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Effect of auxin and cytokinin on growth and galantamine production from *in vitro* shoot cultures of *Narcissus tazetta* var. *chinensis*

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ABSTRACT

In recent years, *in vitro* plant culture techniques have significantly contributed to the large-scale propagation of plants and the production of bioactive compounds. This study investigates the impact of various plant growth regulators, including auxins (IAA, IBA, and NAA) and cytokinins (BAP, Kinetin, and TDZ), on the growth and galantamine production in shoot cultures of *Narcissus tazetta* var. *chinensis* cv. 'Geumjanogdae'. One-year-old *N. tazetta* bulbs, which had been grown in a greenhouse, were used to regenerate shoots and establish *in vitro* cultures. The bulbs were zoomed in (2-3 bulbs were associated together in a flowering phase) exposed to a sterile environment and explants grew on Murashige and Skoog (MS) medium with different concentration of auxins and cytokinins. The objective of the study was to optimize simultaneous shoot growth and galantamine, a pharmacologically active alkaloid with great therapeutic significance as an Alzheimer disease medication, biosynthesis conditions. The culture with fresh weight and the galantamine content from the results showed significant effects of hormone treatments. Among the auxins, 2 mg/L NAA resulted in a maximum of 14.03 g/flask of fresh weight, and 1 mg/L IBA gave the best galantamine content of 277.64 µg/g dry weight (DW), respectively. The highest fresh weight of plant was in TDZ 2 mg/L with 16.33 g/flask, while it had the lowest rank in BAP 4 mg/L. Cytokinin, especially BAP, perform a significant role on galantamine enhancement synthesis by shoot cultures. The treatment BAP 4 mg/L had the highest galantamine content (232.16 µg/g DW) than that of TDZ and Kinetin. The study proved that the use of auxins and cytokinins could be manipulated in a way that not only improved shoot growth but also considerably increased the level of bioactive compounds like galantamine. This study also provides a clearer insight for optimal *in vitro* culture conditions by showing the performance of growth regulators on *N. tazetta*, which can serve as result of the basic information for large scale production of galantamine for medicinal purposes. Moreover, this work serves as an initial step towards the subsequent optimization of other bioactive compounds in different plant species.

KEYWORDS: *In vitro* culture, Auxins, Shoot regeneration, Plant growth regulators, Shoot growth, Pharmacological activity

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INTRODUCTION

Narcissus tazetta L. var. *chinensis* is a perennial monocot from the Amaryllidaceae family. This species of the plant is widely studied for its ornamental as well as pharmacological use. *N. tazetta* bulb is well known for its bioactive alkaloids, including galantamine, which is a critical drug for treating Alzheimer's neurological illness through the inhibition of the enzyme acetylcholinesterase (involved in the degradation of the neurotransmitter acetylcholine in the brain) (Keglevich *et al.*, 2016; Abdel-Rahman *et al.*, 2017).

The alkaloids of this family are extensively researched and many of their biological activities hold significance to the pharmaceutical industry. Applications of these include

Compounds with activity against neurological disorders, cancer and various infections in particular, galantamine is regarded as a first-line treatment for Alzheimer's disease (AD), a type of neuro degenerative disease that is characterized by memory loss and cognitive decline (Nair & van Staden, 2023). While *N. tazetta* has a promising potential in galantamine production, its prevalent extraction from plant source is laborious, time-consuming and not cost-effective causing a great interest towards producing galantamine through tissue culture methods (Ayaz *et al.*, 2022).

Tissue culture, which is a technique to grow plant cells, tissues, or organs *in vitro* under a sterile controlled environment, is one of the most popular tools of plant biotechnological approaches.

Through this process, growth conditions can be manipulated to evoke the production of certain compounds such as secondary metabolites (Pang *et al.*, 2021). Auxins and cytokinins are two of the most widely used plant hormones in tissue culture. Root growth is largely under the control of auxins such as IAA and IBA, which also modulate cell elongation, division and differentiation. In contrast to this type of cytokinin which are predominantly involved in root development, other cytokinins such as benzyl aminopirine (BAP) are major regulators of shoot formation stimulating cell division and growth (Pichersky & Gang, 2000).

