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INFLUENCE OF PLANT REGULATORS ON THE MICROPROPAGATION OF *Echinacea purpurea* 'RASPBERRY TRUFFLE'

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ABSTRACT

A micropropagation protocol was conducted for *Echinacea purpurea* 'Raspberry Truffle' to determine the influence of medium amendment with a plant growth regulator (PGR). The efficacy of meta-Topolin (mT) and benzyladenine (BA) was evaluated during the proliferation stages. Additionally, indole-3-butyric acid (IBA) and naphthaleneacetic acid (NAA) were assessed during the rooting stages. Multiple shoots were initiated and proliferated on a Murashige and Skoog (MS) medium and supplemented with 1 mL·L^{-1} of a Plant Preservative Mixture (PPM) and selected plant growth regulators with concentrations of 0.5, 1 and 2 mg·L^{-1} for shoot proliferation; and 0.05, 0.1, 0.5 and 1 mg·L^{-1}, for rooting performance. The combination of mT and BA in the medium significantly enhanced shoot regeneration and elongation. Both, mT and BA resulted in 100% shoot regeneration. mT at a concentration of 0.5 mg·L^{-1} in the MS medium induced the maximum number of shoots, followed by 0.5 mg·L^{-1} BA. The supplementation of 0.05 mg·L^{-1} and 1 mg·L^{-1} IBA, and 0.1 NAA mg·L^{-1} resulted in a 100% root percentage with the highest number of roots found in the media amended with 1 mg·L^{-1} IBA and 0.1 mg·L^{-1} NAA.

Keywords: cytokinin, auxin, *in vitro*, purple coneflower, proliferation, rooting

INTRODUCTION

Echinacea purpurea, commonly known as purple coneflower, is a perennial herb from the Asteraceae family, celebrated for its medicinal qualities and ornamental appeal. Among the many cultivars of *Echinacea purpurea*, 'Raspberry Truffle' stands out due to its desirable traits, including an upright growth habit, robust basal branching, sturdy stems and peduncles, prolific flowering, and large anemone-like inflorescences [Armitage 2008]. This cultivar has gained significant recognition among horticulturists and herbal enthusiasts, triggering a growing interest in developing effective propagation methods to satisfy the demand for 'Raspberry Truffle' in both commercial and home gardening.

Despite the increasing interest in the micropropagation of Echinacea species for both commercial and conservation efforts, there is a dearth of detailed data on the optimization of propagation protocols, particularly for specific cultivars, like 'Raspberry Truffle'. To address this gap, a tissue culture-based micropropagation experiment was carried out with this cultivar. Micropropagation, a method that involves cultivating plants from small tissue explants under sterile conditions, offers significant advantages over traditional propagation techniques, such as seed germination or the division of mature plants [Ashraf et al. 2014]. This approach enables the rapid production of large numbers of genetically-uniform plants with desirable traits, en-

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suring consistency in attributes like flower color, size, and resistance to diseases. However, the effectiveness of micropropagation protocols is heavily influenced by the choice and optimization of growth regulators, which are essential in controlling key physiological processes, including cell division, elongation, and differentiation [Ault 2007].

Combinations of plant growth regulators (PGR) have been used to promote the growth of purple coneflower species during propagation. For instance, an auxin and cytokinin mixture, such as NAA and BAP, was supplemented to the medium where the leaf explants were cultured to induce the callus and shoot performance. Medium amendment with low concentrations of NA and higher BAP concentrations enabled the development of the adventitious shoot. Although the basic medium without a regulator or supplemented with IBA or IAA will produce roots, various auxin concentrations were applied to boost rooting performance, [Nazir et al. 2022]. However, a high concentration of auxin has been reported to exert an inhibiting effect [Lakshmanan et al. 2002].

An efficient *in vitro* propagation culture system for purple coneflower where adventitious buds were first regenerated from leaf, petiole, and root explants from aseptic seedlings was the one on the MS medium with 0.3 mg·L^{-1} BA and 0.01 mg/L NAA. These adventitious buds were then rooted and grown into more mature plantlets on the MS medium with 0.01 mg·L^{-1} NAA to provide more explant materials. Within a year, this system produced at least one million transplant-ready plantlets [Dahanayake et al. 2011]. In recent years, the popularity of topolins as naturally occurring aromatic compounds has led to their increased usage as an effective alternative for other types of cytokinins in a micropropagation procedure. Among them, meta-topolin is the most popular one and its use in plant tissue culture has amplified swiftly. Its effectiveness in micropropagation, alleviation of various physiological disorders, rooting, and acclimatization of tissue culture-raised plants has been extensively studied over the past few decades [Gantait and Mitra 2021].

The optimal concentration of mT and an aromatic cytokinin, like BAP, in the micropropagation of sweet basil (*Ocimum basilicum* L.) was determined by analyzing plant growth parameters. The results demonstrate that 1 mg·L^{-1} BAP (4.43 M) and 0.5 mg·L^{-1} mT (2.07 M) had the most beneficial effects on new shoot development. Meta-Topolin was shown to improve plant quality and minimize distortions compared to BAP [Kőszeghi et al. 2014].

