



Evaluating the Impact of Exogenous Application of Jasmonic Acid on *in vitro* Growth and Development of Rose Micropropagules

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ABSTRACT

Background: Rose (*Rosa hybrida* L.) micropropagules were studied to assess the impact of jasmonic acid (JA) on their *in vitro* growth, biomass accumulation and biochemical parameters. The study also investigated the effects of two JA incorporation methods-pre-autoclaving and post-autoclaving (filter sterilization).

Methods: Micropropagules were cultivated *in vitro* on Murashige and Skoog (MS) medium supplemented with jasmonic acid at concentrations of (0, 5, 10, 25, 50, 75 and 100 mg L⁻¹). Growth parameters, including shoot number, shoot length, fresh weight and dry weight, were measured. Biochemical analyses included total carbohydrates, proteins, phenols and chlorophyll content. The influence of JA concentration and incorporation method was evaluated.

Result: JA application method (pre or post-autoclaving) did not significantly affect growth parameters. Low concentrations of jasmonic acid (5 and 10 mg L⁻¹) increased total chlorophyll content in rose micropropagules when applied using the pre-autoclaving method, with the highest accumulation observed at 25 mg L⁻¹ (0.46±0.02 mg g⁻¹ fw). In contrast, post-autoclaving application resulted in a marked reduction in chlorophyll content across all concentrations. The highest biochemical improvements were observed at 25 mg L⁻¹ JA, while concentrations above 50 mg L⁻¹ negatively impacted growth and biochemical attributes. These findings highlight JA's effectiveness as a regulator for improving rose micropropagules' growth and biochemical properties, with optimal results achieved at moderate concentrations and slight variations in response based on the incorporation method.

Key words: Carbohydrates, Chlorophyll, Growth parameters, Jasmonic acid, Micropropagation, Rose micropropagules.

INTRODUCTION

The *Rosa hybrida* L. is a highly valued rose grown worldwide due to its significant demand. The initial micropropagation protocol for this cultivar was developed by Skirvin and Chu (1979). Since then, numerous researchers have explored various tissue culture methods and commercial applications for this economically important ornamental plant (Nirmal *et al.*, 2023; Ash *et al.*, 2020). However, despite these efforts, achieving a high multiplication rate and substantial growth improvement has remained challenging (Jakhar and Choudhary, 2023). The involvement of jasmonic acid (JA) in plant growth and development, both *in vitro* and *in vivo*, as well as its impact on ethylene biosynthesis, respiration and stomatal behaviour, has been documented (Nandy *et al.*, 2021). Recently, jasmonic acid (JA) has been recognized as a crucial signalling molecule in plants during pathogen attacks (Teraiya *et al.*, 2023). The role of jasmonic acid (JA) in managing abiotic stresses, such as chilling, heat, heavy metal toxicity, drought and osmotic stress, has been extensively studied in various plants, both *in vitro* and *in vivo* (Wang *et al.*, 2021). The potential use of jasmonic acid (JA) as a plant growth regulator (PGR) has also been previously reported (Liu and Timko, 2021; Nirmal *et al.*, 2024; Teraiya *et al.*, 2023). Jasmonic acid (JA) can regulate the physiological and biochemical functions of plants

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growing under *in vitro* conditions (Vasant *et al.*, 2023). In tomato, it was noted by Scalschi *et al.*, (2020) that exogenous supply of JA induces resistance against *Pseudomonas syringae*. The addition of jasmonic acid (JA) also led to increased production of secondary metabolites in *Oryza sativa* (Nandy *et al.*, 2021). Similarly, Abeed *et al.* (2021) has reported better growth and performance in wheat cultivars grown under the influence of JA. The promoting role of jasmonic acid (JA) under *in vitro* conditions in various plants, such as *Nicotiana attenuata* and *Arabidopsis thaliana*, has also been studied. Additionally, jasmonic acid (JA) can also play a significant role in inducing somatic embryogenesis, as demonstrated in *Phoenix dactylifera* L.

(Al-Qatrani *et al.*, 2021). JA affects photosynthesis-related activities and antioxidants in plants by modulating protein profile. Exogenous application of JA also improves the drought tolerance in *Brassica rapa* genotypes by modulating osmolytes, antioxidants and photosynthetic system (Ahmad Lone *et al.*, 2022). The aim of this study was to investigate the promoting effect of jasmonic acid on rose micropropagules under *in vitro* conditions, aiming to enhance their proliferation and multiplication efficiency.

