Colegio de Postgraduados

Effect of meta-Topolin on the *in vitro* Propagation of Strawberry (*Fragaria*×*ananassa* Duch)

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ABSTRACT

Objective: Strawberry cultivation (*Fragaria* sp.) is globally relevant due to the appeal of its fruit and its organoleptic characteristics, which increase its demand. However, it is necessary to implement new propagation methods that allow the establishment of commercial plantations, such as plant micropropagation, in order to obtain thousands of plants in a reduced period of time. Therefore, the objective of this study was to determine the effect of meta-Topolin (mT) during the *in vitro* multiplication of strawberries.

Design/Methodology/Approach: *In vitro* strawberry plants were used in MS medium with 30 g L^{-1} of sucrose, evaluating concentrations of meta-Topolin (mT: 0, 0.5, 1.0, 1.5 mg L^{-1}) and 6-benzylaminopurine (BAP: 0, 0.5, 1.0, 1.5 mg L^{-1}), under conditions of 24±2 °C and a photoperiod of 16:8 h light/darkness.

Results: mT increased the number of shoots compared to BAP, with 1.0 mg/L yielding 4.72 shoots per explant and 1.5 mg/L yielding 3.50. At 1.5 mg/L, the shoots reached 3.41 cm. The roots formed during multiplication facilitated acclimatization.

Limitations/Implications: Although the use of meta-Topolin showed promising results in the *in vitro* multiplication of strawberries, its performance needs to be evaluated in later developmental stages, field growth, and response to different environmental conditions.

Findings/Conclusions: mT increased the number of shoots compared to BAP, with 1.0 mg L^{-1} yielding 4.72 shoots per explant and 1.5 mg L^{-1} yielding 3.50. At 1.5 mg L^{-1}, the shoots reached 3.41 cm. The roots formed during multiplication facilitated acclimatization.

Keywords: micropropagation, strawberry, commercial propagules, cytokinins.

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INTRODUCTION

The strawberry fruit (*Fragaria* sp.) is globally valued for its organoleptic properties (Carole *et al.*, 2024). Additionally, this fruit contains various nutrients such as proteins, calcium, potassium, iron, copper, and vitamins (Kannaujia & Asrey, 2021). The cultivation of this species contributes to the economy of different countries around the world, including

Mexico, where approximately 443,552.81 tons of strawberries are produced annually, with Michoacán being the leading producer, accounting for 326,191.10 tons (SIAP, 2024).

The propagation of this crop is mainly carried out asexually through cuttings or by seed germination (Li *et al.*, 2020). However, propagation through cuttings does not meet the demand for propagules required for the establishment of commercial orchards (Dhukate *et al.*, 2021). On the other hand, seed germination has low efficiency and promotes genetic segregation, limiting its use. For this reason, it is essential to implement strategies that optimize and increase the propagation capacity of this crop (Valliath & Mondal, 2023). Consequently, plant tissue culture, known as micropropagation, has been successfully implemented in this plant species (Neri *et al.*, 2022). This technique allows the production of a large number of plants in relatively short periods and in limited spaces. Additionally, it generates plants with greater genetic uniformity, vigor, and high phytosanitary quality. However, to reach its commercial potential, it is necessary to develop protocols that maximize plant production (Abdalla *et al.*, 2022). In this context, the proper selection of the type and concentration of plant growth regulators (PGRs) is crucial to ensure the success of micropropagation (Papafotiou *et al.*, 2023). The 6-Benzylaminopurine (BAP) is one of the most commonly used PGRs to stimulate shoot formation in various plant species (Krishna *et al.*, 2021; Monthony *et al.*, 2021; Nugrahani *et al.*, 2024). However, other cytokinins can be evaluated to determine their efficacy in *in vitro* shoot formation. One such alternative is meta-Topolin (mT, 6-(3-hydroxybenzylamino) purine), a synthetic cytokinin that activates different morphogenetic regeneration pathways in *in vitro* cultures (Gantait & Mitra, 2021). mT has been successfully used in the micropropagation of species such as vanilla (*Vanilla planifolia* Andrews) (Manokari *et al.*, 2021) and gerbera (*Gerbera jamesonii* Bolus ex Hook.f.) (Mahanta *et al.*, 2023), proving to be more efficient than BAP in shoot generation. Therefore, the objective of this study was to evaluate the effect of meta-Topolin on the *in vitro* multiplication of strawberry (*Fragaria*×*ananassa* Duch).

MATERIALS AND METHODS

Plant Material

In vitro strawberry plants (*Fragaria*×*ananassa* Duch), previously established from apical meristems, were used. These plants, cultivated at the National Center for Genetic Resources, served as the source of explants for the study.