This study focused on evaluating the influence of PGRs with different concentrations in the micropropagation of *Echinacea purpurea* 'Raspberry Truffle'. The goal was to determine the optimal concentrations of these PGRs, including natural and synthetic auxins and cytokinins, for shoot proliferation and root formation, that would ultimately contribute to the development of an efficient and reliable micropropagation protocol for this valuable medicinal plant.

MATERIAL AND METHODS

The plant material was collected from stabilized cultures of *Echinacea purpurea* 'Raspberry Truffle', procured from an *in vitro* laboratory of the Ornamental Plant Section, Faculty of Horticulture, Warsaw University of Life Sciences, Poland. Apical parts were taken from established plants, with the leaves cut off as the starting explants for *in vitro* experiments. All explants were put on the basic medium used in this research; MS (Murashige and Skoog, 1962), with 30 g·L^{-1} sugar, 8 g·L^{-1} agar, and 1 ml·L^{-1}, and supplemented with 1 mL·L^{-1} of a Plant Preservative Mixture (PPM), BA and mT for multiplication stages and IBA and NAA for rooting stages. The pH of the media was adjusted to 5.8 with HCl and NaOH, before autoclaving. Jars (250 mL) filled with 50 mL of the medium were closed with transparent plastic covers and autoclaved at 121 °C and 110 kPa for 18 minutes. Afterwards, the jars were transferred into the phytotron under 22 ±1 °C, 16-hday with the light of 35 mmol m^{-2} s^{-1} PPF, provided by fluorescent lamps.

Shoot proliferation stage

A shoot proliferation experiment was carried out following the inoculation of approximately 1-cm apical shoots of the explant in plant growth regulator (PGR)-free MS medium (served as a control) and MS media supplemented with cytokinin; BA and mT in various concentrations (0.5, 1 and 2 mg·L^{-1}) and inoculated in jars (250 mL capacity, filled with 50 mL of the basic culture medium) under aseptic conditions in a laminar low chamber. The shoot proliferation expe-

Table 1. Effects of different concentrations of cytokinin mT on shoot proliferation of *Echinacea purpurea* 'Raspberry Truffle'

mT concentration ($mg \cdot L^{-1}$)	Shoot regeneration (%)	Average number of shoots	Average length of shoots (cm)
0	88.3[a]	3.13[a]	3.23[b]
0.5	100[b]	6.76[c]	3.0[b]
1	100[b]	4.3[b]	2.04[a]
2	100[b]	3.88[ab]	1.7[a]

* Means followed by the same letter do not differ significantly at $\alpha = 0.05$

riment consisted of 8 jars with 5 plants each. The percentage of regenerating explants, the number of regenerated shoots, and their length were evaluated after four weeks. After all the data had been recorded, all explants were transplanted into the rooting medium.

Rooting stage

A root induction experiment was performed via inoculating 4-week *in vitro* shoots approximately 3 cm in length in the MS medium as control and the MS medium amended with IBA and NAA in various concentrations (0.05 $mg \cdot L^{-1}$, 0.1 $mg \cdot L^{-1}$, 0.5 $mg \cdot L^{-1}$) for rooting evaluation. This stage consisted of 8 jars with 5 plants each. The percentage and number of rooted explants were determined four weeks after transplanting the explants.

Data analysis

All results were subjected to a one-way analysis of variance (ANOVA) using Statgraphics plus 4.1, and Duncan's *t*-test was used to compare the mean values obtained. Differences were found significant at $\alpha = 0.05$.

RESULTS

Effects of different concentrations of cytokinin mT on *in vitro* shoot proliferation of *Echinacea purpurea* 'Raspberry Truffle'

The observation of shoot formation on explants revealed that the growing medium amendment with mT significantly influenced shoot regeneration. The percentage of regenerating explants, lengths of regenerated shoots, and the number of shoots per explant were evaluated four weeks after placing the explants on the medium. As shown in Table 1, MS medium supplemented with mT resulted in 100% regeneration across all treatments, with no significant differences observed among various concentrations, indicating that the lowest concentration tested (0.5 $mg \cdot L^{-1}$) was as effective as the higher concentrations in promoting regeneration. Additionally, the number of shoots per explant was significantly higher with 0.5 $mg \cdot L^{-1}$ mT, which led to a 50% increase compared to the control group. However, the higher concentrations of mT tested resulted in shorter shoots, as the control group recorded the greatest average shoot length (3.23 cm). This finding suggests that, while mT promotes shoot regeneration, it may also inhibit elongation when used at higher concentrations. The above results indicate that mT is effective in promoting shoot proliferation in *Echinacea purpurea* 'Raspberry Truffle', but further optimization is needed to balance shoot number and elongation.