Tissue culture conditions can also influence plant growth and the production of secondary metabolites (e.g., alkaloids like galantamine) by adjusting the ratio of auxins to cytokinins. Among many factors, hormone levels are one of the most critical for establishing conditions to optimize tissue culture parameters for galantamine production (Piasecka *et al.*, 2015) and hormonal balance is important in terms as both efficient shoot induction and bioactive compounds accumulation. Recent attempts to develop *in vitro* cultures of *C. chinensis* have demonstrated the potential for high production levels of secondary metabolites, including other phenolic compounds, which also contribute to medicinal characteristics (Kilgore *et al.*, 2016; Fan *et al.*, 2021), besides galantamine.

Tissue regeneration is one of the classic examples for a proof of concept for shoot culture strategy, employed in massive propagation of plants and also the production of secondary metabolites. Analysis of the shoot culture of *N. tazetta* showed a wide spectrum of bioactive compounds, some of which are possibly involved in galantamine biosynthesis. Among these methods, the culture on MS medium *in vitro*, with or without different combinations and concentrations of growth regulators (auxins and cytokinin) (Murashige & Skoog, 1962) may be mentioned. This strategy has various advantageous features including scalability, stable genetic homogeneity, and less dependence on natural plant reservoirs (Guerriero *et al.*, 2018).

This work is to evaluate the concentrations of several hormones to find the best number of shoots but also the suitable growth conditions for maximal galantamine contents. Since the regulatory role of auxiliary metabolites is well established, this makes understanding these two groups of key compounds easier, to eventually drive the mechanistic functionality of hormones in plant tissue biosynthesis which will contribute highly to functionalizing the respective biotechnological processes for producing galantamine (Chandran *et al.*, 2020).

Besides functionally interrogating the effect of growth regulators on galantamine biosynthetic profile, this study aims to expand known generalities of hormonal regulation of secondary metabolites to the level of metabolic pathway during their accumulation in *N. tazetta* cultures. These findings can help to overcome existing obstacles in the optimization of galantamine yield and can also be exploited for the development of *in vitro* systems that are better suited to propagation prior to large-scale production of this important pharmaceutical compound (Sivanesan & Jeong, 2009). More importantly, success in these strategies may be necessary for the commercial generation of other more valuable metabolites from *N. tazetta* and close relatives, given that they may presumably reduce environmental impact compared to classical plant cultivation (Rameshk *et al.*, 2018).

This study demonstrates a detailed biochemical approach toward gaining insight into galantamine production by developing optimal culture conditions and investigating the link between growth regulators and the accumulation of secondary metabolites. This study may assist with the biotechnological use of *N. tazetta* and other medicinal plants, which represents sustainable alternatives for drug production with reduced natural plant resource over-exploitation (Ferri *et al.*, 2017).

MATERIALS AND METHODS

Plant Materials

A 1-year-old bulb of *Narcissus tazetta* var. *chinensis* cv. 'Geumjanogdae' was purchased from Xplant Co. (Seoul, Korea). They were grown in a Chungnam National University greenhouse. The bulbs were used as callus or shoot induction materials when they were one-year-old.

Shoot Induction

In *N. tazetta* sterile bulbs the meristems were dissected into parts 0.5 cm × 0.5 cm × 0.2 cm in size. Explants were cultured in 100 × 25 mm Petri dishes on medium (around 25 mL). The basal medium contained MS medium, adjusted to pH 5.8 before solidification with 0.8% (w/v) Phytagar (Sigma, St. Louis, MO., USA). Autoclaving at 1.1 kg cm^{-2} (121 °C) for 20 min was used for the sterilization of media. MS medium supplemented with 2 mg/L BAP (6-benzylaminopurine) and 0.1 mg/L NAA (Naphthaleneacetic acid) for shoot regeneration from meristem explants. Plant hormones were from Sigma (St. Louis, MO, USA). The cultures were placed at 25±1 °C in the growth chamber (with a 16-hr photo period under standard cool white, fluorescent tubes (35 mmol s^{-1} m^{-2}) for 6 weeks.

