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In vitro Propagation of Ornamental Snapdragon (*Antirrhinum majus* L.) Revisited: An Analysis on the Effects of Plant Growth Regulators

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Abstract: The effects of N^6-(2-isopentenyl) adenosine (IP) and p-chlorophenoxyacetic acid (CPA) on the propagation of *Antirrhinum majus* have yet to be elucidated. This study compares plant growth regulators' effects on enhancing propagation efficiency in snapdragon. In the seedling development phase, IP at 1.0 mg L^{-1} provided the highest germination ratio (91.11 ± 9.30%). The 6-benzylaminopurine (BAP) treatment at 1.0 mg L^{-1} gave the highest number of shoots (4.42 ± 0.30) whereas the medium with 0.50 mg L^{-1} IP triggered shoot elongation (3.80 ± 0.28 cm) from germinating seeds. The medium with 1.0 mg L^{-1} IP gave the highest number of leaves (12.33 ± 1.77) and roots (2.96 ± 0.32). IP treatment at 0.50 mg L^{-1} produced the greatest increase in seedling root lengths (2.50 ± 0.31 cm). In the shoot multiplication phase, thidiazuron (TDZ) at 1.0 mg L^{-1} gave the maximum number of shoots (10.04 ± 2.42 per nodal explant) while BAP treatment at the same concentration triggered shoot elongation (5.99 ± 0.29 cm). In the rooting phase, 3-indoleacetic acid (IAA) treatment at 0.50 mg L^{-1} induced the highest rooting rate (100%), root production (4.93 ± 0.48 per shoot), and root length (7.16 ± 0.97 cm). IAA treatments did not trigger callus production. However, the CPA treatments induced consistently higher callogenesis responses (96% and 100%), resulting in a minimal rooting response. The findings suggested using IP to increase seedling development without causing a restriction in root development, TDZ to improve shoot multiplication efficiency, and CPA to produce high-frequency calli production in ornamental snapdragon.

Keywords: Callogenesis, ornamental plants, rhizogenesis, seed germination, shoot multiplication.

Aslanağzının (*Antirrhinum majus* L.) *In vitro* Çoğaltımı Yeniden Gözden Geçirildi: Bitki Büyüme Düzenleyicilerinin Etkileri Üzerine Bir Analiz

Öz: N^6-(2-izopentenil)adenozin (IP) ve p-klorofenoksiasetik asidin (CPA) *Antirrhinum majus*'un çoğaltılması üzerindeki etkileri henüz aydınlatılmamıştır. Bu çalışma, bitki büyüme düzenleyicilerinin aslanağzı bitkisinde çoğaltım verimliliğini artırma üzerindeki etkilerini karşılaştırmaktadır. Fide gelişim aşamasında, 1.0 mg L^{-1}'deki IP en yüksek çimlenme oranını (%91.11 ± 9.30) sağlamıştır. En yüksek sürgün sayısını (4.42 ± 0.30) 1.0 mg L^{-1}'deki 6-benzilaminopürin (BAP) uygulaması verirken, 0.50 mg L^{-1} IP içeren ortam çimlenen tohumlardan sürgün uzamasını (3.80 ± 0.28 cm) tetiklemiştir. En yüksek yaprak (12.33 ± 1.77) ve kök (2.96 ± 0.32) sayısını 1.0 mg L^{-1} IP ile zenginleştirilmiş ortam vermiştir. Fide kök uzunluklarında en büyük artışı 0.50 mg L^{-1} IP uygulaması sağlamıştır (2.50 ± 0.31 cm). Sürgün çoğaltımı aşamasında, 1.0 mg L^{-1}'deki thidiazuron (TDZ) en fazla sürgün sayısını verirken (nodal eksplant başına 10.04 ± 2.42), aynı konsantrasyondaki BAP uygulaması sürgün uzamasını tetiklemiştir (5.99 ± 0.29 cm). Köklenme aşamasında, 0.50 mg L^{-1} 3-indol asetik asit (IAA) uygulaması en yüksek köklenme oranını (%100), kök üretimini (sürgün başına 4.93 ± 0.48) ve kök uzunluğunu (7.16 ± 0.97 cm) vermiştir. IAA uygulamaları kallus üretimini uyarmamıştır. Bununla birlikte, CPA uygulamaları sürekli olarak daha yüksek kallus oluşumu (%96 ve %100) teşvik etmiş ve son derece az bir köklenme yanıtı ile sonuçlanmıştır. Bulgular, kök gelişiminde bir kısıtlamaya neden olmadan fide gelişimini artırmak için IP, sürgün çoğaltımı verimliliğini artırmak için TDZ ve aslanağzında yüksek miktarda kallus üretimi için CPA kullanılmasını önermektedir.

Anahtar kelimeler: Kallogenez, rizogenez, sürgün çoğaltımı, süs bitkileri, tohum çimlenmesi.

1. Introduction

Snapdragon (*Antirrhinum majus* L.), a subshrub that grows mainly in the temperate biome, belongs to the Plantaginaceae (plantain) family. The species is native to France and Spain but has been introduced in many countries from different geographical regions (POWO, 2023). The species possesses medicinal, ornamental, and culinary properties. In recent reports, the antimicrobial properties of its aerial parts (Saqallah et al., 2022), the anti-proliferative and anti-metastatic properties of its flower extracts on several cancer cell lines (Seo et al., 2020), its value for the cut flower industry due to its straight stem, long vase life and colourful flowers (Lewis et al., 2021), and the value of its flowers due to their edible and nutritional properties in the food industry (Stefaniak & Grzeszczuk, 2019) have been reported. Snapdragon is also considered a model plant for genetic studies (Mizzotti et al., 2014).

Plant tissue culture techniques are the most widely used season-independent plant propagation methods in ornamentals since they allow the producers to generate genetically identical, uniform, and disease-free plants (Mehbub et al., 2022). The roles of plant growth regulators (PGRs) have long been recognized in plant tissue culture studies. Among the main groups of PGRs, cytokinins and auxins are commonly used to control plant growth and development in plant tissue culture by modulating cell

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division, elongation, differentiation, and organogenesis (Perrot-Rechenmann, 2010; Hill & Schaller, 2013). In this context, the effects of several cytokinins such as 6-benzylaminopurine (BAP), kinetin (KIN), zeatin (ZTN), and thidiazuron (TDZ), and auxins such as 2,4-dichlorophenoxyacetic acid (2,4-D), indole-3-butyric acid (IBA), 3-indoleacetic acid (IAA), 2-naphthoxyacetic acid (NOA), and 1-naphthaleneacetic acid (NAA) have been tested on *in vitro* cultures of *A. majus* in propagation-oriented studies (Sangwan & Harada, 1975; Sheyab et al., 2010; Hamza et al., 2013). In these studies, the researchers reported that high callogenesis response in the presence of 2,4-D but limited callus development in response to IAA and NAA can be recorded and the cytokinins cannot provide organ formation without being combined with auxins (Sangwan & Harada, 1975). In addition, high shoot multiplication ratios were reached when BAP and KIN were used and auxin treatment was required for rooting (Sheyab et al., 2010). Another study in *A. majus* showed the high efficacy of BAP and IBA treatments in shoot multiplication and rooting, respectively (Hamza et al., 2013).