Shoot Proliferation

Individual 1 cm long shoots of strawberry (*Fragaria*×*ananassa* Duch) were selected and transferred to MS (Murashige & Skoog, 1962) culture medium supplemented with 30 g L^{-1} of sucrose. Different concentrations of meta-Topolin (mT: 0, 0.5, 1.0, and 1.5 mg L^{-1}) and 6-benzylaminopurine (BAP: 0, 0.5, 1.0, and 1.5 mg L^{-1}) were evaluated. The medium was adjusted to a pH of 5.8±0.1, and 7 g L^{-1} of agar was added as a gelling agent. A total of 35 mL of the medium was dispensed into 250 mL glass jars, which were sterilized in an autoclave at 1.5 kg cm^2 and 121 °C for 15 min. The plants were maintained under controlled conditions of 24±2 °C, with a photoperiod of 16 h light and 8 h darkness, under an irradiation of 50 μmol/m^2/s. After 45 days of culture, the following parameters were evaluated: number of shoots per explant, shoot length, number of leaves, as well as number and length of roots.

Acclimatization

Shoots measuring 3 cm in length, with a well-developed root system, were transferred to plastic domes measuring 24×16×7 cm, containing a sterile substrate composed of peat moss and perlite in a 1:1 (v/v) ratio. The plants were maintained under controlled greenhouse conditions with 50% shade, a temperature of 30±5 °C, and relative humidity gradually adjusted: 90±5% during the first week, 80±5% in the second week, and 65±5% in the third week. After 30 days, the plant survival rate was recorded.

Statistical Analysis

All experiments were conducted in duplicate, following a completely randomized design. The data obtained were statistically analyzed using IBM SPSS Statistics software (version 21). An analysis of variance (ANOVA) was performed, followed by Tukey's multiple comparison test ($p \leq 0.05$) to identify statistically significant differences between treatments.

RESULTS AND DISCUSSION

Sprouting Proliferation

After 45 days of cultivation, statistically significant differences were observed among the treatments evaluated (Table 1). In general, mT produced a higher number of shoots compared to BAP (Figure 1). A total of 4.72 shoots per explant were obtained in the culture medium containing 1.0 mg L^{-1} of mT, followed by 3.50 shoots in 1.5 mg L^{-1} of mT and 3.17 shoots in 1.0 mg L^{-1} of BAP. The lowest number of shoots per explant (1.20) was observed in the control treatment (0 mg L^{-1} of BAP or mT). Regarding shoot length, 1.5 mg L^{-1} of mT produced shoots of 3.41 cm, followed by 2.59 cm obtained in the control treatment and 1.68 cm in 1.0 mg L^{-1} of mT. The shortest shoots were observed with 1.0 and 1.5 mg L^{-1} of BAP, measuring 0.83 and 0.85 cm, respectively. The highest number of leaves (5.10) was recorded in the control treatment, while the lowest (1.87) was observed in 1.5 mg L^{-1} of mT. Strawberry shoots generated roots during the multiplication stage.

Table 1. Effect of 6-Benzylaminopurine and meta-Topolin on the proliferation of strawberry shoots (*Fragaria*×*ananassa* Duch) after 45 days of cultivation.

Treatment (mg L^{-1})	Number of shoots	Length of shoots (cm)	Number of sheets	Number of roots	Length of roots (cm)
0 BAP/mT	1.20±0.37d	2.59±0.75ab	5.10±0.09a	5.40±0.63a	1.08±0.29a
0.5 BAP	2.75±0.33bc	1.27±0.15c	2.02±0.11c	0.60±0.19bc	0.23±0.08bc
1.0 BAP	3.17±0.32bc	0.83±0.09c	2.05±0.10c	0.89±0.26bc	0.33±0.08bc
1.5 BAP	1.83±0.30cd	0.85±0.09c	2.16±0.11c	0.00±0.00c	0.00±0.00c
0.5 mT	2.41±0.39bc	1.23±0.22c	3.67±0.08b	1.50±0.45b	0.36±0.11b
1.0 mT	4.72±0.22a	1.68±0.30bc	2.79±0.13bc	0.54±0.31bc	0.13±0.08bc
1.5 mT	3.50±0.26ab	3.41±0.27a	1.87±0.09c	0.00±0.00c	0.00±0.00c

The data are presented as mean±standard error. Different letters indicate significant differences (Tukey, $p \leq 0.05$).