Effects of different concentrations of cytokinin BA on *in vitro* shoot proliferation of *Echinacea purpurea* 'Raspberry Truffle'

The addition of BA to the growth medium exerted significant effects on the in vitro proliferation of *Echinacea purpurea* 'Raspberry Truffle'. The MS medium supplemented with BA resulted in 100% regeneration, similar to the findings with mT, with no significant differences observed among the various BA concentrations. As shown in Table 2, the number of shoots per explant showed significant variations, with the 2 $mg \cdot L^{-1}$ BA group recording the lowest number (2.4 shoots per explant), which did not differ significantly from the control (3 shoots per explant). In contrast, medium amendment with 0.5 mg/L BA

Table 2. Effects of different concentrations of cytokinin BA on shoot proliferation of *Echinacea purpurea* 'Raspberry Truffle'

BA concentration ($mg \cdot L^{-1}$)	Shoot regeneration (%)	Average number of shoots	Average length of shoots (cm)
0	88.3[a]	3.0[a]	3.23[c]
0.5	100[b]	5.4[c]	2.0[b]
1	100[b]	4.0[b]	1.9[b]
2	100[b]	2.4[a]	1.27[a]

* Means followed by the same letter do not differ significantly at $\alpha = 0.05$

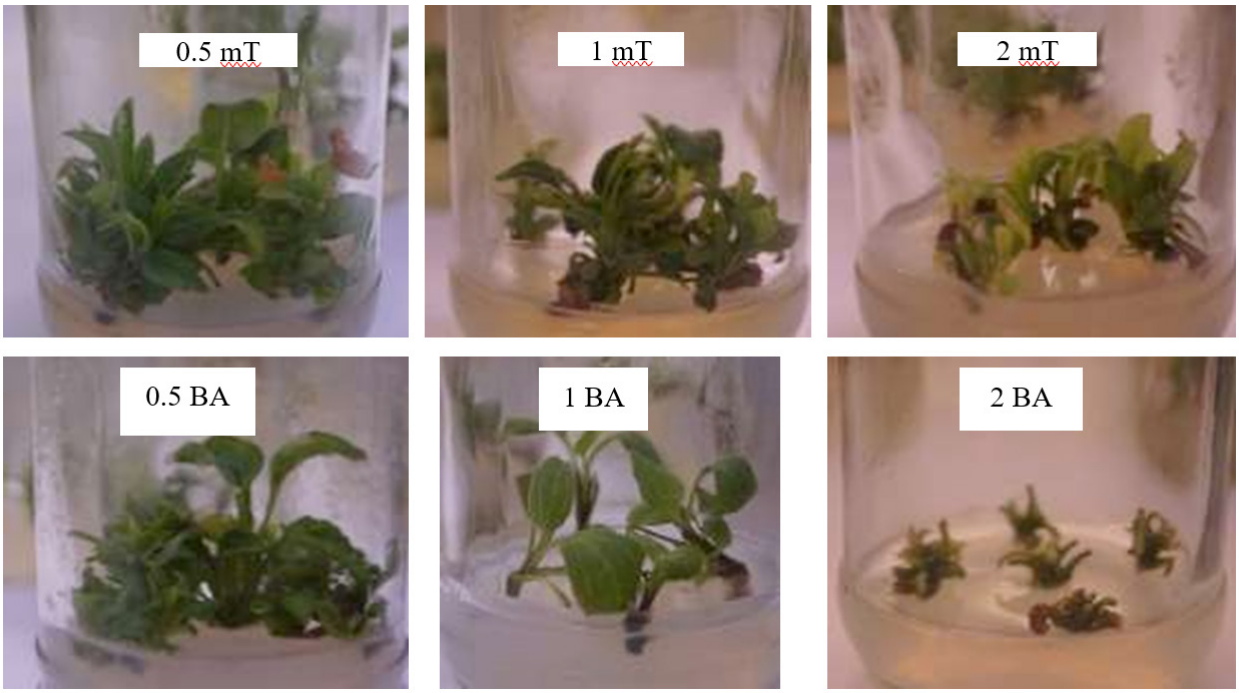

Fig. 1. *In vitro* shoot regeneration on *Echinacea purpurea* 'Raspberry Truffle' explant. mT – meta-Topolin, BA – benzyladenine

increased the shoot number compared to the control, indicating its effectiveness in enhancing shoot proliferation. The length of shoots was also significantly influenced by BA concentrations, with the control group exhibiting the longest average shoot length (3.23 cm), and shorter roots recorded with increasing BA concentrations. These results highlight a potential trade-off between shoot number and length when using BA. The findings suggest that BA is effective in promoting shoot regeneration and proliferation but may require careful concentration adjustments to optimize shoot characteristics.

Figure 1 shows the appearance of shoot regeneration at each concentration of mT and BA given. It can be seen that the 0.5 $mg \cdot L^{-1}$ concentration of both cytokinins produced more regenerated shoots compared with other concentrations.

Effects of different concentrations of auxin IBA on *in vitro* root regeneration of *Echinacea purpurea* 'Raspberry Truffle'

The application of IBA in varying concentrations had a significant impact on root regeneration in *Echinacea purpurea* 'Raspberry Truffle'. As shown in

Table 3. Effects of different concentrations of auxin IBA on root regeneration of *Echinacea purpurea* 'Raspberry Truffle'

IBA Concentration (mg·L^{-1})	Root regeneration (%)	Average number of roots
0	23.33^{a}	1^{a}
0.05	100^{c}	2.44^{ab}
0.5	88.89^{bc}	2.11^{a}
0.1	44.44^{ab}	1.56^{a}
1	100^{c}	4.22^{b}

* Means followed by the same letter do not differ significantly at α = 0.05

Table 4. Effects of different concentrations of auxin NAA on root regeneration of *Echinacea purpurea* 'Raspberry Truffle'