Establishment of Shoot Cultures

Shoots from the meristems in the sterilized bulbs of *N. tazetta* maintained on agar solidified MS (Murashige & Skoog, 1962)

media containing BAP and NAA to 2 and 0.1 mg/L were initiated. For fast-growing shoot cultures, 3 g (fresh weight) of regenerated shoots were placed into 125 mL Erlenmeyer flasks containing 30 mL of 30 g/L sucrose-supplemented MS liquid media. To determine the inclusion of different types and concentrations (0, 1, 2 and 4 mg/L) of auxin (IAA, IBA, and NAA) and cytokinin (BAP, Kinetin, and TDZ) on MS medium for shoot cultures. The medium was pH 5.8 and autoclaved at 1.1 kg cm^{-2} (121 °C) for 20 min. Growth conditions: Shoots were cultured under standard cool white, fluorescent tubes at a flux rate of 35 µmol $s^{-1}m^{-2}$, which was supplied for 16 h a day, at 25 °C on a gyratory shaker (100 rpm). The culture was harvested after 4 weeks and frozen in liquid nitrogen, freeze-dried at -80 °C for 72 h and then ground with a pestle for galantamine analysis. Experiment was performed in triplicate.

HPLC Analysis of Galantamine

Galantamine was extracted according to a previously described procedure with slight modifications (Park *et al.*, 2020). For the different organs (leaf, bulb and root) of *L. radiata*, the materials (100 mg each) were fine powder, then 2 mL of 0.1% trifluoroacetic acid in water was added to the powdered materials. The samples were sonicated for 30 min and kept overnight at 4 °C, followed by a second cycle of 30 min of sonication and 10 min of 13,000 rpm centrifugation. The supernatant was then filtered into the vials through a 0.45 µm Acrodisc syringe filter (Pall Corporation, Port Washington, NY, USA). Galantamine was analysed using an NS-4000 HPLC system equipped with a ND-6000 auto-sampler and a UV-Vis detector (Futecs Corporation, Daejeon, Korea). Separation of galantamine was performed on an OptimaPak C18 column (250 × 4.6 mm, 5 µm, RStech Corporation, Daejeon, Korea) with mobile phase solvents consisting of eluent (A) 50 mM ammonium formate aqueous buffer and eluent (B) acetonitrile at a flow rate of 1 mL/min. The oven was maintained, with the thermostat at 30 °C, the gradient program was as follows: 0-15 min, 2% B; 15-30 min, 2-65% B; 30-31 min, 65-100% B; 31-35 min, 100% B; 35-36 min, 100-2% B; and 36-38 min, 2% B (total time 38 min). Sample was injected (20 µL) per run with the following detection wavelength: 285 nm. Galantamine commercial standard was obtained from ChemFace (China). A calibration curve was plotted based on the concentrations of six known galantamine concentrations. The linear equation was $y = 12.7704 \times -12.5000$ ($R2=0.9996$). The values were represented as means±standard deviation.

Statistical Analysis

Data were analyzed by Duncan's multiple range test, with a significance level of $p<0.05$, using SAS 9.4 (SAS Institute, Inc., Cary, NC, USA).

RESULTS

N. tazetta shoots regenerated on agar-solidified MS medium are shown in Figure 1a. The solid medium provides nutritional support and physical stability, which is necessary for the initial shoot proliferation and root formation in tissue culture. It provides a controlled environment that enables successful plant growth in the early stages.

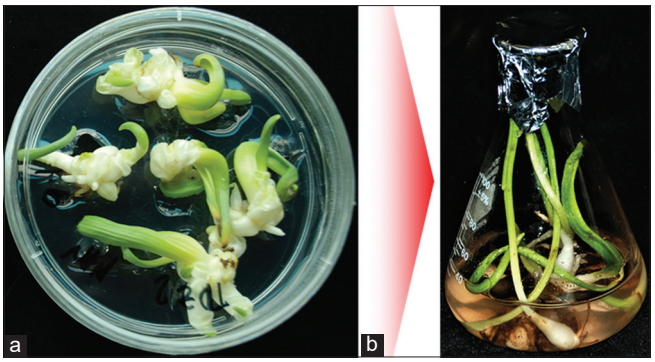

Figure 1: a) Regenerated shoots of *N. tazetta* maintained on agar-solidified MS medium and b) Shoots of *N. tazetta* cultured in liquid MS medium

Liquid MS medium is designed to fully immerse plant tissues in a manner that allows for optimal nutrient and gas transfer and as shown in Figure 1b featuring *N. tazetta* shoots. A liquid medium improves growth, diminishes the risk of desiccation, and is typically employed during more advanced phases of the propagation process or during large-scale biomass production.