MATERIALS AND METHODS

Establishment of cultures

Shoot cultures of *Rosa hybrida* L. cv. Ashwini were initiated using mature nodal segments sterilized with ethanol and sodium hypochlorite. Explants were aseptically cultured on MS medium with BAP, NAA, agar and sucrose. Cultures were sub-cultured every three weeks under controlled conditions ($28 \pm 2^\circ\text{C}$, 16/8-hour light/dark cycle), (Carelli and Echeverrigaray (2002); and Joshi and Purohit (2011)). The present study was carried out at the Department of Biotechnology, Atmiya University, Rajkot, India. The research work was conducted during the period July 2022 to June 2023.

Mode of jasmonic acid supplementation

Jasmonic acid (JA) was applied using two different methods: Pre-autoclaving and post-autoclaving (filter sterilization). For the pre-autoclaving method, JA was dissolved in a small volume of ethanol and added directly to the culture medium before autoclaving at 121°C and 15 psi for 20 minutes. For the post-autoclaving method, JA was first dissolved in ethanol, sterilized using a $0.22 \mu\text{m}$ syringe filter and then aseptically added to the autoclaved and cooled medium (approximately $45\text{--}50^\circ\text{C}$) inside a laminar airflow cabinet. In both methods, the final JA concentrations in the medium were maintained at 0 (control), 5, 10, 25, 50, 75 and 100 mg L^{-1} .

Experimental design

Jasmonic acid was tested at concentrations of 0, 5, 10, 25, 50, 75 and 100 mg L^{-1} in the shoot multiplication medium along with standard plant growth regulators (PGRs). The concentrations of jasmonic acid (JA) used in this study were selected based on prior literature demonstrating dose-dependent effects on *in vitro* morphogenesis, particularly in enhancing shoot proliferation and root induction in woody and ornamental species (Singh *et al.*, 2016).

JA was incorporated either pre-autoclaving or post-autoclaving. Shoots were cultured on semi-solid medium, sub-cultured every three weeks for 126 days and growth parameters and biochemical analyses were measured. Shoot number, average length and biomass (fresh and dry weight) were measured; dry weight was obtained by oven-drying shoots at 62°C for 48 hours.

Biochemical analyses

Biochemical analyses were conducted to measure chlorophyll, phenols, carbohydrates and proteins.

Chlorophyll content was determined using Arnon's (1949) method, grinding 500 mg of green shoots in 80% acetone under dark conditions, followed by centrifugation and spectrophotometric readings at 663, 652 and 645 nm. Phenol content was assessed using methanol extracts and Folin-Ciocalteu's reagent, with absorbance measured at 650 nm (Chauhan *et al.*, 2018). Total carbohydrates were estimated using Anthrone method, with absorbance at 610 nm. Protein content was determined by Bradford's method using Coomassie Brilliant Blue G-250 dye at 595 nm. Data validity was confirmed using XLSTAT.

RESULTS AND DISCUSSION

The present study evaluated the effect of incorporating varying concentrations of jasmonic acid (JA) into the standard rose multiplication medium during culture. Two modes of JA application were tested: pre-autoclaving and post-autoclaving *via* filter sterilization. The outcomes demonstrated no significant variation in key growth parameters, such as shoot number, shoot length, fresh weight and dry weight, between the two application modes. This indicates that both methods of JA addition are equally effective for rose micropropagation, providing flexibility in experimental protocols.

At a lower jasmonic acid (JA) concentration of 5 mg L^{-1} , rose micropropagules exhibited reduced shoot number (9.51) and shoot length (2.85 cm) compared to the control (13.32 shoots and 3.51 cm, respectively). However, increasing the JA concentration to 10 mg L^{-1} led to an improvement in shoot number (15.30) and shoot length (3.45 cm), both of which further increased significantly at 25 mg L^{-1} (19.28 shoots and 3.65 cm). Correspondingly, fresh and dry weights also improved, reaching 10.14 g and 2.05 g, respectively, at 25 mg L^{-1} compared to the control (10.78 g fresh and 2.17 g dry weight). In contrast, at higher JA concentrations of 50 mg L^{-1} and above, growth parameters declined sharply. At 100 mg L^{-1} , shoot number dropped to 6.46, shoot length to 1.65 cm, fresh weight to 3.15 g and dry weight to 0.54 g, indicating a strong inhibitory effect of elevated JA levels on micropropagule growth (Table 1).