NAA concentration (mg·L^{-1})	Root regeneration (%)	Average number of roots
0	23.33^{a}	1^{a}
0.05	88.89^{bc}	2.89^{bc}
0.5	55.56^{ab}	1.33^{ab}
0.1	100^{c}	3.56^{c}
1	88.89^{bc}	2.78^{bc}

* Means followed by the same letter do not differ significantly at α = 0.05

Table 3, in the non-amended MS medium (0 mg·L^{-1} IBA), root regeneration was minimal, with only 23.33% of the explants showing any signs of root development. However, as the concentration of IBA increased to 0.05 mg·L^{-1}, root regeneration boosted to 100%, indicating that even low levels of IBA can effectively stimulate root formation. This trend continued, albeit with some fluctuations in efficiency, i.e., regeneration rate decrease to 88.89% with IBA concentration increasing to 0.5 mg·L^{-1}.A further reduction in the regeneration rate was observed at 0.1 mg·L^{-1}, where only 44.44% of the explants exhibited root regeneration. Despite this decline, the overall findings indicate that IBA is effective in promoting root development, particularly at lower concentrations. Furthermore, the number of roots formed per explant was the highest at 1 mg·L^{-1}, reaching an average of 4.22 roots. This finding suggests that, while higher IBA concentrations may lead to lower regeneration percentages, they can enhance the number of roots per explant, making IBA a viable option for optimizing root regeneration in tissue culture.

Effects of different concentrations of auxin NAA on *in vitro* root tegeneration of *Echinacea purpurea* 'Raspberry Truffle'

NAA exerted a pronounced effect on root regeneration in *Echinacea purpurea* 'Raspberry Truffle', with varying concentrations leading to significant differences in both the percentage of root regeneration and the number of roots formed, as shown in Table 4. Likewise with IBA, at 0 mg·L^{-1} NAA, the root regeneration was low, with only 23.33% of the explants regenerating roots. However, when its concentration was increased to 0.05 mg·L^{-1}, there was a notable improvement, producing a remarkable 88.89% regeneration rate. The optimal concentration for root regeneration was observed at 0.1 mg·L–1, where 100% of the explants showed successful root formation. At this concentration, the number of roots per explant also peaked to 3.56 roots on average, indicating NAA's efficacy in enhancing both the percentage and quantity of root regeneration. In comparison to IBA, NAA was particularly effective at lower concentrations, ensuring higher regeneration rates without fluctuations

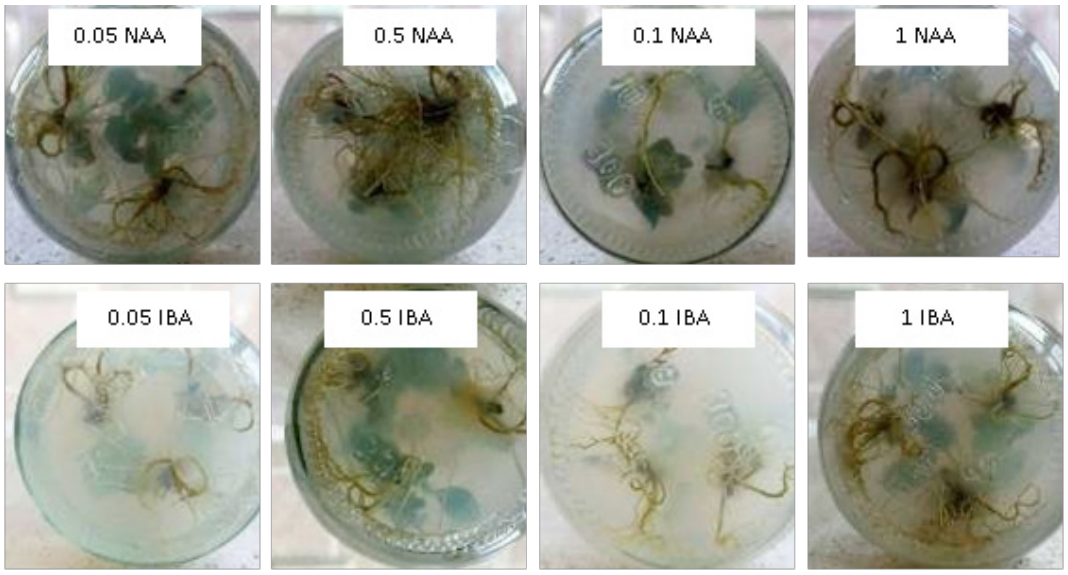

Fig. 2. *In vitro* root regeneration on *Echinacea purpurea* 'Raspberry Truffle' explant. IBA – indole-3-butyric acid, NAA – naphthaleneacetic acid

observed in IBA's performance (Figure 2). These results underscore the potential of NAA as a key regulator in tissue culture protocols for promoting robust root development in *Echinacea purpurea* 'Raspberry Truffle'.