In vitro shoot regeneration using both solid and liquid media (solid for initial growth, liquid for enhancing nutrient uptake and shoot multiplication) is essential for scalable production of bioactive compounds, such as galantamine, from *N. tazetta* cultures.

Effect of Auxin and Cytokinin on the Growth of *in vitro* Shoot Culture of *N. tazetta*

The influence of different treatments (control, IAA, IBA, and NAA at different concentrations) on the fresh weight (g/flask) of plant samples varied significantly (Figure 2). All the treatments significantly enhanced the growth pattern in comparison with the control. It was observed that the production of *N. tazetta* increased by increasing concentration, irrespective of auxin treatment. In all the cases the highest production was found at the concentration of 2 mg/L. The maximum fresh weight (14.03 g/flask) was obtained at NAA with the concentration of 2 mg/L, which was significantly higher than the control (7.85 g/flask) and other concentrations. Here in this study, the performance of both IAA and IBA, irrespective of concentrations, were almost the same. Of the growth regulators tested, IAA and NAA performed the best at 2 mg/L. From the data, it can be concluded that IAA and NAA at 2 mg/L promote plant growth compared to IBA.

Impact of cytokinin (BAP, Kinetin, and TDZ) concentrations on *N. tazetta in vitro* shoot culture growth as determined by fresh weight (g/flask) cultured for a period of four weeks on MS medium (Figure 3). Fresh weight significantly varied among the cytokinin treatments. The fresh weight increased with the increase in the concentration of cytokinin except for TDZ 2 mg/L. From the

results of cytokinin, it was observed that TDZ performed the best, followed by BAP and then Kinetin for the higher production of fresh hairy cultures. The range of fresh weight was 7.85 to 16.33/flask which indicates that fresh weight boosted more than twice compared to the control treatment. The highest fresh weight/flask (16.33±0.65 g/flask) was obtained from the treatment of TDZ 2 mg/L followed by the treatment of TDZ 1 mg/L (14.56±0.72 g/flask). The lowest fresh weight was noted in the control treatment (7.85±0.51 g/flask). Through the treatment of BAP 4 mg/L, BAP 2 mg/L and BAP 1 mg/L, produced a fresh weight of 14.09±0.71, 13.46 ±0.71 and 11.58±0.57 g/flask, respectively). Kinetin 1 mg/L and Kinetin 2 mg/L had a fresh weight of 10.41±0.48 g/flask and 12.1±0.43 g/flask, whereas Kinetin 4 mg/L showed a fresh weight of 12.93±0.59 g/flask. The comparison indicates that fresh weight was significantly improved by TDZ 2 mg/L in comparison with all other treatments.

Effect of Auxin and Cytokinin on Galantamine Content of *in vitro* Shoot Culture of *N. tazetta*

The impact of auxin (IAA, IBA, and NAA) concentrations on *N. tazetta* shoot culture growth was assessed by measuring hormone treatments for galantamine contents (µg/g DW) after a four-week culture period on MS medium (Figure 4). Significant variation in galantamine contents was observed across the auxin treatments. The accumulation of galantamine generally decreased with increasing the concentrations of auxin except concentration of NAA. Among the auxin treatments, IBA was found to be the most effective, followed by IAA, in promoting higher galantamine production in cultures. The range of galantamine concentrations varied from 145.71 µg/g DW to 277.64 µg/g DW, which indicates that hormone treatment especially with IBA of 1µg/g enhanced almost double compared to control treatment. The highest accumulation of galantamine (277.64±6.37 µg/g DW) was recorded in the IBA 1 µg/g DW treatment, followed by IAA 1 µg/g DW with a concentration of 178.29±10.14 µg/g DW. The lowest galantamine content was found in the IAA 2 treatment (118.88±3.77 µg/g DW). Additionally, IBA 2 and IBA 4 treatments had galantamine concentrations of 119.70±4.93 µg/g DW and 123.18±15.64 µg/g DW, respectively, while NAA treatments (NAA 1, NAA 2, and NAA 4) resulted in galantamine content of 126.66±6.66 µg/g DW, 137.73±1.45 µg/g DW, and 136.70±6.95 µg/g DW, respectively. Overall, the comparison of these treatments