Biomass production followed a trend similar to that of shoot growth. At 5 mg L^{-1} JA, fresh and dry weights were 6.10 g and 1.16 g, respectively, showing no significant increase compared to the control (10.78 g fresh and 2.17 g dry weight). A steady rise in biomass was observed at 10 mg L^{-1} (7.77 g fresh and 1.66 g dry weight) and peaked at 25 mg L^{-1} , where fresh and dry weights reached 10.14 g and 2.05 g, respectively. These values were comparable to, though slightly lower than, the control. However, beyond 25 mg L^{-1} , a marked decline in biomass was recorded. At the highest concentration of 100 mg L^{-1} JA, fresh and dry weights dropped significantly to 3.15 g and 0.54 g, respectively (Table 1).

No abnormal phenotypic changes were observed across any of the jasmonic acid (JA) treatments. Biochemical indices such as chlorophyll and phenol content

showed slight variations among treatments, but these were not substantial enough to significantly affect overall growth performance. For example, total chlorophyll content in the pre-autoclaving method increased from 0.33 mg g⁻¹ fw in the control to 0.46 mg g⁻¹ fw at 25 mg L⁻¹ JA, indicating a mild enhancement. However, phenol and carbohydrate contents remained relatively stable, with no consistent trends across concentrations or application modes. The control cultures grown on standard MS medium with recommended plant growth regulators (PGRs) served as a consistent baseline, showing balanced growth and biochemical composition. These findings underscore the potential of jasmonic acid in improving rose micropropagation outcomes when used at an optimal concentration, particularly

25 mg L⁻¹, where both growth and biochemical responses were favorable.

Interestingly, the addition of jasmonic acid (JA) before autoclaving resulted in a progressive increase in total chlorophyll content, with the highest value observed at a concentration of 25 mg L⁻¹ (0.46 mg g⁻¹ fw). Beyond this concentration, a sharp decline in total chlorophyll content was recorded, dropping significantly at 50 mg L⁻¹ and higher concentrations. Despite this decline, the total chlorophyll content at these higher JA concentrations (≥50 mg L⁻¹) remained comparable to or slightly higher than the control propagules.

In contrast, when sterilized JA was added post-autoclaving, no significant improvement in total chlorophyll content was observed at concentrations of 25 mg L⁻¹ or less.

Table 1: Effect of jasmonic acid on *in vitro* growth of rose micropropagules.

Mode of JA application	Jasmonic acid concentration mg L ⁻¹	No. of shoots mean±SD	Length of shoots (cm)±SD	Fresh weight (g)±SD	Dry weight (g)±SD
Pre-autoclaving	0	13.32±0.82c	3.51±0.06c	10.78±0.03g	2.17±0.02f
	5	9.51±0.99b	2.85±0.08b	6.10±0.06d	1.16±0.03c
	10	15.30±1.21d	3.45±0.10c	7.77±0.02e	1.66±0.02d
	25	19.28±1.17e	3.65±0.10d	10.14±0.03f	2.05±0.02e
	50	14.60±0.85cd	3.43±0.02c	5.70±0.05c	1.15±0.03c
	75	8.36±1.17b	1.75±0.03a	3.64±0.05b	0.64±0.03b
	100	6.46±1.23a	1.65±0.03a	3.15±0.03a	0.54±0.03a
Post-autoclaving (filter sterilized)	0	13.32±0.82c	3.51±0.06c	10.78±0.03g	2.17±0.02f
	5	7.43±0.95b	2.81±0.02b	7.25±0.02c	1.45±0.03d
	10	11.17±1.06c	3.47±0.02d	8.75±0.02e	1.57±0.03e
	25	15.05±1.45d	4.10±0.02f	11.36±0.10f	2.34±0.03f
	50	8.16±1.57b	3.65±0.03e	7.89±0.04d	1.26±0.02c
	75	7.20±1.62b	3.16±0.02c	3.88±0.03b	0.65±0.03b
	100	4.46±0.71a	2.15±0.03a	2.84±0.03a	0.45±0.03a

Means in the same columns followed by different letters are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test, SD- Standard deviation.