However, the effects of N^6-(2-isopentenyl)adenosine (IP) as a cytokinin on seed germination, organ development, and shoot multiplication and the effects of p-chlorophenoxyacetic acid (4-CPA) as an auxin on rooting of *in vitro* shoots in *A. majus* have not been elucidated in comparison with other commonly-used PGRs. IP is considered a potent natural cytokinin in shoot proliferation, whereas 4-CPA is a herbicide with auxin properties that affects root development and fruit set. Thus, we hypothesized that these PGRs could give different results than the previously tested ones in seed germination, organ development, shoot multiplication, or callus production which could enhance the efficacy of *in vitro* propagation methods of the species for ornamental plant production and genetic transformation studies. In addition, an improved callogenesis response would be beneficial for establishing cell cultures to harvest bioactive compounds from the species.

2. Material and Methods

2.1. Plant material and disinfection treatment

Seeds of a commercial variety of *Antirrhinum majus* L. (*Antirrhinum majus pumilum* 'Magic Carpet' Mix, as declared by Genta Tarım, Türkiye) were purchased from a local supplier in Kocaeli, Türkiye. The seeds were maintained in their original packaging at 4 °C until use. To disinfect the seeds before inoculation, they were placed in a seed envelope (5 × 5 cm) prepared from filter paper and treated with 1% (*v:v*) NaOCl solution (40 mL) for 8 min with gentle agitation in a 50-mL conical centrifuge tube. The disinfectant was changed multiple times with sterilized distilled water to rinse the seeds.

2.2. Medium preparation, cytokinin treatment, and culture initiation

Murashige and Skoog's (MS) medium (Murashige & Skoog, 1962) fortified with 6-γ-γ-(dimethylallylamino) purine riboside (N^6-(2-isopentenyl)adenosine) (IP; Duchefa Biochemie, D0934), 6-benzylaminopurine (N^6-benzyladenine) (BAP; Caisson Labs, B001), or thidiazuron (1-phenyl-3-(1,2,3-thiadiazol-5-yl)urea) (TDZ; Duchefa Biochemie, T0916) at a concentration range from 0.25 mg L^{-1} to 1.0 mg L^{-1} was used in the seed germination and organ development experiments. The culture medium was supplemented with 30 g L^{-1} sucrose and the pH was adjusted between 5.7 and 5.8. The medium was solidified using 7 g L^{-1} agar (Duchefa Biochemie, P1001). The disinfected seeds were then placed onto the cytokinin-fortified medium and left for incubation.

2.3. Shoot multiplication and rooting

Shoots of seedlings from *in vitro* germinated seeds were excised at the end of the incubation period and inoculated into the shoot multiplication medium. Shoots with at least one node were employed in the experiment. The same MS medium with 1.0 mg L^{-1} of cytokinins tested previously (IP, BAP, and TDZ) was used for shoot multiplication. The multiplied shoots were then excised from their basal ends and inoculated into the same MS medium but fortified with p-chlorophenoxyacetic acid (4-CPA, abbreviated herein as CPA for clarity in the figures; Duchefa Biochemie, C0909), 3-indoleacetic acid (IAA; Sigma-Aldrich, 57333) or 1-naphthaleneacetic acid (NAA; Sigma-Aldrich, N0640) at 0.25, 0.50, or 1.0 mg L^{-1} for rooting experiments.

2.4. Acclimatization

The caps of the culture vessels were substituted with filtered caps and left for incubation at the same previous culture conditions for 1 week. The plants with developed roots were removed from the culture vessels and the medium residues on their roots were washed out. The plants were then sown into pots filled with a peat and perlite mixture (70:30; *v:v*). The hardening process was performed by covering the pots with transparent plastic bags with holes for ventilation for 1 week.

2.5. Culture conditions and parameters measured

GA-7 (Magenta LLC, USA) vessels were used for the cultures. The cultures were maintained in a growth chamber with a photon flux density of 60 mol m^{-2} s^{-1} and a photoperiod of 16 h at 23 ± 1 °C. The seed germination percentage, callus induction ratio, rhizogenesis percentage, mean number and length of shoots and roots, and/or leaf numbers were assessed at the end of a 30-day incubation period at the appropriate study phases.

2.6. Statistical comparison

The effects of cytokinins on seed germination and organ development and the effects of auxins on rhizogenesis were assayed over 45 seeds and shoots equally distributed into 5 culture vessels, respectively. The parameters related to shoot multiplication were measured over 27 shoots cultured in 3 culture vessels for each cytokinin. Each culture vessel having 9 shoots represented an independent repeat. The data are represented as the mean value of the parameter ± standard deviation (SD). The statistical differences between the mean value of the parameter measured from the control and treatment groups were compared using Tukey's honestly significant difference (HSD) test after conducting a one-way analysis of variance (ANOVA) test at a significance level of $P < 0.05$ using IBM SPSS Statistics 22 software. The data were then subjected to min-max normalization into [0, 1] interval and a hierarchical cluster analysis based on Euclidean distance and the complete-linkage clustering method was performed. The relationships among the data were

visualized through a heat map using the Interactive Clustered Heat Map Builder (Ryan et al., 2020).

3. Results

3.1. Effects of cytokinins on seed germination and organ development

The seeds started to germinate following the 4th day of culture. The control medium gave a germination rate of 71.11 ± 20.18%. The medium supplemented with 1.0 mg L^{-1} IP increased the germination rate, giving the maximum value for the parameter (91.11 ± 9.30%). The lowest germination rate (46.67 ± 9.30%) was found in seeds cultured in medium containing 1.0 mg L^{-1} TDZ. All BAP treatments induced statistically the same germination rates (Fig. 1A). A general view of the germinated seeds in culture is shown in Figure 2A.

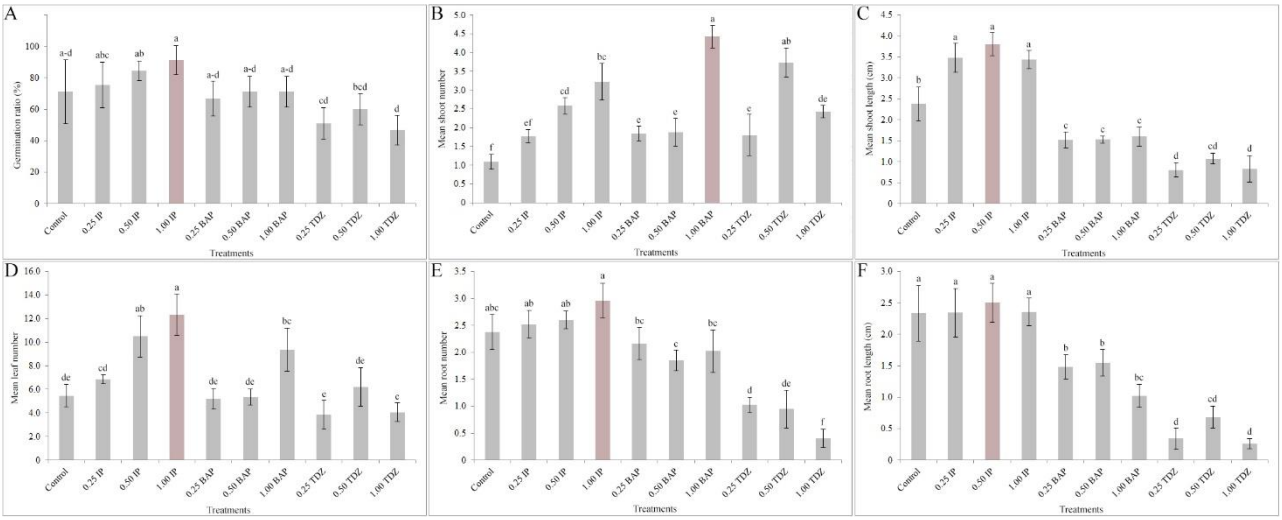

Figure 1. Effects of cytokinins on *in vitro* seed germination and organ development in *Antirrhinum majus* 'Magic Carpet' seedlings. The graphs in panels represent the germination ratio of the seeds (A), shoot number (B), shoot length (C), leaf number (D), root number (E), and root length (F) of the seedlings. Data represent mean ± SD. The means having the same superscript letters are not significantly different by Tukey's HSD test ($P < 0.05$). The bars in red colour indicate the maximum value for the parameter.