DISSCUSION

Effects of cytokinins on shoot proliferation of *Echinacea purpurea* 'Raspberry Truffle'

Cytokinins are essential in plant tissue culture, particularly in regulating cell division, differentiation, and shoot proliferation. This study highlights the significant role of cytokinins, specifically BA and mT, in enhancing the *in vitro* proliferation of *Echinacea purpurea* 'Raspberry Truffle'. The findings align with the established understanding that cytokinins promote cell division in the shoot apical meristem, which is crucial for shoot regeneration and proliferation [George et al. 2008, Preece and Read 1993].

A notable outcome of this study is that growing medium amendment with both BA and mT, at a concentration of 0.5 mg·L^{-1}, produced in the highest number of shoots per explant, with a 100% regeneration rate. This underscores the effectiveness of these cytokinins in inducing shoot proliferation even at relatively low concentrations. The superior performance of mT at 0.5 mg·L^{-1} may be attributed to its natural origin, which allows it to more closely mimic endogenous cytokinins compared to the synthetic variants, like BA [Amoo et al. 2011]. Meta-Topolin, a derivative of zeatin, has been reported to have a higher affinity for cytokinin receptors in plant cells, which leads to more effective stimulation of cell division and shoot initiation [Strnad 2021, Werbrouck et al. 1996, Demirci 2022].

The comparable performance of BA at the same concentration suggests that, despite being synthetic, it effectively activates cytokinin signaling pathways essential for shoot proliferation. BA is a widely used cytokinin in plant tissue culture, known for activating cytokinin-responsive genes that promote cell cycle progression, particularly the transition from the G1 to S phase, which is indispensable for cell division [Cortleven et al. 2019, Howell et al. 2003]. The fact that both BA and mT produced similar results highlights their capability to modulate the hormonal balance within the explants, optimizing conditions for shoot regeneration.

The study also uncovers an interesting concentration-dependent effect, where an increase in the concentration of both BA and mT to 2 mg·L^{-1} resulted in reduced shoot proliferation. This finding aligns with

the hormesis theory, which posits that while low doses of a substance can promote biological processes, its higher doses might inhibit them [Calabrese and Baldwin 2002, Jones and Green 2019].

This inhibition observed at higher cytokinin concentrations could also be linked to the interaction between cytokinins and other plant hormones, particularly auxins. The balance between cytokinins and auxins is crucial for regulating various aspects of plant growth and development, including shoot initiation and elongation [Sakakibara 2006]. An excess of cytokinins might upset this balance, leading to reduced shoot elongation and overall shoot proliferation. The observation that shoot length was not significantly affected by cytokinin application suggests that, while they are effective in promoting shoot initiation, they may not substantially influence the elongation phase [Werner et al. 2001, Tyub et al. 2021].

Furthermore, the findings from this study enhance our comprehension of the physiological and molecular processes that drive cytokinin-induced shoot proliferation. Cytokinins are recognized for their role in modulating the expression of crucial genes, such as CYCD3 and ARR (Arabidopsis Response Regulators), which are vital in the regulation of the plant cell cycle [Riou-Khamlichi et al. 1999, To et al. 2004, Trinh et al. 2023]. When activated by cytokinins, these genes increase the number of cells within the meristematic tissue, thereby encouraging new shoot formation. The differing impacts observed at various cytokinin concentrations are likely linked to the intricate regulatory networks governing cytokinin biosynthesis, signaling, and degradation [Hwang et al. 2012].

The application of cytokinins in tissue culture is not only about enhancing shoot proliferation but also about maintaining the quality and physiological stability of regenerated plants. Cytokinin-induced shoots must retain their ability to grow and develop normally when transferred to soil or other growth conditions. High cytokinin concentrations, while initially promoting shoot formation, may lead to physiological abnormalities, such as hyperhydricity, characterized by excessive water accumulation in tissues, resulting in poor plant quality [Debergh et al. 1992]. The lower shoot proliferation at 2 $mg \cdot L^{-1}$ concentrations of BA and mT observed in this study may partly be due to such physiological stress factors, which can negatively impact the overall growth and development of the shoots.

The present study's results are consistent with earlier research on the role of cytokinins in plant tissue culture. For instance, investigations into species like Eucalyptus and Populus have demonstrated that the optimal concentration of cytokinins is species-specific and dependent on the particular physiological requirements of the plants [Coleman and Ernst 1989, Nakhoda 2011]. Identifying 0.5 $mg \cdot L^{-1}$ as the optimal concentration for *Echinacea purpurea* 'Raspberry Truffle' underscores the importance of species-specific optimization in tissue culture protocols. This finding is critical for developing efficient micropropagation techniques, particularly for medicinal plants like *Echinacea purpurea*, which hold significant economic and therapeutic value [Burlou-Nagy et al. 2022].

In addition to promoting shoot proliferation, cytokinins influence other aspects of plant development, such as leaf formation, chloroplast differentiation, and delaying senescence [Rashotte 2013, Wu et al. 2021]. Their use in the tissue culture of *Echinacea purpurea* 'Raspberry Truffle' may have broader implications for the overall health and vigor of regenerated plants. For instance, cytokinin-induced delay of senescence could enhance the longevity and productivity of the plants, which is particularly important for commercial cultivation [Choffe 2000].