Table 2: Effect of jasmonic acid on chlorophyll contents in rose micropropagules grown under *in vitro* conditions.

Mode of JA application	Jasmonic acid concentration mg L ⁻¹	Total chlorophyll content (mg g ⁻¹ fw±SD)	Chlorophyll a content (mg g ⁻¹ fw±SD)	Chlorophyll b content (mg g ⁻¹ fw±SD)
Pre-autoclaving	0	0.33±0.02c	0.18±0.02c	0.15±0.01b
	5	0.24±0.03ab	0.11±0.02ab	0.12±0.02ab
	10	0.43±0.01d	0.22±0.02d	0.20±0.02c
	25	0.46±0.02d	0.27±0.02e	0.20±0.02c
	50	0.20±0.03a	0.09±0.02a	0.10±0.02a
	75	0.25±0.03b	0.14±0.02b	0.11±0.02a
	100	0.20±0.03a	0.09±0.02a	0.11±0.02a
Post-autoclaving (filter sterilized)	0	0.33±0.02c	0.18±0.02c	0.15±0.01b
	5	0.17±0.02d	0.08±0.02b	0.10±0.02d
	10	0.14±0.04cd	0.06±0.02ab	0.07±0.02cd
	25	0.11±0.01bc	0.06±0.01ab	0.05±0.02bc
	50	0.14±0.04cd	0.07±0.02ab	0.07±0.03cd
	75	0.06±0.00a	0.04±0.01a	0.02±0.01a
	100	0.07±0.04ab	0.04±0.02a	0.03±0.02ab

Means in the same columns followed by different letters are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test, SD- Standard deviation.

However, a notable increase in chlorophyll content occurred at 50 mg L⁻¹ JA (0.14 mg g⁻¹ fwt), which was higher than at lower concentrations but still less than the control. At concentrations above 50 mg L⁻¹, the total chlorophyll content decreased sharply, reaching its lowest values at 75 and 100 mg L⁻¹. These findings underscore the differential effects of JA concentrations and application modes on chlorophyll biosynthesis, with pre-autoclaving at 25 mg L⁻¹ showing the most favorable results for enhancing chlorophyll content in rose micropropagules (Table 2).

Similarly, the effect of jasmonic acid (JA) on the biochemical content of rose micropropagules was evaluated under two modes of application: Pre-autoclaving and post-autoclaving (filter sterilized). When JA was added before autoclaving, a gradual increase in total carbohydrate, total phenol and total protein content was observed at lower concentrations (5.0 and 10.0 mg L⁻¹). The carbohydrate content increased from 10.473 mg g⁻¹ fwt (control) to 15.880 mg g⁻¹ fwt at 5 mg L⁻¹ and further to 21.413 mg g⁻¹ fwt at 10 mg L⁻¹. Similarly, protein content increased significantly, reaching 90.243 mg g⁻¹ fwt and 94.457 mg g⁻¹ fwt at 5 and 10 mg L⁻¹ JA, respectively. However, phenol content decreased to 1.733 and 1.267 mg g⁻¹ fwt at these concentrations. At a moderate JA concentration of 25 mg L⁻¹, the biochemical response peaked, with total carbohydrate, phenol and protein content reaching their highest values-113.827, 0.467 and 118.753 mg g⁻¹ fwt, respectively. This suggests that 25 mg L⁻¹ JA provides optimal conditions for enhanced biochemical accumulation (Fig 1, 2 and 3).

Conversely, higher JA concentrations (50, 75 and 100 mg L⁻¹) resulted in a decline in biochemical content. Carbohydrates decreased from 97.270 mg g⁻¹ fwt at 50 mg L⁻¹ to 54.377 mg g⁻¹ fwt at 100 mg L⁻¹. Similarly, protein content dropped sharply, reaching 52.820 mg g⁻¹ fwt at the highest concentration. Interestingly, phenol content showed a slight recovery at 100 mg L⁻¹ (2.567 mg g⁻¹ fwt), approaching the control level. In contrast, when JA was added after autoclaving (filter sterilized), the biochemical responses were somewhat different. At lower concentrations (5 and 10 mg L⁻¹), carbohydrate and protein content increased, although the values were slightly lower than those observed in the pre-autoclaving treatment. Higher JA concentrations (50, 75 and 100 mg L⁻¹) added post-autoclaving resulted in a sharp decline in biochemical parameters. Carbohydrate content decreased to 62.110 mg g⁻¹ fwt at 50 mg L⁻¹ and further to 36.910 mg g⁻¹ fwt at 100 mg L⁻¹. Protein content followed a similar trend, dropping to 53.783 mg g⁻¹ fwt at the highest concentration. Interestingly, phenol content increased slightly at 100 mg L⁻¹ (2.167 mg g⁻¹ fwt) but remained below the control.