Figure 2. Appearance of *Antirrhinum majus* 'Magic Carpet' treated with plant growth regulators at different phases of *in vitro* propagation. The panels from A to E represent general views of seed germination (A), organ development in the control medium (B) and the effects of IP (C), BAP (D) and TDZ (E) at 1 mg L^{-1} on organ development in seedlings. The panels from F to J represent the general views of the culture at the shoot multiplication phase (F), organ development in the control medium (G), and the effects of cytokinins: IP (H), BAP (I), and TDZ (J) at 1 mg L^{-1} on shoot regeneration from nodal explants. The panels from K to M represent the effects of auxin treatments: CPA (K), IAA (L), and NAA (M) at 0.5 mg L^{-1} on rooting, and the acclimatized plants (N). The photographs were taken at the end of the incubation period. The bars in the panels correspond to 1 cm.

Shoot production greatly varied in response to different cytokinin treatments in a concentration-dependent manner. The control group had the lowest mean shoot number (1.09 ± 0.20) whereas BAP treatment at 1.0 mg L^{-1} significantly increased shoot production to the maximum value (4.42 ± 0.30). The IP treatments gradually increased the shoot production with increasing concentrations. The lowest concentration of TDZ and BAP treatments gave statistically the same results as that of BAP treatment at 0.50 mg L^{-1} concentration (Fig. 1B). Shoot

elongation was significantly triggered in the presence of IP in the medium. All the IP treatments gave statistically the same shoot lengths but at 0.50 mg L^{-1} IP treatment produced the most elongated shoots (3.80 ± 0.28 cm). Shoots from the control group had longer shoots (2.38 ± 0.41 cm) than those from the medium with BAP or TDZ. The shortest shoots (0.81 ± 0.16 cm) were produced in the presence of TDZ at 0.25 mg L^{-1}. All media with BAP supplement produced shoots at statistically the same length (Fig. 1C).

Leaf production exhibited a similar trend to shoot production (Fig. 1D). However, IP treatment at 1.0 mg L^{-1} gave the highest leaf number per shoot (12.33 ± 1.77). The control medium (5.47 ± 0.94), the 0.50 mg L^{-1} concentration of BAP and TDZ, and the lowest concentration of TDZ gave statistically the same results in leaf production. The medium with 0.25 mg L^{-1} TDZ gave limited leaf production and minimum value (3.82 ± 1.22).

Root production increased in the presence of IP in the culture medium (Fig. 1E). The maximum number of roots (2.96 ± 0.32) was produced in the medium with 1.0 mg L^{-1} IP whereas the control group gave 2.38 ± 0.32 roots per seedling. BAP and TDZ treatments decreased root production. TDZ treatment at 1.0 mg L^{-1} induced the minimum number of roots (0.40 ± 0.17). The lowest and medium concentrations of IP treatment gave statistically the same results. Cytokinin treatments affected root elongation, similar to root production (Fig. 1F). The IP treatments gave statistically the same results as the control. Maximum root elongation (2.50 ± 0.31 cm) was found in the medium supplemented with 0.50 mg L^{-1} while the control group had a mean root length of 2.33 ± 0.44 cm. Similar to root production, the TDZ treatments drastically decreased root elongation compared with the control. The shortest mean root length was recorded from plants developed in the medium with 1.0 mg L^{-1} TDZ. The visual developmental responses of the cultures to the cytokinin treatments are shown in Figure 2B-E.

3.2. Effects of cytokinins on shoot multiplication

The effects of the cytokinins used at 1.0 mg L^{-1} were also tested on shoot multiplication in nodal explants of *A. majus*. In the control group, each explant produced an average of 2.08 ± 0.14 shoots with a mean length of 4.30 ± 0.49 cm. The efficacy of TDZ treatment on shoot multiplication was superior to that of IP and BAP. BAP treatment induced 3.78 ± 0.67 shoots while the TDZ gave 10.04 ± 2.42 shoots per explant as the most efficient cytokinin treatment in shoot multiplication. Regarding shoot multiplication, the control, IP, and BAP treatments produced statistically the same results (Fig. 3A). However, the efficacy of TDZ was not recorded in the elongation of multiplied shoots. The TDZ treatment resulted in the shortest shoots (3.07 ± 1.09 cm). The BAP treatment increased the shoot lengths and gave the highest mean shoot length (5.99 ± 0.29 cm). Statistically, the IP and BAP treatments showed the same shoot elongation results (Fig. 3B). The multiple shoot induction responses of the nodal explants to the cytokinin treatments are shown in Figure 2F-J.

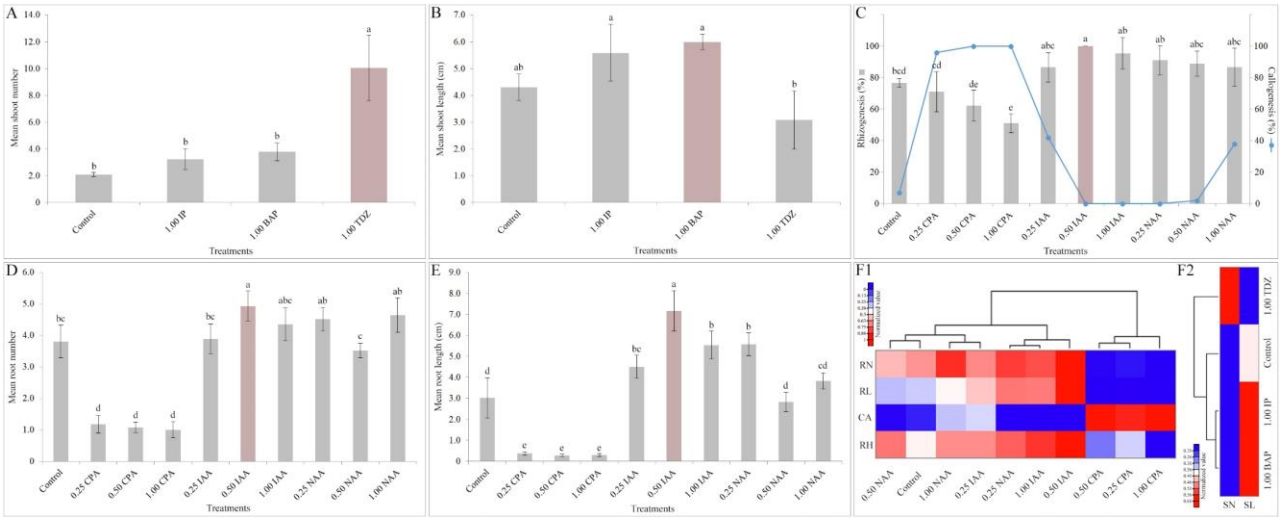

Figure 3. Effects of plant growth regulators on *in vitro* shoot multiplication, callogenesis, and rhizogenesis in *Antirrhinum majus* 'Magic Carpet' cultures. The graphs in panels A and B represent the effects of cytokinins on shoot formation from nodal explants (A) and the mean lengths of the shoots (B). The graphs in panels from C to E represent the effects of auxins on callus and root formation ratios (C), mean root numbers (D), and lengths of the roots (E). The heatmaps in panel F separately represent the similarities among the auxin treatments (F1) and cytokinin treatments (F2) based on their effects on the developmental parameters. Data represent mean ± SD. The means having the same superscript letters are not significantly different by Tukey's HSD test ($P < 0.05$). The bars in red colour indicate the maximum value for the parameter. RN: Root number, RL: Root length, CA: Callogenesis, RH: Rhizogenesis, SN: Shoot number, SL: Shoot length