Effects of auxins on root regeneration of *Echinacea purpurea* 'Raspberry Truffle'

Root formation and development in plants are heavily influenced by auxins. This research examined how different concentrations of auxins, particularly IBA and NAA, affected the *in vitro* rooting of *Echinacea purpurea* 'Raspberry Truffle'. The results showed that IBA was generally more effective than NAA in promoting root formation, with significant rooting percentages observed even at low IBA concentrations. These findings align with the established role of auxins in facilitating cell elongation, differentiation, and the initiation of adventitious roots [Taiz and Zeiger 2010, Choffe 2000].

IBA's performance in this study may be attributed to its stability and efficiency in inducing root initiation. Unlike other auxins, such as indole-3-acetic acid (IAA), IBA is more stable and less susceptible to de-

gradation by light or enzymes, making it particularly effective in tissue culture applications [Nissen and Sutter 1990]. Its ability to induce high rooting percentages at low concentrations (0.05 mg·L^{-1}) suggests it effectively activates auxin signaling pathways crucial for root development. Auxin signaling involves the hormone's perception by specific receptors, leading to downstream gene expression that promotes root primordia formation [Ljung 2013].

The molecular mechanisms underlying auxin-induced root formation involve a complex interplay between auxin transport, perception, and signaling. Auxins are transported within plants through polar auxin transport, mediated by auxin efflux carriers like PIN proteins [Petrásek and Friml 2009, Gao et al. 2024]. The localization and activity of these carriers are critical for establishing auxin gradients that determine root initiation sites. Due to its structural similarity to IAA, IBA is likely efficiently transported within plant tissues, establishing optimal auxin concentrations at root formation sites. This efficient transport and localization may explain why IBA outperforms NAA, which might be less efficiently transported or metabolized within the plant.

The present study's findings also suggest that IBA may be more effective than NAA in modulating the expression of key genes involved in root initiation. Auxin-responsive genes, such as ARF (Auxin Response Factor) and GH3 (Gretchen Hagen 3), are essential in mediating auxins' effects on root development [Guilfoyle and Hagen 2007]. Their activation by IBA could lead to more efficient root primordia initiation and development, resulting in higher rooting percentages. In contrast, NAA, despite being a potent synthetic auxin, may not activate these pathways as effectively, leading to lower rooting efficiency.

The study also highlighted the importance of optimizing auxin levels for successful root formation, as both IBA and NAA exhibited concentration -dependent effects. While low IBA concentrations (0.05 mg·L^{-1}) were sufficient to induce significant rooting, higher concentrations (1 mg·L^{-1}) of both IBA and NAA resulted in 100% root formation. This finding suggests that auxin concentration becomes a critical factor beyond a certain threshold in ensuring successful rooting. Higher auxin concentrations may enhance root primordia differentiation and increase the number of roots per explant, as observed in this study. However, balancing auxin concentration is essential to avoid potential negative effects like excessive root elongation or abnormal root morphology, which are likely at very high auxin levels [Teale et al. 2006, Zhang et al. 2022].

Additionally, the study found differences in root length between control and treated explants, indicating that while auxins are crucial for root initiation, they may not significantly influence subsequent root elongation. The greatest average root length of the control explants suggests that endogenous auxin levels within the plant may be sufficient for root elongation once roots are initiated. This observation supports the idea that auxin concentration gradients, rather than absolute levels, are more critical in regulating root elongation [Adamowski and Friml 2015]. Excessive exogenous auxin application might disrupt these gradients, leading to altered root elongation patterns.

The study also provides valuable insights into the physiological responses of *Echinacea purpurea* 'Raspberry Truffle' to auxin application, particularly regarding rooting efficiency and plantlet quality. Rooting is a critical step in the micropropagation process, as a robust root system is essential for the successful establishment of plantlets in soil. The high rooting percentages observed with IBA, especially at lower concentrations, suggest that this auxin effectively promotes root initiation without compromising plantlet quality. In contrast, while effective at higher concentrations, NAA may require careful management to avoid potential adverse effects on root morphology and overall plantlet health [Pasternak and Steinmacher 2024].

Moreover, the study emphasizes the potential for optimizing routing protocols for *Echinacea purpurea* 'Raspberry Truffle' by selecting appropriate auxin types and concentrations. The different effects of IBA and NAA observed in this study suggest that natural auxins, like IBA, may be better suited for promoting root development in this species. This finding is particularly relevant for the commercial propagation of *Echinacea purpurea*, where efficient and reliable rooting is crucial for producing high-quality plantlets that can be successfully established in the field [Parsons et al. 2018].

CONCLUSIONS

This study successfully established a micropropagation protocol for Echinacea purpurea 'Raspberry Truffle' by assessing the effects of PGRs on shoot proliferation and rooting performance. The results demonstrate that both cytokinins tested, i.e., mT and BA, are effective in promoting shoot regeneration, with mT at a concentration of 0.5 $mg \cdot L^{-1}$ inducing the highest number of shoots. This finding suggests that mT, given its natural origin and effectiveness in tissue culture applications, could be a preferable alternative to BA.

During the rooting phase, both IBA and NAA significantly influenced root regeneration. IBA demonstrated its efficacy at lower concentrations (0.05 $mg \cdot L^{-1}$) for maximizing regeneration rates, while higher concentrations enhanced root numbers per explant. NAA, however, showed consistent performance, achieving optimal regeneration (100%) and root formation at 0.1 $mg \cdot L^{-1}$ without the fluctuations seen with IBA. IBA's performance shows its effectiveness in root initiation, likely due to its stability and efficient transport within plant tissues.