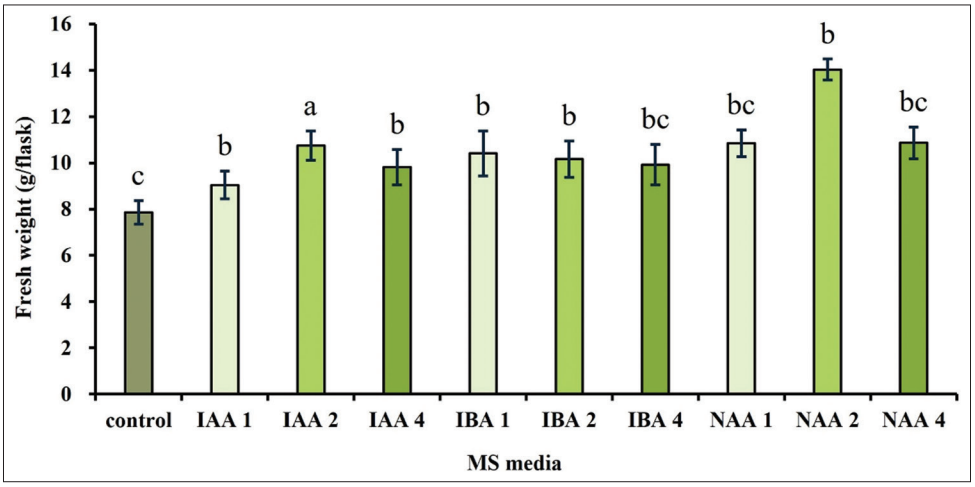

Figure 2: Effect of different concentrations of auxins on the growth of *in vitro* shoot culture of *N. tazetta* after 4 weeks in MS medium

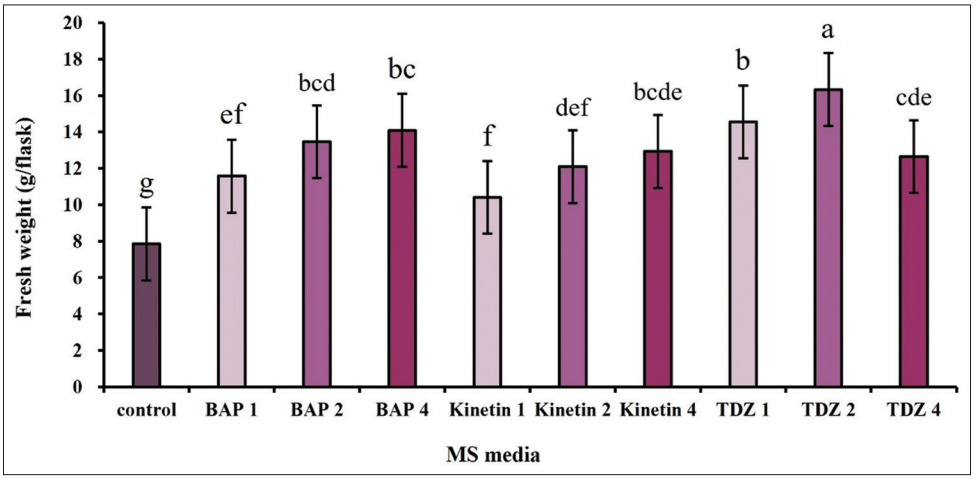

Figure 3: Effect of different concentrations of cytokinin on the growth of *in vitro* shoot culture of *N. tazetta* after 4 weeks in MS medium

indicates that the highest content of galantamine was achieved with IBA 1 µg/g DW, outperforming all other treatments.