3.3. Effects of auxins on rhizogenesis

The IAA and NAA treatments induced better rhizogenesis responses than the CPA treatments. A gradual decrease was observed in rooting at elevated concentrations of CPA. The highest rooting rate (100 ± 0.0%) was recorded from the plants cultured in the medium with 0.50 mg L^{-1} IAA, whereas the CPA treatment at 1.0 mg L^{-1} induced the minimum rooting response (51.11 ± 6.09%) (Fig. 3C). However, CPA treatments induced consistently higher callogenesis responses (96% and 100%) than the other auxins tested (38% and 42%). The higher concentrations of IAA than 0.25 mg L^{-1} and the lower concentrations of NAA than 1.0 mg L^{-1} did not induce any callus response (Fig. 3C). Better root production was recorded from plants

cultured in the presence of IAA and NAA. The roots produced in the presence of NAA were thicker than those cultured in the medium with IAA. The IAA treatment at 0.50 mg L^{-1} gave the highest number of roots (4.93 ± 0.48 per shoot). The control group had a mean root number of 3.80 ± 0.52 and a mean root length of 3.02 ± 0.97 (Fig. 3D). The IAA treatment also supported root elongation more than NAA and CPA. The maximum mean root length (7.16 ± 0.97 cm) was recorded from the medium with 0.50 mg L^{-1} IAA, whereas the CPA treatment at the same concentration gave the shortest mean root length (0.28 ± 0.07 cm) (Fig. 3E). The CPA treatments significantly decreased root development and induced statistically the same results in root production and elongation. The root production and elongation responses of the shoots to auxin treatments are depicted in Figure 2K-M. The appearance of the *in vitro*-propagated plants planted into the soil is shown in Figure 2N.

3.4. Hierarchical cluster analyses

The effects of cytokinin treatments on seeds and developmental parameters of seedlings were grouped into two main clusters (Fig. 4). All TDZ treatments and high-concentration BAP (1.0 mg L^{-1}) were placed into the same main cluster, while BAP treatments at 0.25 and 0.50 mg L^{-1}, IP treatment at 0.25 mg L^{-1}, and the control group were presented under the same main cluster. IP treatments at 0.25 and 0.50 mg L^{-1} were found as a distinct subcluster. The heatmap suggested that the optimum IP treatment was found superior over other cytokinin treatments tested except its effects on shoot number parameter.

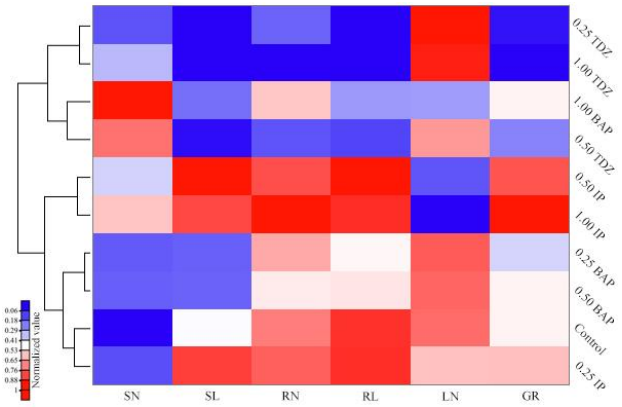

Figure 4. Similarities among the cytokinin treatments shown on a hierarchical clustering heatmap generated based on the normalized developmental data. SN: Shoot number, SL: Shoot length, RN: Root number, RL: Root length, LN: Leaf number, GR: Germination ratio

The influence of auxin treatments on root development and callogenesis from nodal explants were placed into two main clusters. The first cluster consisted of two main subclusters. The first main subcluster included NAA treatments at 0.50 and 1.0 mg L^{-1}, IAA treatments at 0.25 mg L^{-1} and the control group while NAA treatment at 0.25 mg L^{-1} and IAA treatments at 0.50 and 1.0 mg L^{-1} were placed into the other main subcluster. All CPA treatments were grouped into the same main cluster distant from the other treatments (Fig. 3F1). The heatmap showed the efficacy of optimal IAA treatments on rhizogenesis response while CPA treatments were different from other auxin treatments by their callogenesis-triggering effect.

In the shoot multiplication phase, the effects of the BAP and IP treatments were grouped into the same subcluster, whereas the TDZ treatment was shown in a distant cluster (Fig. 3F2).

4. Discussion

Biotechnological techniques, such as gene transfer, mutagenesis, and micropropagation are commonly used in the ornamental plant industry (Ochatt et al., 2022). Plant tissue culture techniques are required especially for the mass production of plants after their improvement through genetic manipulation (Jaiswal et al., 2022). In this context, PGRs are used to enhance the efficacy of *in vitro* production protocols of such plants by testing their effects on explants or calli in culture.

Among the PGRs tested in our study, IP and BAP are adenine-type cytokinins while TDZ is a phenylurea-type cytokinin (Arya et al., 2022). In plant tissue culture studies, BAP is a more commonly used cytokinin than IP and TDZ because of its enhancing activity on cell division and lateral branching in shoot multiplication (Phillips & Garda, 2019). However, their effects on *in vitro* seed germination and organ development may differ among species. BAP treatments did not result in statistically significant changes in our seed germination experiments compared with the control group. On the other hand, IP treatment increased the germination rate of the seeds as its concentration was elevated, whereas TDZ treatments suppressed germination regardless of their concentration. These results suggested that among adenine-type cytokinins, IP, in the culture medium can trigger germination. In contrast, phenylurea-type cytokinins should not be favored in the seed culture of *A. majus*. However, another phenylurea-type cytokinin, *N*-(2-chloro-4-pyridyl)-*N*'-phenylurea (CPPU), should also be tested on *A. majus* seed cultures to prove this hypothesis. The enhanced *in vitro* seed germination ratio has been previously reported for *Lotus corniculatus* in the presence of cytokinins in culture medium (Nikolić et al., 2006). The researchers found TDZ and ZTN as the most active cytokinins in seed germination while IP and KIN increased the germination ratio at higher concentrations. In line with our findings, Kim et al. (2019) reported that TDZ treatments above the optimal concentration (2 µM) decreased seed germination in *Pecteilis radiata*. Thus, even the lowest concentration of TDZ tested (0.25 mg L^{-1}) may be above a possible optimal concentration for *A. majus* seeds. However, TDZ treatments between 0.25 and 2.0 mg L^{-1} were found to increase seed germination in *Pancratium maritimum* (Redhwan et al., 2023). As a result of the literature review, we could not find any study comparing the effects of cytokinins on the *in vitro* germination of *A. majus* seeds. In a report, Kang and Choi (2006) reported higher germination rates in *A. majus* seeds when treated with BAP and gibberellic acid (GA_3) in combination. Furthermore, Silva et al. (2017) stated that the seeds of *A. majus* are small and usually have a low germination potential. Thus, various priming treatments have been conducted to enhance seed germination in *A. majus* (Kepczynski, 1979; Bhargava et al., 2015). Consequently, our findings indicated that a proper cytokinin treatment could increase the seed germination ratio in *A. majus* without needing any priming treatments.