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REFERENCES

Adamowski, M., Friml, J. (2015). PIN-dependent auxin transport: action, regulation, and evolution. Plant Cell, 27(1), 20–32. https://doi.org/10.1105/tpc.114.134874.

Amoo, S.O., Finnie, J.F., Van Staden, J. (2011). The role of meta-topolins in alleviating micropropagation problems. Plant Growth Reg., 63(2), 197–206. https://doi.org/10.1007/s10725-010-9504-7

Armitage, A.M. (2008). Herbaceous perennial plants: A treatise on their identification, culture, and garden attributes. Quarto Publishing Group, USA.

Ashraf, M.F., Aziz, M.A., Kemat, N., Ismail, I. (2014). Effect of cytokinin types, concentrations and their interactions on *in vitro* shoot regeneration of *Chlorophytum borivilianum* Sant. & Fernandez. Electr. J. Biotechnol., 17(6), 275–279. https://doi.org/10.1016/j.ejbt.2014.08.004

Ault, J.R. (2007). Coneflower: Echinacea species. In: N.O. Anderson (ed.), Flower breeding and genetics: Issues, challenges and opportunities for the 21st century. Springer Netherlands, 801–824. https://doi.org/10.1007/978-1-4020-4428-1_29

Burlou-Nagy, C., Bănică, F., Jurca, T., Vicas, L. G., Marian, E., Mureșan, M. E., Bácskay, I., Kiss, R., Fehér, P., & Pallag, A. (2022). *Echinacea purpurea* (L.) Moench: Biological and pharmacological properties – a review. Plants, 11(9), 1244. https://doi.org/10.3390/plants11091244

Calabrese, E.J., Baldwin, L.A. (2002). Defining hormesis. Human Exp. Toxicol., 21(2), 91–97. https://doi.org/10.1191/0960327102ht217oa

Choffe, K.L. (2000). Micropropagation of *Echinacea purpurea* L. Doctoral dissertation, University of Guelph.

Coleman, G.D., Ernst, S.G. (1989). *In vitro* shoot regeneration of *Populus deltoides*: effect of cytokinin and genotype. Plant Cell Rep., 8, 459–462. https://doi.org/10.1007/BF00269048

Cortleven, A., Leuendorf, J.E., Frank, M., Pezzetta, D., Bolt, S., Schmülling, T. (2019). Cytokinin action in response to abiotic and biotic stresses in plants. Plant, Cell Environ., 42(3), 998–1018. https://doi.org/10.1111/pce.13494

Dahanayake, N., Chen, X.L., Zhao, F.C., Yang, Y.C. (2011). An efficient in vitro propagation system for purple cone-flower (*Echinacea purpurea* L.). Trop. Agric. Res. Extension, 13(2), 29–32. http://dx.doi.org/10.4038/tare.v13i2.3135

Debergh, P.C., Aitken-Christie, J., Cohen, D., Grout, B., Von Arnold, S., Zimmerman, R., Ziv, M. (1992). Reconsideration of the term 'vitrification' as used in micropropagation. Plant Cell, Tiss. Organ Cult., 30(2), 135–140. https://doi.org/10.1007/BF00034381

Demirci, T. (2022). Determination of secondary metabolite production efficiency in *Echinacea purpurea* callus, shoot, and root *in vitro* cultures with methyl jasmonate applications. Acta Physiol. Plant., 44(12), 128. https://doi.org/10.1007/s11738-022-03468-6

Gantait, S., Mitra, S. (2021). Role of *meta*-topolin on *in vitro* shoot regeneration: An insight. In: N. Ahmad, M. Strnad (eds). Meta-topolin: A Growth Regulator for Plant Biotechnology and Agriculture. Springer, Singapore. https://doi.org/10.1007/978-981-15-9046-7_12

Gao, J., Zhuang, S., Zhang, W. (2024). Advances in plant auxin biology: Synthesis, metabolism, signaling, interaction with other hormones, and roles under abiotic stress. Plants, 13(17), 2523. https://doi.org/10.3390/plants13172523

George, E.F., Hall, M.A., De Klerk, G.-J. (eds), (2008). Plant propagation by tissue culture (3rd ed.). Springer. https://doi.org/10.1007/978-1-4020-5005-3

Guilfoyle, T.J., Hagen, G. (2007). Auxin response factors. Curr. Opin. Plant Biol., 10(5), 453–460. https://doi.org/10.1016/j.pbi.2007.08.014

Howell, S.H., Lall, S., Che, P. (2003). Cytokinins and shoot development. Trends Plant Sci., 8(9), 453–459. https://doi.org/10.1016/S1360-1385(03)00191-2

Hwang, I., Sheen, J., Müller, B. (2012). Cytokinin signaling networks. Ann. Rev. Plant Biol., 63, 353–380. https://doi.org/10.1146/annurev-arplant-042811-105503