The effects of cytokinin (BAP, Kinetin, and TDZ) concentrations on *N. tazetta in vitro* shoot culture growth was evaluated by measuring fresh weight (µg/g DW) after a four-week culture period on MS medium. The hormone treatment for the accumulation of galantamine exhibited significant variation among the different cytokinin treatments. The accumulation of galantamine was higher in any of the treatments than that of control. It was observed that the galantamine concentration increased with increasing the concentration of cytokinin up to certain and then declined slightly. Among the cytokinin treatments, BAP 2 µg/g DW showed the most effective treatment, followed by TDZ 2 µg/g DW, for promoting the production of galantamine in the shoot cultures. The range of galantamine concentrations varied from 145.71 µg/g DW to 232.16 µg/g DW, indicating a higher amount of galantamine levels increased in the cytokinin treatment compared to the control treatment. The highest galantamine content (232.16±0.58 µg/g DW) was observed in the BAP 2 µg/g DW treatment, closely followed by TDZ 2 µg/g DW with a galantamine content of 231.34±5.79 µg/g DW. In contrast, the control treatment had the lowest galantamine content, with 145.71±5.21 µg/g DW. Further analysis revealed that the BAP 1 µg/g DW and BAP 4 µg/g DW treatments resulted in galantamine contents of 189.14±1.74 µg/g DW and 136.29±2.90 µg/g DW, respectively. The Kinetin treatments (Kinetin 1 µg/g DW, Kinetin 2 µg/g DW, and Kinetin 4 µg/g DW) produced galantamine contents of 175.42±2.03 µg/g DW, 192.63±3.77 µg/g DW, and 220.90±4.35 µg/g DW, respectively. TDZ 1 µg/g DW and TDZ 4 µg/g DW resulted in galantamine contents of 211.47±37.37 µg/g DW and 229.09±3.19 µg/g DW. The comparison of cytokinin treatments revealed that BAP 2 µg/g DW significantly enhanced the galantamine content more than any other treatment, followed closely by TDZ 2 µg/g DW.

DISCUSSION

In the current study, we evaluated the effects of several auxins and cytokinins on the growth and galantamine production by *N. tazetta in vitro* shoot cultures. These findings are essential in optimizing culture conditions to facilitate increased growth as well as production of metabolites for large-scale cultivation for production of galantamine, a pharmaceutical of importance as reported earlier (Abdel-Rahman *et al.*, 2017; Ayaz *et al.*, 2022).

Three different auxins, IAA, IBA, and NAA (each at three different concentrations) were applied in our experiment to evaluate their effects on shoot growth and galantamine. The highest fresh weight was observed in the NAA 2 mg/L treatments (14.03 g/flask) with significant increase over control (7.85 g/flask). More probably, NAA (especially of 2 mg/L) was the best plant growth promoter under the conditions employed (Guerriero *et al.*, 2018; Nair & van Staden, 2023). Of the IAA treatments, 2 mg/L was the most effective in promoting cell division and elongation, both necessary for shoot growth in *N. tazetta*, and it led to the greatest fresh weight. This reinforces the role of exogenous auxins in enhancing *in vitro* growth as the control group had the lowest fresh weight (Piasecka *et al.*, 2015; Fan *et al.*, 2021).

IAA and NAA treatments, indicating that IBA is perhaps less effective in promoting a shoots growth. This finding is in line with other research where IAA and NAA proved more effective in encouraging shoot proliferation for different plant species (Guerriero *et al.*, 2018).

Relating to the galantamine content, the maximum possible galantamine content (277.64±6.37 µg/g DW) was obtained from IBA 1 mg/L with a significant difference from other treatments including the normal (145.71±5.21 µg/g DW) (Figure 5). The above result shows that the efficiency of IBA kinds in increasing the production of galantamine in *N. tazetta* cultures is very high, especially at 1 mg/L. At 1 mg/L, IAA also produced a significant increase in galantamine content (178.29±10.14 µg/g DW) over higher concentrations of the same hormone, 2 mg/L and 4 mg/L, which produced declines in galantamine accumulation (Kilgore *et al.*, 2016; Nair & van Staden, 2023). It indicates that auxin concentration must be balanced, and lower concentrations of IAA were more favorable for the production of galantamine, and a similar finding was also