The IP treatments also triggered organ development and gave the best results in shoot elongation, rhizogenesis,

and leaf production in the seedlings. The enhancing activity of BAP on organogenesis was limited to shoot production which is the only parameter for which BAP was superior to IP treatment. Therefore, the enhancing effect of IP on biomass production in *A. majus* is superior to that of BAP and TDZ treatments in terms of shoot development from germinating seeds. In the study of Stirk et al. (2005), it was shown that the concentrations of active cytokinins in germinating seeds of *Tagates minutia* may change in a time-dependent manner. The authors found that 80% of the total cytokinins in the germinating seeds 144 h after sowing were from the isopentenyladenine group, of which isopentenyladenine-9-glucoside was the most abundant cytokinin. In our study, a possible similar cytokinin composition in germinating seeds may explain IP's ability to trigger organ development in *A. majus*. However, further research is required to prove this hypothesis.

TDZ is an herbicide generally classified as a phenyl-urea with cytokinin-like effects (Erland et al., 2020). Nevertheless, it can have auxin- and cytokinin-like effects on various plant species; even though, its chemical formula is not closely related to those PGRs that are frequently used (Govindaraj, 2018). In *Rosa hybrida*, TDZ was found to reduce shoot length mainly by suppressing bioactive GA biosynthesis genes (Çelikel et al., 2021). Thus, the physiological mechanism behind the stunted shoot growth due to TDZ treatments in *A. majus* might involve a GA-dependent pathway similar to that in *R. hybrida* which should be further investigated. In *A. majus*, TDZ has not been tested on *in vitro* organ development from germinating seeds. However, Hoshino and Mii (1998) tested its effects at 1 and 5 mg L^{-1} on root growth and callus formation in transformed hairy root cultures of *A. majus*. The researchers found that 9 and 8 out of 10 explants treated with TDZ at the concentrations mentioned above formed green callus, respectively, but only 1 and 2 of them showed growth without any shoot formation in the culture. In our study, TDZ treatments significantly reduced root development in seedlings. TDZ treatment was found to induce root formation from hypocotyl and cotyledon explants of *Glycine max* when cultured in B5 medium (Gamborg et al., 1968). The same treatments failed to induce roots from the same explants when cultured in MS medium (Radhakrishnan et al., 2009). Thus, the culture medium can play a role in organ development in addition to the PGR treatments in *in vitro* cultures.

BAP has long been recognized as a bud-breaking agent in tissue culture studies (Mulwa & Bhalla, 2000; Al-Ali et al., 2023). However, BAP treatments at non-optimal concentrations may result in stunted growth of the seedling shoots. Similar results have been found for *Origanum vulgare* (Premi et al., 2021). The increase in leaf number after BAP treatment at 1.0 mg L^{-1} can be attributed to the higher shoot number induced by cytokinin. Exogenous cytokinin treatment decreases root development and lowers root meristem size without affecting stem-cell activity or cell division in the primary root meristem (Del Bianco et al., 2013) but low concentrations may enhance adventitious root formation (Arya et al., 2022). However, IP treatments did not inhibit root formation and elongation but BAP and TDZ treatments significantly reduced the parameters measured in our experiments on *A. majus*. Thus, isoprenoid group cytokinins, such as IP, may not inhibit or reduce *in vitro* root development in *A. majus* seedlings like aromatic cytokinins, such as BAP, and phenyl-urea type synthetic cytokinin analogs such as TDZ. A recent study focused on comparing the molecular effects of BAP and IP on modulating the molecular mechanism of shoot proliferation and root development in *Cymbopogon citratus* showed a different pattern of gene expression between BAP- and IP-treated plants. In brief, the researchers found that BAP treatment upregulates the genes related to shoot production and represses root development, whereas IP treatment activates genes related to root production and represses shoot proliferation (Cárdenas-Aquino et al., 2023). Likewise, our findings suggest that the effects of cytokinins on root development in *A. majus* may differ according to their chemical structures.

In the shoot multiplication phase, TDZ treatment induced the highest number of shoots from nodal explants in *A. majus* but the treatment failed to elongate the lateral shoots. The efficacy of TDZ in triggering multiple shoot formation has also been reported in *Vitex trifolia* (Ahmed & Anis, 2012), *Physalis peruviana* (Yücesan et al., 2015), and *Monstera deliciosa* (Sivanesan et al., 2023). To elucidate the efficacy of TDZ in multiple shoot formation in *A. majus*, the interaction of TDZ treatment with the synthesis of related phytohormones and molecular mechanisms should be investigated. In another study, KIN and BAP were tested in combinations on shoot multiplication in *A. majus*. The researchers found that KIN alone in the medium (0.5 mg L^{-1}) did not induce new shoot formation but gave slightly longer shoots than the control. In contrast, BAP alone in the medium at the same concentration slightly increased new shoot formation and decreased shoot length. Both the results of KIN treatment and the shoot formation result of BAP remained statistically the same with the control at the end of a 6-week period (Sheyab et al., 2010). Therefore, our results in the shoot multiplication phase indicated that shoot length can be increased in *A. majus* when the optimal concentration of BAP is reached. In a recent study in *A. majus*, Ahmed et al. (2022) found that 1.0 mg L^{-1} BAP treatment gave 3.2 and 3.4 shoots per nodal explant at the initiation and multiplication stages, respectively. The researchers also reported an increase of up to 7.2 and 10.2 shoots per nodal explant after 0.75 mg L^{-1} BAP treatment in these stages, respectively. In our experiments, 1.0 mg L^{-1} BAP treatment gave 3.78 shoots per explant in the shoot multiplication phase, whereas TDZ treatment gave 10.04 shoots per explant. These results suggest that cytokinin analogs and widely used cytokinins should also be tested on shoot multiplication in *A. majus*. Contrary to our report, Hamza et al. (2013) reported that BAP treatments at 0.5 and 1.0 mg L^{-1} gave higher shoot numbers from nodal explants in *A. majus* 'Apple Blossom'. However, the researchers also found that BAP and TDZ treatments reduced shoot lengths compared with the control. Shimasaki and Fukumoto (1998) also reported the stimulatory effects of BAP on adventitious shoot formation in 'Apple Blossom'. The cultivar difference may explain variations in the shoot production results in the literature related to *A. majus*. In this context, Newbury (1986) compared the multiplication rate of shoots of nine *A. majus* varieties cultured in MS medium fortified with BAP (0.5 and 1.0 mg L^{-1}), KIN (0.5 and 1.0 mg L^{-1}), and IBA (0.1 mg L^{-1} along with 0.5 mg L^{-1} BAP). The researcher reported that 1.0 mg L^{-1} BAP

treatment formed transferable shoots in all the varieties, whereas only four varieties responded to 0.5 mg L^{-1} BAP and BAP+IBA treatments and a single variety responded to the KIN treatments. Similarly, Cui et al. (2004) tested the effects of NAA and IAA along with ZTN on shoot regeneration frequency from hypocotyls and stem segments of six different *A. majus* cultivars. The researchers found that ZTN at 2.0 mg L^{-1} combined with 0.25 mg L^{-1} NAA gave higher shoot regeneration rates in all cultivars. Lee et al. (2023), who tested the effects of NAA and ZTN on shoot regeneration from hypocotyl explants of *A. majus* 'Maryland Apple Blossom', found ZTN at 2.0 and 2.5 mg L^{-1} as the best treatments to enhance shoot regeneration frequency, while the latter treatment produced the highest number of shoots per explant. The researchers also reported silver nitrate as a hyperhydricity-reducing agent in *A. majus* shoots regenerated from hypocotyl explants. Schroeder and Stimart (1999) also stated that genotype and PGR treatment duration can significantly affect adventitious shoot formation from the hypocotyls of *A. majus*. Thus, the effects of PGRs on organ formation in *A. majus* may vary depending on the cultivar.