Jasmonic acid (JA) is widely recognized for its role in regulating plant growth, development, stress response and interactions with microbes (Wang *et al.*, 2021; Ali and Baek, 2020). Many studies have highlighted its beneficial effects under *in vitro* conditions, such as improved plant growth and development (Nabi *et al.*, 2021; Chauhan *et al.*, 2018;

Cirak *et al.*, 2020). Our study sought to explore the regulatory role of exogenously applied JA in promoting the *in vitro* growth and development of rose micropropagules.

Previous research, such as that by Cirak *et al.* (2020), demonstrated JA's regulatory effects in *Hypericum* species when added after sterilization, while Demirci *et al.* (2022) investigated its influence on stem elongation and water

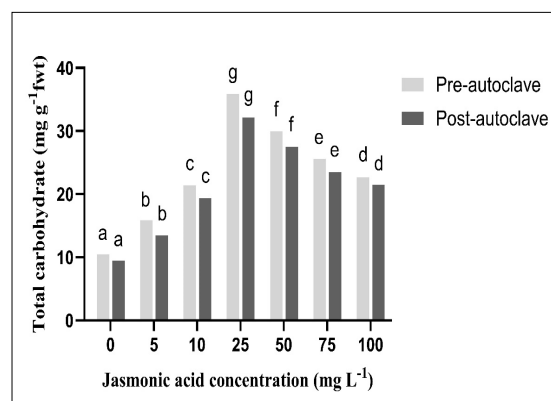


Fig 1: Effect of jasmonic acid on accumulation of total carbohydrate contents in rose micropropagules grown under *in vitro* conditions.

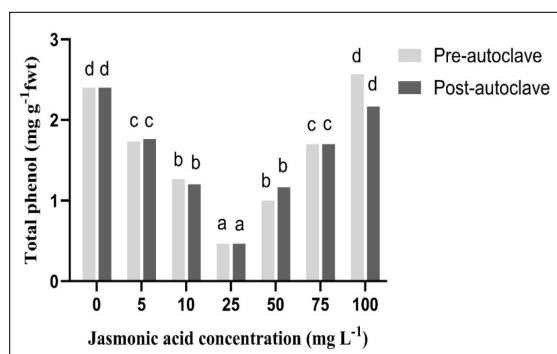


Fig 2: Effect of jasmonic acid on accumulation of total phenol contents in rose micropropagules grown under *in vitro* conditions.

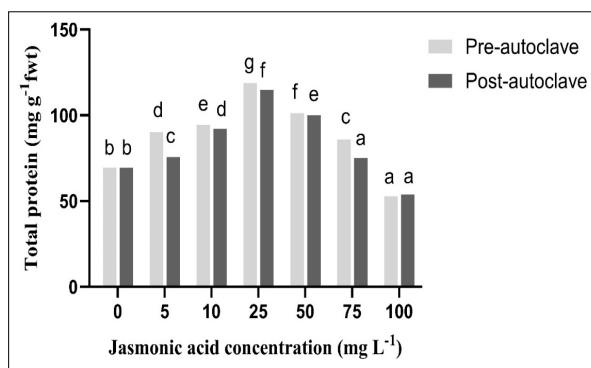


Fig 3: Effect of jasmonic acid on accumulation of total protein contents in rose micropropagules grown under *in vitro* conditions.

stress responses. Conversely, other studies incorporated JA into the medium prior to autoclaving (Pisitpaibool *et al.*, 2021; Rawat *et al.*, 2020). While a few studies compare the impact of these methods (Pervaiz *et al.*, 2023; Jeyasri *et al.*, 2023), our findings indicate that the mode of JA application did not significantly alter shoot growth or biomass production in rose micropropagules.