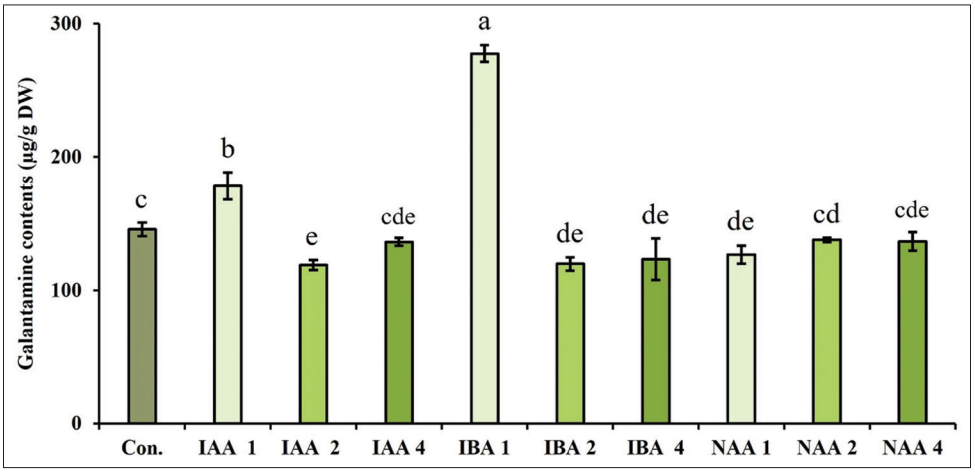

Figure 4: Effect of different concentrations of auxins on galantamine content of *in vitro* shoot culture of *N. tazetta* after 4 weeks in MS medium

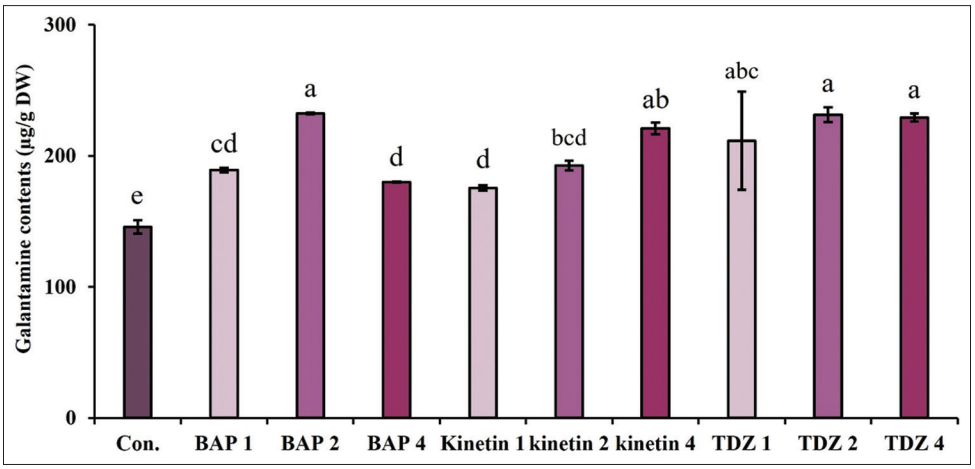

Figure 5: Effect of different concentrations of cytokinin on galantamine content of *in vitro* shoot culture of *N. tazetta* after 4 weeks in MS medium

reported in previous research concerning the effects of auxins on alkaloid production in other species (Fan *et al.*, 2021).

All NAA treatments produced moderate increases in galantamine content but values (126.66±6.66 µg/g DW–137.73±1.45 µg/g DW) remained lower than those reported for optimal treatments using IBA and IAA (Ferri *et al.*, 2017). Consequently, although NAA may promote general growth, it is generally less effective than IBA in increasing galantamine level, which is consistent with previous observations on growing cultures of *N. tazetta* (Kilgore *et al.*, 2016; Abdel-Rahman *et al.*, 2017).

All three different cytokinins (BAP, Kinetin and TDZ) were examined for their effect on both growth and galantamine content. TDZ 4 mg/L showed the maximum fresh weight (16.33±0.65 g/flask) which was higher than all other treatments such as BAP 4 mg/L (14.09±0.30 g/flask) and TDZ 2 mg/L (14.56±0.72 g/flask). The results show that TDZ was superior compared to other hormones inducing shoot initiation. Moreover, TDZ, especially at 4 mg/L, appears to be the key to attaining vigorous growth of the tissue culture systems, as cell division is one of the most critical determinant parameters (Pichersky & Gang, 2000; Piasecka *et al.*, 2015). Cultures treated with TDZ likewise showed the highest relative growth rate, thereby corroborating its efficiency as a potent cytokinin for shoot proliferation (Guerriero *et al.*, 2018).