In our study, the auxins IAA and NAA significantly triggered the rhizogenesis response. In contrast, CPA treatments did not increase rooting in *A. majus* shoots, and the control treatment was better than the CPA treatments in triggering rooting responses. However, CPA treatments resulted in great callusing at the basal ends of the shoots. Hasemi & Daneshvar (2016) found that the control group did not produce roots and reported IBA and NAA at 2.0 mg L^{-1} as the best auxin treatments for triggering the rooting response of *A. majus* shoots. The authors also found that IBA at 1.0 mg L^{-1} gave the highest root number (14.86), while NAA at 1.0 mg L^{-1} gave the maximum root length (5.16 cm). The same study found the maximum callus induction ratio after 2,4-D (1.0 mg L^{-1}) + BAP (0.1 mg L^{-1}) treatment in cotyledon explants of *A. majus*. In another study, Hamza et al. (2013) tested the effects of IAA, IBA, and NAA on rooting the shoots of *A. majus* 'Apple Blossom'. The researchers found IBA to be the best root formation-triggering auxin when used at 0.5 and 1.0 mg L^{-1} and they also reported that the treatments NAA at 0.5 mg L^{-1} and IBA at 1.0 mg L^{-1} gave the highest results for root production (12.83 roots) and elongation (4.58 cm) in *A. majus*, respectively. On the other hand, Sheyab et al. (2010) tested the effects of IAA, IBA, and NAA on the *in vitro* rooting of *A. majus*. They found IBA treatment at 1.2 mg L^{-1} and NAA treatment at 0.8 mg L^{-1} optimal for root production (15.78 roots) and elongation (6.07 cm), respectively. The effect of *in vitro* IBA treatment on rhizogenesis in shoots regenerated from nodes has also been shown by Ahmed et al. (2022) in *A. majus*. The authors reported that treatment at 0.6 mg L^{-1} gave the highest root number (8.8 roots) and length (10 cm). Gonzalez-Benito et al. (1996) found that the addition of auxins to the medium did not improve the rooting of *A. majus* ssp. *barrelieri*, *A. microphyllum,* and *A. majus* 'Floral Carpet' shoots. In our study, IBA treatments were not tested but IAA treatment reached a maximum root number of 4.93. Thus, IBA treatments may be more effective than IAA treatments on root production in *A. majus*. However, the IAA treatment tested in our study showed a better mean shoot length result in roots (7.16 cm) than the previous reports summarized above, except that of Ahmed et al. (2022). In the same study, the root length was indicated as the "highest root length", meaning that it is not the mean value but a single root among the samples. Contrary to the previous reports, Atkinson et al. (1991) tested the effects of IAA treatments on 5 different *A. majus* cultivars. The researchers reported a significant variation among the plant's root production and elongation results, depending not only on the concentration but also on the differences in cultivar, physical phase of the medium, and explant source. The maximum root number (14.7) was found in 'Victory' after the treatment at 11.4 µM (2.0 mg L^{-1}), whereas 'Wisley Golden Fleece' did not produce any roots at the same treatment. The authors reached the maximum root length (11.3 cm) in 'Yellow Freedom' after 0.28 µM (0.05 mg L^{-1}). Our results were reached on 'Magic Carpet' cultivar of *A. majus*, while a cultivar name was not mentioned in the reports of Sheyab et al. (2010), Hasemi & Daneshvar (2016), and Ahmed et al. (2022). Therefore, these variations in the results are cultivar-dependent.

Callus cultures have significant roles in green biotechnology such as supplying plant material for genetic modification studies in the production of ornamentals and establishing cell suspension cultures for sustainable and cost-effective production of phytochemicals (Efferth, 2019). CPA is not only an herbicide used in agriculture but also a PGR in tissue culture studies since it has auxin-like properties in plants. The main difference between CPA treatments and other auxins tested in our study was the strong effect of CPA on callus production. Xiaofang et al. (1992) stated the importance of NAA on callogenesis in *A. majus*. However, we found CPA more effective than NAA. The effectiveness of CPA treatment in callus growth has been reported from *Securinega suffruticosa* (Raj et al., 2015). Similar results on callus induction and growth have also been reported from *Ipomoea batatas* (Yugay et al., 2023). CPA is used specifically as a root growth inhibitor in mung beans during seed germination (Price, 1988). Accordingly, CPU treatments tested in our study decreased root production and drastically reduced root elongation in *A. majus*. A similar finding regarding the inhibition of root growth was also reported from *Oryza sativa* after the exogenous application of CPA (Wang et al., 2023). Therefore, we advise using CPA in *A. majus* 'Magic Carpet' cultures only for callus production but not for organ development.

5. Conclusion

The previous *in vitro* studies on *A. majus* discussed above mainly employed conventional PGRs. However, these reports do not include the use of two effective cytokinin and auxin, IP and CPU, respectively. Also, there needs to be a comprehensive study among previous reports testing the comparative effects of cytokinins on seed germination and organ development in seedlings of *A. majus*. In this concept, our study presents a holistic approach to *in vitro A. majus* propagation by comparatively testing the effects of cytokinins not only on seedling development but also on shoot multiplication and by comparing the effects of auxins on rhizogenesis and callogenesis. Furthermore, the differences between organ development results at the seedling growth and shoot multiplication phases in response to cytokinin treatments revealed that the cytokinin type and balance required by the plant may change in the different phases of *in vitro* propagation. Our

findings showed that using suitable cytokinins in *A. majus* can enhance seed germination rates without the need for priming treatments. Our study also demonstrated the efficacy of synthetic herbicides TDZ and CPA in shoot multiplication and callogenesis, respectively, in *A. majus*. These findings can be used to enhance commercial *A. majus* production in the ornamental horticulture industry and its propagation for future scientific studies.

Ethics committee approval: Ethics committee approval is not required for this study

Conflict of interest: The authors declare that there is no conflict of interest.

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References

Ahmed, M.R., & Anis, M. (2012). Role of TDZ in the quick regeneration of multiple shoots from nodal explant of *Vitex trifolia* L.—an important medicinal plant. *Applied Biochemistry and Biotechnology*, 168(5), 957-966. https://doi.org/10.1007/s12010-012-9799-0

Ahmed, Z.S., Salim, A.M., & Ahmed, H.N. (2022). Regeneration of *Antirrhinum majus* by nodes culture. *International Journal of Agricultural and Statistical Sciences*, 18(S1), 1487-1490.