Kőszeghi, S., Bereczki, C., Balog A., Benedek, K. (2014). Comparing the effects of benzyladenine and meta-topolin on sweet basil (*Ocimum basilicum*) micropropagation. Notulae Sci. Biol. 6(4), 422–427. https://doi.org/10.15835/nsb649464

Lakshmanan, P., Danesh, M., Taji, A. (2002). Production of four commercially cultivated Echinacea species by different methods of in vitro regeneration. J. Hortic. Sci. Biotechnol., 77, 158–163. https://doi.org/10.1080/14620316.2002.11511473

Ljung, K. (2013). Auxin metabolism and homeostasis during plant development. Development, 140(5), 943–950. https://doi.org/10.1242/dev.086363

Nakhooda, M. (2011). The actions of, and interactions between, auxins and cytokinins and their effect on *in vitro* rooting of selected Eucalyptus clones. Doctoral dissertation in the School of Biological and Conservation Sciences, Faculty of Science and Agriculture, University of KwaZulu-Natal, Durban, South Africa.

Nazir, U., Gul, Z., Shah, G.M., Khan, N.I. (2022). Interaction effect of auxin and cytokinin on *in vitro* shoot regeneration and rooting of endangered medicinal plant *Valeriana jatamansi* Jones through tissue culture. Am. J. Plant Sci., 13(2), 223–240. https://10.4236/ajps.2022.132014

Nissen, S., Sutter, E. (1990). Stability of IAA and IBA in nutrient medium to several tissue culture procedures. DigitalCommons @University of Nebraska-Lincoln. Retrieved from https://digitalcommons.unl.edu.

Parsons, J.L., Cameron, S.I., Harris, C.S., Smith, M.L. (2018). Echinacea biotechnology: advances, commercialization and future considerations. Pharmaceut. Biol., 56(1), 485–494. https://doi.org/10.1080/13880209.2018.1501583

Pasternak, T.P., Steinmacher, D. (2024). Plant growth regulation in cell and tissue culture *in vitro*. Plants, 13(2), 327. https://doi.org/10.3390/plants13020327

Petrásek, J., Friml, J. (2009). Auxin transport routes in plant development. Development, 136(16), 2675–2688. https://doi.org/10.1242/dev.030353

Preece, J.E., Read, P.E. (1993). The biology of horticulture: An introductory textbook. John Wiley & Sons.

Riou-Khamlichi, C., Huntley, R., Jacqmard, A., Murray, J.A.H. (1999). Cytokinin activation of Arabidopsis cell division through a D-type cyclin. Science, 283(5407), 1541–1544. https://doi.org/10.1126/science.283.5407.1541

Sakakibara, H. (2006). Cytokinins: Activity, biosynthesis, and translocation. Ann. Rev. Plant Biol., 57, 431–449. https://doi.org/10.1146/annurev.arplant.57.032905.105231

Strnad, M. (2021). History of meta-topolin and the aromatic cytokinins. In: N. Ahmad, M. Strnad, Meta-topolin: A Growth Regulator for Plant Biotechnology and Agriculture. Spronger, Singapore, 1–10. https://doi.org/10.1007/978-981-15-9046-7_1

Taiz, L., Zeiger, E. (2010). Plant Physiology. 5th ed. Sinauer Associates, Sunderland, MA.

Tyub, S., Dar, S.A., Lone, I.M., Mir, A.H., Kamili, A.N. (2021). A robust *in-vitro* protocol for shoot multiplication of *Echinacea angustifolia*. Curr. Plant Biol., 28, 100221. https://doi.org/ 10.1016/j.cpb.2021.100221

Teale, W.D., Paponov, I.A., Palme, K. (2006). Auxin in action: Signaling, transport and the control of plant growth and development. Nat. Rev. Mol. Cell Biol., 7(11), 847–859. https://doi.org/10.1038/nrm2020

To, J.P., Haberer, G., Ferreira, F.J., Deruère, J., Mason, M.G., Schaller, G.E., Alonso, J.M., Ecker, J.R., Kieber, J.J. (2004). Type-A Arabidopsis response regulators are partially redundant negative regulators of cytokinin signaling. Plant Cell, 16(3), 658–671. https://doi.org/10.1105/tpc.018978

Werner, T., Motyka, V., Strnad, M., Schmülling, T. (2001). Regulation of plant growth by cytokinin. Proc. Nation. Acad. Sci., 98(18), 10487–10492. https://doi.org/10.1073/pnas.171304098

Werbrouck, S.P., Strnad, M., Van Onckelen, H.A., Debergh, P.C. (1996). Meta-topolin, an alternative to benzyladenine in tissue culture?. Physiol. Plant., 98(2), 291–297. https://doi.org/10.1034/j.1399-3054.1996.980210.x

Wu, W., Du, K., Kang, X., Wei, H. (2021). The diverse roles of cytokinins in regulating leaf development. Hortic. Res., 8. https://doi.org/10.1038/s41438-021-00558-3

Zhang, Y., Yu, J., Xu, X., Wang, R., Liu, Y., Huang, S., Li, Y., Zhao, H., Zhang, X., Chen, Z., Li, Z., Wei, Z. (2022). Molecular mechanisms of diverse auxin responses during plant growth and development. Int. J. Mol. Sci., 23(20), 12495. https://doi.org/10.3390/ijms232012495