BAP 4 mg/L significantly expressed the accumulation of galantamine content. Interestingly, despite slight improvements over those of the control, Kinetin treatments had a lower effective role for both growth and galantamine content in contrast to BAP and TDZ. The highest fresh weight with Kinetin was 12.93±0.59 g/flask (at 4 mg/L), and the galantamine content was also modest (192.63±3.77 µg/g DW at 4 mg/L). This indicates that Kinetin also promotes growth, but to a lesser extent than BAP or TDZ, which appeared to enhance not only plant growth but also galantamine accumulation together (Pichersky & Gang, 2000; Nair & van Staden, 2023). Our finding regarding the relative effect of Kinetin on metabolite content was also supported by a previous study where it was shown that Kinetin, when compared to other cytokinins, may not be as effective in triggering secondary metabolite biosynthesis (Pichersky & Gang, 2000; Nair & van Staden, 2023).

Notably, Kinetin also produced some improvement, relative to controls, but it was consistently less effective than BAP and TDZ over both growth and galantamine content. Kinetin was concluded to give the most elevated crisp weight (12.93±0.59 g/flask, at 4 mg/L) and lower levels of galantamine (192.63±3.77 µg/g DW, at 4 mg/L) were found. Kinetin, however, contributed considerably less to plant growth and galantamine accumulation than did BAP or TDZ (Pichersky & Gang, 2000; Nair & van Staden, 2023). Similar is the observation in a report that shows Kinetin probably less effective compared to other cytokinins in induction of secondary metabolite biosynthetic pathways (Pichersky & Gang, 2000; Nair & van Staden, 2023).

The present findings are of great relevancy for *in vitro* propagation as well as for galantamine production from *N. tazetta*. Moreover, optimal IBA 1 mg/L for galantamine and TDZ 4 mg/L for shoot growth with improved efficiencies of auxins and cytokinins also enhance the semiconsolid culture systems which enhance biomass and secondary metabolites contributes to plant productivity. This is especially relevant since it highly affects some pharmaceutical industries that benefit from the exploitation of *N. tazetta* in order to obtain galantamine, Alzheimer disease treatment drug (Ayaz *et al.*, 2022).

They also suggest that medium formulations are to be optimized so that growth can be maximized without compromising the production of bioactive compounds, as is the case with the auxins + cytokinins combination in which plants were induced in. Hence, it should be optimized by using other plant growth regulators and their combinations for maximum growth and galantamine content (DiCosmo & Misawa, 1995; Pang *et al.*, 2021), and need further investigation.

Based on the findings of this study, we provide novel data into the role of auxins and cytokinins in *N. tazetta* growth *in vitro* and galantamine content. The utilization of IBA 1 mg/L that stimulates content of galantamine together with TDZ 4 mg/L to promote robust growth of shoots can be a step forward in

rendering efficient culture systems for *N. tazetta*. This work not only expands the concept of plant tissue cultural techniques by providing options for high-volume production of galantamine but also expands the therapeutic possibilities of this important compound (DiCosmo & Misawa, 1995; Abdel-Rahman *et al.*, 2017; Guerriero *et al.*, 2018).

CONCLUSION

Finally, an efficient *in vitro* system for *N. tazetta* shoot culture has been established for optimizing their growth and the galantamine content under the effect of various auxins and cytokinins. These conclusions confirmed that auxins and cytokinins have a strong impact on shoot growth and galantamine accumulation and the values providing the significantly best results. In contrast, the highest fresh weight of plant was in TDZ 2 mg/L, and BAP 4 mg/L among all the treatments. The different scenarios were observed for the accumulation of galantamine, where IBA 1 mg/L boosted the highest accumulation of galantamine, followed by BAP and TDZ 2 mg/L. These results verify that the type and concentration of auxin and cytokinin are mostly case sensitive for the production of shoots and accumulation of galantamine. Among the auxin treatments, IBA and, for cytokinin, BAP and TDZ are important factors in promoting galantamine production, and future work is needed for large-scale production of the compound. This study provides a valuable baseline for further research, targeting the *in vitro* culture growth conditions, which are positive specifically for pharmaceutical applications.

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