Al-Ali, A.M., Dewir, Y.H., & Al-Obeed, R.S. (2023). Influence of cytokinins, dark incubation and air-lift bioreactor culture on axillary shoot proliferation of Al-Taif rose (*Rosa damascena trigintipetala* (Diek) R. Keller). *Horticulturae*, 9(10), 1109. https://doi.org/10.3390/horticulturae9101109

Arya, A., Sharma, V., Kumar Tyagia, P., Gola, D., & Husen, A. (2022). Role of cytokinins in adventitious root formation. In A. Husen (Ed.), *Environmental, physiological and chemical controls of adventitious rooting in cuttings* (pp. 239-249). Academic Press. https://doi.org/10.1016/B978-0-323-90636-4.00017-9

Atkinson, N.J., Newbury, H.J., & Ford-Lloyd, B.V. (1991). *In vitro* adventitious root induction in *Antirrhinum majus* L. *Plant Cell, Tissue and Organ Culture*, 27(1), 77-79. https://doi.org/10.1007/bf00048210

Bhargava, B., Gupta, Y.C., Dhiman, S.R., & Sharma, P. (2015). Effect of seed priming on germination, growth and flowering of snapdragon (*Antirrhinum majus* L.). *National Academy Science Letters*, 38(1), 81-85. https://doi.org/10.1007/s40009-014-0298-4

Cárdenas-Aquino, M.D., Camas-Reyes, A., Valencia-Lozano, E., López-Sánchez, L., Martínez-Antonio, A., & Cabrera-Ponce, J.L. (2023). The cytokinins BAP and 2-iP modulate different molecular mechanisms on shoot proliferation and root development in lemongrass (*Cymbopogon citratus*). *Plants*, 12(20), 3637. https://doi.org/10.3390/plants12203637

Cui, M., Takayanagi, K., & Handa, T. (2004). High frequency of shoot regeneration from hypocotyls and stem segments of *Antirrhinum majus* (Snapdragon). *Plant Cell, Tissue and Organ Culture*, 78(1), 51-53. https://doi.org/10.1023/b:ticu.0000020394.74766.af

Çelikel, F.G., Zhang, Q., Zhang, Y., Reid, M.S., & Jiang, C. (2021). A cytokinin analog thidiazuron suppresses shoot growth in potted rose plants via the gibberellic acid pathway. *Frontiers in Plant Science*, 12, 639717. https://doi.org/10.3389/fpls.2021.639717

Del Bianco, M., Giustini, L., & Sabatini, S. (2013). Spatiotemporal changes in the role of cytokinin during root development. *New Phytologist*, 199(2), 324-338. https://doi.org/10.1111/nph.12338

Efferth, T. (2019). Biotechnology applications of plant callus cultures. *Engineering*, 5(1), 50-59. https://doi.org/10.1016/j.eng.2018.11.006

Erland, L.A., Giebelhaus, R.T., Victor, J.M., Murch, S.J., & Saxena, P.K. (2020). The morphoregulatory role of thidiazuron: Metabolomics-guided hypothesis generation for mechanisms of activity. *Biomolecules*, 10(9), 1253. https://doi.org/10.3390/biom10091253

Gamborg, O., Miller, R., & Ojima, K. (1968). Nutrient requirements of suspension cultures of soybean root cells. *Experimental Cell Research*, 50(1), 151-158. https://doi.org/10.1016/0014-4827(68)90403-5

Gonzalez-Benito, M.E., Tapia, J., Rodriguez, N., & Iriondo, J.M. (1996). Micropropagation of commercial and wild genotypes of snapdragon (*Antirrhinum* spp.). *Journal of Horticultural Science*, 71(1), 11-15. https://doi.org/10.1080/14620316.1996.11515377

Govindaraj, S. (2018). Thidiazuron: a potent phytohormone for *in vitro* regeneration. In N. Ahmad & M. Faisal (Eds.), *Thidiazuron: From urea derivative to plant growth regulator* (pp. 393-418). Springer. https://doi.org/10.1007/978-981-10-8004-3_22

Hamza, A., Abd El-Kafie, O., Helaly, A., & EL-Mongy, M. (2013). *In vitro* propagation methodes of snapdragon (*Antirrhinum majus* L.) plant. *Journal of Plant Production*, 4(11), 1621-1637. https://doi.org/10.21608/jpp.2013.74484

Hasemi, M., & Daneshvar, M.H. (2016). Regeneration from callus which is produced from cotyledon of *Antirrhinum majus*. *Indo-American Journal of Agricultural and Veterinary Sciences*, 4(1), 20-24.

Hill, K., & Schaller, G.E. (2013). Enhancing plant regeneration in tissue culture. *Plant Signaling & Behavior*, 8(10), e25709. https://doi.org/10.4161/psb.25709

Hoshino, Y., & Mii, M. (1998). Bialaphos stimulates shoot regeneration from hairy roots of snapdragon (*Antirrhinum majus* L.) transformed by *Agrobacterium rhizogenes*. *Plant Cell Reports*, 17(4), 256-261. https://doi.org/10.1007/s002990050388

Jaiswal, J., Vikram Singh, A., Kumar Yadav, V., & Kumari, N. (2022). Plant tissue culture in tree species. In A. C. Rai, A. Kumar, A. Modi, & M. Singh (Eds.), *Advances in plant tissue culture: Current developments and future trends* (pp. 345-356). Academic Press. https://doi.org/10.1016/B978-0-323-90795-8.00020-5

Kang, J-S., & Choi, I. (2006). Effect of plant growth regulators and seed priming treatment on the germination and early growth of snapdragon (*Antirrhinum majus* L.). *Journal of Life Science*, 16(3), 493-499. https://doi.org/10.5352/jls.2006.16.3.493

Kepczynski, J. (1979). The improving of *Antirrhinum majus* seed germination. *Acta Horticulturae*, 91, 511-516. https://doi.org/10.17660/actahortic.1979.91.63

Kim, D., Kang, K., Enkhtaivan, G., Jan, U., & Sivanesan, I. (2019). Impact of activated charcoal, culture medium strength and thidiazuron on non-symbiotic *in vitro* seed germination of *Pecteilis radiata* (Thunb.) RAF. *South African Journal of Botany*, 124, 144-150. https://doi.org/10.1016/j.sajb.2019.04.015

Lee, J., Naing, A.H., Park, K.I., & Kim, C.K. (2023). Silver nitrate reduces hyperhydricity in shoots regenerated from the hypocotyl of snapdragon cv. Maryland Apple Blossom. *Scientia Horticulturae*, 308, 111593. https://doi.org/10.1016/j.scienta.2022.111593

Lewis, M., Stock, M., Maughan, T., Black, B., & Drost, D. (2021). Snapdragon Cut Flower Production in Utah. *Utah State Universiy Extension Publications, Paper 2187*. Published on the Internet; https://digitalcommons.usu.edu/extension_curall/2187 [Retrieved 05 January 2024].

Mehbub, H., Akter, A., Akter, M.A., Mandal, M.S., Hoque, M.A., Tuleja, M., & Mehraj, H. (2022). Tissue culture in ornamentals: Cultivation factors, propagation techniques, and its application. *Plants*, 11(23), 3208. https://doi.org/10.3390/plants11233208

Mizzotti, C., Galliani, B.M., & Masiero, S. (2014). The backstage of the ABC model: The *Antirrhinum majus* contribution. *Plant Biosystems*, 148(1), 176-186. https://doi.org/10.1080/11263504.2013.877531

Mulwa, R., & Bhalla, P. (2000). *In vitro* shoot multiplication of *Macadamia tetraphylla* L. Johnson. *The Journal of Horticultural Science and Biotechnology*, 75(1), 1-5. https://doi.org/10.1080/14620316.2000.11511192

Murashige, T., & Skoog, F. (1962). A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiologia Plantarum*, 15(3), 473-497. https://doi.org/10.1111/j.1399-3054.1962.tb08052.x

Newbury, H.J. (1986). Multiplication of *Antirrhinum majus* L. by shoot-tip culture. *Plant Cell, Tissue and Organ Culture*, 7(1), 39-42. https://doi.org/10.1007/bf00043919

Nikolić, R., Mitić, N., Miletić, R., & Nešković, M. (2006). Effects of cytokinins on *in vitro* seed germination and early seedling morphogenesis in *Lotus corniculatus* L. *Journal of Plant Growth Regulation*, 25(3), 187-194. https://doi.org/10.1007/s00344-005-0129-4

Ochatt, S.J., Beruto, M.I., Chan, M., Doyle Prestwich, B.M., Eimert, K., Lambardi, M., & Winkelmann, T. (2022). Biotechnology of ornamental plants: When beauty joins science—preface from the editors. *Plant Cell, Tissue and Organ Culture*, 149(3), 497-502. https://doi.org/10.1007/s11240-022-02330-4

Perrot-Rechenmann, C. (2010). Cellular responses to auxin: Division versus expansion. *Cold Spring Harbor Perspectives in Biology*, 2(5), a001446-a001446. https://doi.org/10.1101/cshperspect.a001446

Phillips, G.C., & Garda, M. (2019). Plant tissue culture media and practices: An overview. *In Vitro Cellular & Developmental Biology - Plant*, 55(3), 242-257. https://doi.org/10.1007/s11627-019-09983-5

POWO (2023), "*Antirrhinum majus* L." Plants of the World Online. *The Royal Botanic Gardens, Kew*. Published on the Internet; https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:799180-1 [Retrieved 05 January 2024].

