'Amato, G., Smith, G.D. (2024). Cryopreservation, cryoprotectants, and potential risk of epigenetic alteration. Journal of Assisted Reproduction and Genetics, 41(11): 2953-2967. https://doi.org/10.1007/s10815-024-03287-3

[7] Khanam, M.N., Anis, M., Javed, S.B., Mottaghipisheh, J., Csupor, D. (2022). Adventitious root culture—An alternative strategy for secondary metabolite production: A review. Agronomy, 12(5): 1178. https://doi.org/10.3390/agronomy12051178

[8] Razzaq, A., Zafar, M.M., Ali, A., Ihsan, L., Qadir, F., Khan, M.N., Qiao, F. (2025). Elicitor-mediated enhancement of secondary metabolites in plant species: A review. Frontiers in Plant Science, 16: 1706600. https://doi.org/10.3389/fpls.2025.1706600

[9] Wang, J., Zhang, L., Qi, L., Zhang, S. (2022). Integrated transcriptomic and metabolic analyses provide insights into the maintenance of embryogenic potential and the biosynthesis of phenolic acids and flavonoids involving transcription factors in Larix kaempferi (Lamb.) Carr. Frontiers in Plant Science, 13: 1056930. https://doi.org/10.3389/fpls.2022.1056930

[10] Rao, M.J., Zheng, B. (2025). The role of polyphenols in abiotic stress tolerance and their antioxidant properties to scavenge reactive oxygen species and free radicals. Antioxidants, 14(1): 74. https://doi.org/10.3390/antiox14010074

[11] Sarmah, D., Mohapatra, P.P., Seleiman, M.F., Mandal, T., Mandal, N., Pramanik, K., Wasonga, D.O. (2024). Efficient regeneration of in vitro derived plants and genetic fidelity assessment of Phalaenopsis orchid. Frontiers in Sustainable Food Systems, 8: 1359486. https://doi.org/10.3389/fsufs.2024.1359486

[12] Zaman, F., Zhang, E., Ihtisham, M., Ilyas, M., Khattak, W.A., Guo, F., Zhao, H. (2023). Metabolic profiling, pigment component responses to foliar application of Fe, Zn, Cu, and Mn for tea plants (*Camellia sinensis*). Scientia Horticulturae, 319: 112149. https://doi.org/10.1016/j.scienta.2023.112149

[13] Zhang, X., Liu, L., Luo, S., Ye, X., Wen, W. (2023). Research advances in aluminum tolerance and accumulation in tea plant (*Camellia sinensis*). Beverage Plant Research, 3(1): 18. https://doi.org/10.48130/BPR-2023-0018

[14] Carsono, N., Juwendah, E., Liberty, L., Sari, S., Damayanti, F., Rachmadi, M. (2021). Optimize 2, 4-D concentration and callus induction time enhance callus proliferation and plant regeneration of three rice genotypes. Biodiversitas Journal of Biological Diversity, 22(7): d220702. https://doi.org/10.13057/biodiv/d220702

[15] Anjalani, T.R., Rasmi, S.A., Rahayu, A.E., Ramadhani, M.R.N., Sholihah, M.F., Puspaningtyas, I., Jadid, N. (2024). Methyl jasmonate stimulates growth and upregulates the expression of Phenylalanine Ammonia-Lyase (PAL) gene in Gynura pseudochina in vitro micropropagation. Biodiversitas Journal of Biological Diversity, 25(5): d250512. https://doi.org/10.13057/biodiv/d250512

[16] Weeks, Z., Chaturvedi, G., Day, E., Kelly, S., Moody, L.A. (2025). A FLOE-related protein regulates the two-dimensional to three-dimensional growth transition in the moss Physcomitrium patens. Development, 152(16): dev204508. https://doi.org/10.1242/dev.204508

[17] Mulyati, Y., Zubaidah, S., Adiredjo, A.L., Rofiq, M., Jadid, N., Saputro, T.B., Sari, G.M. (2025). Salicylic acid treatment induced change in the morphological, chlorophyll content, and phytochemical traits in two rice genotypes. Biodiversitas Journal of Biological Diversity, 26(2): d260226. https://doi.org/10.13057/biodiv/d260226

[18] El-Beltagi, H.S., Mohamed, H.I., Aldaej, M.I., Al-Khayri, J.M., Rezk, A.A., Al-Mssallem, M.Q., Ramadan, K.M. (2022). Production and antioxidant activity of secondary metabolites in Hassawi rice (Oryza sativa L.) cell suspension under salicylic acid, yeast extract, and pectin elicitation. Vitro Cellular & Developmental Biology-Plant, 58(4): 615-629. https://doi.org/10.1007/s11627-022-10264-x

[19] Jain, D., Bisht, S., Parvez, A., Singh, K., Bhaskar, P., Koubouris, G. (2024). Effective biotic elicitors for augmentation of secondary metabolite production in medicinal plants. Agriculture, 14(6): 796. https://doi.org/10.3390/agriculture14060796

[20] Kısa, D., Ceylan, Y., İmamoğlu, R. (2023). Accumulation of phenolic compounds and expression of phenylpropanoid biosynthesis-related genes in leaves of basil transformed with A. rhizogenes strains. Physiology and Molecular Biology of Plants, 29(5): 629-640. https://doi.org/10.1007/s12298-023-01320-w

[21] Elsherif, D.E., Safhi, F.A., Khalifa, A.M., Ragab, G.A. (2024). Upregulated synthesis and production of bioactive compounds in Lotus arabicus L. by in vitro feeding with dried powder of date palm seeds. BMC Plant Biology, 24(1): 225. https://doi.org/10.1186/s12870-024-04919-7

[22] Balilashaki, K., Martinez-Montero, M.E., Vahedi, M., Cardoso, J.C., Silva Agurto, C.L., Leiva-Mora, M., Musharof Hossain, M. (2023). Medicinal use, flower trade, preservation and mass propagation techniques of Cymbidium orchids—An overview. Horticulturae, 9(6): 690. https://doi.org/10.3390/horticulturae9060690

[23] Sivanesan, I., Nayeem, S., Venkidasamy, B., Kuppuraj, S.P., Rn, C., Samynathan, R. (2022). Genetic and epigenetic modes of the regulation of somatic embryogenesis: A review. Biologia Futura, 73(3): 259-277. https://doi.org/10.1007/s42977-022-00126-3

[24] Pathak, A., Haq, S., Meena, N., Dwivedi, P., Kothari, S. L., Kachhwaha, S. (2023). Multifaceted role of nanomaterials in modulating in vitro seed germination, plant morphogenesis, metabolism and genetic engineering. Plants, 12(17): 3126. https://doi.org/10.3390/plants12173126

[25] Sharma, P., Jha, A.B., Dubey, R.S. (2025). Utilizing manganese-based nanoparticles for enhancing environmental stress resilience and productivity of plants. Environmental Science: Nano, 12(5): 2580-2602. https://doi.org/10.1039/D5EN00292C

[26] Elhiti, M., Stasolla, C. (2022). Transduction of signals during somatic embryogenesis. Plants, 11(2): 178. https://doi.org/10.3390/plants11020178

[27] Chen, X., Zhang, L., Liu, C., Wang, R., Dai, J., Kong, L., Zhao, J. (2025). The transcription factor LpWRKY65 enhances embryogenic capacity through reactive oxygen species scavenging during somatic embryogenesis of larch. Plant Physiology, 198(3): kiaf286. https://doi.org/10.1093/plphys/kiaf286