Jasmonates function within intricate signaling networks, modulating growth, development and hormone pathways. Endogenous JA levels are particularly high in young, actively dividing tissues, influencing cytokinin levels and cell cycle progression. For example, Dermastia *et al.* (1994) showed that JA increased active cytokinin levels in *S. tuberosum*, while Avalbaev *et al.* (2016) reported doubled cytokinin accumulation in *T. aestivum* without altering auxin or ABA levels. However, high JA and ABA levels disrupt the cell cycle, with JAs arresting cells in the G2 phase (Świątek *et al.*, 2002).

The analysis of biomolecule composition during *in vitro* culture provides critical insights into the physiological and metabolic shifts governing rose micropropagule development. Our findings demonstrate that jasmonic acid (JA) supplementation at different concentrations significantly alters carbohydrate, protein and phenolic compound accumulation, reflecting its role as a key metabolic regulator. The observed 3-fold increase in carbohydrate content at 25 mg L⁻¹ JA aligns with reports that JA enhances sucrose metabolism, likely to meet energy demands for shoot proliferation. For instance, Zhang *et al.* (2022) reported similar carbohydrate accumulation in *Isatis indigotica* under methyl jasmonate treatment, supporting our findings. The elevated protein levels (~1.7-fold higher than controls at 25 mg L⁻¹ JA) correlate with JA's known activation of phenylpropanoid pathways. This mirrors results in *Perilla frutescens*, where doubled phenolic acid production (Tavan *et al.*, 2023). In our study, the increase in ferulic acid derivatives suggests enhanced defense metabolism, crucial for *in vitro* stress adaptation. Notably, the highest concentration (100 mg L⁻¹) showed reduced efficacy, indicating a potential threshold for positive metabolic responses. Manivannan *et al.* (2016) observed flavonoid enhancement in *Scrophularia kakudensis* when supplemented with JA, reinforcing our results. However, the growth inhibition observed at 100 mg L⁻¹ suggests a hormetic response, where excessive JA may trigger stress signaling over growth promotion. These concentration-dependent effects emphasize the need for precise JA dosing in micropropagation protocols to balance metabolic enhancement with growth requirements.

Jasmonic acid (JA) also influences chlorophyll synthesis. At low concentrations, it promotes chlorophyll production by regulating genes involved in the biosynthetic pathway. However, at higher concentrations, JA can trigger stress responses that lead to the generation of reactive oxygen species (ROS) and subsequent chlorophyll degradation. Sadeghipour (2017), who reported that seed treatment with methyl jasmonate significantly improved

salinity tolerance in cowpea plants. This supports the notion that exogenous application of jasmonates can enhance plant stress resilience. However, the effects of jasmonic acid (JA) can be species-specific and may involve trade-offs. For instance, Khataee *et al.* (2020) observed that JA increased alkaloid production in *Catharanthus roseus* but simultaneously reduced chlorophyll content due to induced stress. These findings align with our observations, where a similar trend in chlorophyll biosynthesis was noted enhancement at lower JA levels and degradation at higher levels, indicating a dose-dependent regulatory effect.

In our study, low concentrations of JA enhanced shoot number, length, biomass and biomolecule accumulation in rose micropropagules. Conversely, higher JA levels had adverse effects, possibly due to ethylene stimulation, which inhibits growth (Kumlay, 2016). Total chlorophyll, carbohydrates, proteins, phenols and biomass increased at lower JA levels, aligning with similar findings in potato (Kumlay, 2016). This highlights JA's concentration-dependent dual role in promoting or inhibiting growth and biomolecule synthesis under *in vitro* conditions.

CONCLUSION

Jasmonic acid (JA) significantly influences plant growth, development and stress responses, including *in vitro* cultures. Our study on rose micropropagules revealed that JA application alters biochemical parameters like chlorophyll, phenols, proteins and carbohydrates, without adversely affecting growth at lower concentrations. Higher JA concentrations, however, inhibited growth, likely due to ethylene biosynthesis. Optimal JA concentrations are crucial for enhancing rose micropropagation under controlled conditions. These findings align with studies on other plants, such as strawberries and *Nardostachys jatamansi*, supporting the use of JA in *in vitro* cultures to improve growth and biochemical activity when carefully regulated.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

This study did not involve human participants or animals. Therefore, informed consent and ethical approval were not required. All experimental procedures involving plant materials were conducted in accordance with institutional and standard scientific guidelines for plant research.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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