Premi, N., Acemi, A., & Ozen, F. (2021). Cytokinin-like effects of chitosan on *in vitro* culture of *Origanum vulgare* L. *Italus Hortus*, 28(1), 100. https://doi.org/10.26353/j.itahort/2021.1.100108

Price, T. (1988). Seed sprout production for human consumption — A review. *Canadian Institute of Food Science and Technology Journal*, *21*(1), 57-65. https://doi.org/10.1016/s0315-5463(88)70718-x

Radhakrishnan, R., Ramachandran, A., & Ranjitha Kumari, B.D. (2009). Rooting and shooting: Dual function of thidiazuron in *in vitro* regeneration of soybean (*Glycine max*. L). *Acta Physiologiae Plantarum*, 31(6), 1213-1217. https://doi.org/10.1007/s11738-009-0356-6

Raj, D., Kokotkiewicz, A., Drys, A., & Luczkiewicz, M. (2015). Effect of plant growth regulators on the accumulation of indolizidine alkaloids in *Securinega suffruticosa* callus cultures. *Plant Cell, Tissue and Organ Culture*, 123(1), 39-45. https://doi.org/10.1007/s11240-015-0811-6

Redhwan, A., Acemi, A., & Özen, F. (2023). Effects of plant growth regulators on *in vitro* seed germination, organ development and callogenesis in *Pancratium maritimum* L. *Plant Cell, Tissue and Organ Culture*, 154(1), 97-110. https://doi.org/10.1007/s11240-023-02514-6

Ryan, M.C., Stucky, M., Wakefield, C., Melott, J.M., Akbani, R., Weinstein, J.N., & Broom, B.M. (2020). Interactive clustered heat map builder: An easy web-based tool for creating sophisticated clustered heat maps. *F1000Research*, *8*, 1750. https://doi.org/10.12688/f1000research.20590.2

Sangwan, R.S., & Harada, H. (1975). Chemical regulation of callus growth, organogenesis, plant regeneration, and somatic embryogenesis in *Antirrhinum majus* tissue and cell cultures. *Journal of Experimental Botany*, 26(6), 868-881. https://doi.org/10.1093/jxb/26.6.868

Saqallah, F.G., Hamed, W.M., Talib, W.H., Dianita, R., & Wahab, H.A. (2022). Antimicrobial activity and molecular docking screening of bioactive components of *Antirrhinum majus* (Snapdragon) aerial parts. *Heliyon*, 8(8), e10391. https://doi.org/10.1016/j.heliyon.2022.e10391

Schroeder, K.R., & Stimart, D.P. (1999). Adventitious shoot formation on excised hypocotyls of *Antirrhinum majus* L. (Snapdragon) *in vitro*. *HortScience*, 34(4), 736-739. https://doi.org/10.21273/hortsci.34.4.736

Seo, J., Lee, J., Yang, H.Y., & Ju, J. (2020). *Antirrhinum majus* L. flower extract inhibits cell growth and metastatic properties in human colon and lung cancer cell lines. *Food Science & Nutrition*, 8(11), 6259-6268. https://doi.org/10.1002/fsn3.1924

Sheyab, S., Shatnawi, M.A., Shibli, R.A., Obeidat, M., Al-Shadaideh, A.N., Alhussaen, K.M., Abu-Zahra, T. (2010). Micropropagation and medium term conservation of *Antirrhinum majus* L. *Jordan Journal of Agricultural Sciences*, 6(2), 171-182.

Shimasaki, K., & Fukumoto, Y. (1998). Effects of B vitamins and benzylaminopurine on adventitious shoot formation from hypocotyl segments of snapdragon (*Antirrhinum majus* L.). *Plant Biotechnology*, 15(4), 239-240. https://doi.org/10.5511/plantbiotechnology.15.239

Silva, V.N., Dotto, L., Hajar, A.D., Bittencourt, K.C., & Stella, M.R. (2017). Production of *Antirrhinum majus* seedlings on different substrates and containers. *Científica*, 45(2), 169. https://doi.org/10.15361/1984-5529.2017v45n2p169-174

Sivanesan, I., Lee, Y.K., Kang, K.W., & Park, H.Y. (2023). Micropropagation of *Monstera deliciosa* Liebm. 'Thai Constellation'. *Propagation of Ornamental Plants*, 23(2), 31-38.

Stefaniak, A., & Grzeszczuk, M.E. (2019). Nutritional and biological value of five edible flower species. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, 47(1), 128-134. https://doi.org/10.15835/nbha47111136

Stirk, W.A., Gold, J.D., Novák, O., Strnad, M., & Staden, J.V. (2005). Changes in endogenous cytokinins during germination and seedling establishment of *Tagetes minuta* L. *Plant Growth Regulation*, 47(1), 1-7. https://doi.org/10.1007/s10725-005-1767-z

Wang, W., Rui, H., Yu, L., Jin, N., Liu, W., Guo, C., Cheng, Y., & Lou, Y. (2023). Four-chlorophenoxyacetic acid treatment induces the defense resistance of rice to white-backed planthopper *Sogatella furcifera*. *International Journal of Molecular Sciences*, 24(21), 15722. https://doi.org/10.3390/ijms242115722

Xiaofang, Z., Yafen, X., & Hongwei, L. (1992). Regulation of phytohormons on the regeneration of shoot-tips of *Antirrhinum majus* into plantlet *in vitro*. *Journal of Northeast Forestry University*, 3(1), 16-22. https://doi.org/10.1007/bf02874882

Yugay, Y.A., Grishchenko, O.V., Vasyutkina, E.A., Grigorchuk, V.P., Chukhlomina, E.N., Tsydeneshieva, Z.L., Kudinova, O.D., Yaroshenko, Y.L., Degtyarenko, A.I., Subbotin, E.P., Bulgakov, V.P., Kulchin, Y.N., & Shkryl, Y.N. (2023). Influence of growth regulators and different spectra of monochromatic radiation on the growth and biosynthetic characteristics of callus culture of *Ipomoea batatas* (L.) Lam. *Russian Journal of Plant Physiology*, 70(7). https://doi.org/10.1134/s1021443723603105

Yücesan, B.B., Mohammed, A., Arslan, M., & Gürel, E. (2015). Clonal propagation and synthetic seed production from nodal segments of Cape gooseberry (*Physalis peruviana* L.), a tropical fruit plant. *Turkish Journal of Agriculture And Forestry*, 39, 797-806. https://doi.org/10.3906/tar-1412-86