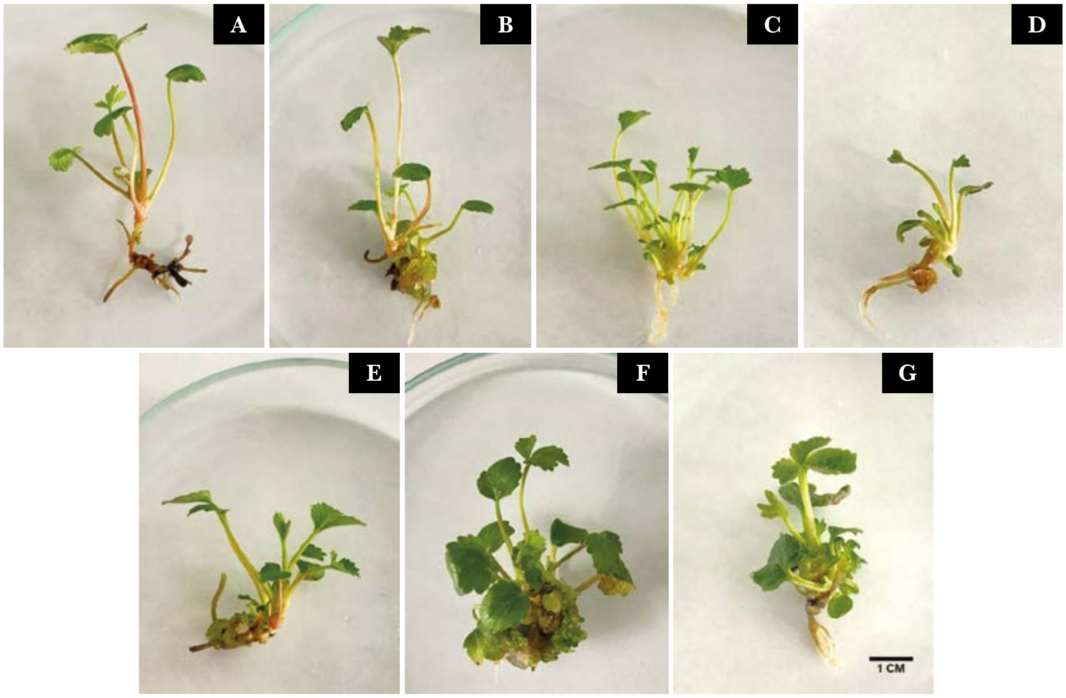

Figure 1. Effect of 6-Benzylaminopurine (BAP) and meta-Topolin (mT) on the proliferation of strawberry shoots (*Fragaria*×*ananassa* Duch). A) Control treatment, B-D) 0.5, 1.0, and 1.5 mg L^{-1} of BAP, left to right, E-G) 0.5, 1.0, and 1.5 mg L^{-1} of mT, left to right.

However, the highest number of roots (5.40) was observed in the control treatment. The treatment with 1.5 mg L^{-1} of mT did not produce roots. Roots with a length of 1.08 cm were obtained in the control treatment, whereas the root length in the other treatments was shorter.

Acclimatization

Strawberry plants (*Fragaria*×*ananassa* Duch) subjected to the acclimatization process showed an 85% survival rate after 30 days of cultivation (Figure 2). The plants were transferred to plastic bags.

The micropropagation of various varieties derived from *Fragaria* sp. crosses has proven to be an effective method for obtaining a large number of disease-free plants with high

Figure 2. Acclimatization process of strawberry (*Fragaria*×*ananassa* Duch). A) *In vitro* plants used and B and C) Plants after 30 days of acclimatization.

genetic uniformity (Quiroz *et al.*, 2017; Neri *et al.*, 2022). In this study, an increase in the *in vitro* proliferation of strawberry (*Fragaria*×*ananassa* Duch) shoots was confirmed through the use of meta-Topolin (mT), an aromatic cytokinin characterized by a benzyl ring with a hydroxyl group in the meta position (Strnad, 2021). This plant growth regulator (PGR) is widely used in the *in vitro* propagation of plants, standing out for its high efficiency based on criteria such as a high multiplication rate, vigorous shoots, optimal rooting, and greater acclimatization capacity (Zaytseva *et al.*, 2021). In this study, the addition of meta-Topolin to the culture medium resulted in a higher number of shoots per explant compared to the addition of 6-benzylaminopurine (BAP). The concentration of 1.0 mg L^{-1} of mT significantly promoted the proliferation of strawberry (*Fragaria*×*ananassa* Duch) shoots, while 1.5 mg L^{-1} of mT produced longer shoots. Although BAP is the most commonly used cytokinin in plant tissue culture due to its positive response in most species (Krishna *et al.*, 2021), in this study, mT demonstrated greater efficiency in generating shoots per explant. These results align with those reported by Kumar *et al.* (2024), who observed a similar response in winter cherry (*Withania somnifera* (L.) Dunal), where the use of 2.5 mg L^{-1} of mT outperformed various concentrations of BAP in the production of shoots per explant. The advantages offered by mT compared to other cytokinins include counteracting the effects of hyperhydricity caused by the use of BAP, preventing shoot necrosis by containing a hydroxyl group that allows the reversal of O-glycosides generated by BAP use, avoiding the formation of chimeras, and not inhibiting root formation during acclimatization (Cardoso, 2021; Werbrouck, 2021). Although it is mentioned that the use of topolins is common in laboratories dedicated to commercial micropropagation, these protocols are industrial and therefore confidential (Ahmad & Strnad, 2021; Werbrouck, 2021).

Meta-Topolin has proven to be highly effective in the micropropagation of various plant species, to the point of being considered, a few years ago, a panacea in plant tissue culture (Aremu *et al.*, 2012). In vanilla (*Vanilla planifolia* Andrews), the use of mT produced a record 62 shoots per explant, the highest reported for this species (Manokari *et al.*, 2021). In sweet potato (*Ipomoea batatas* (L.) Lam.), mT achieved the highest number of shoots compared to other plant growth regulators (PGRs) (Bansal *et al.*, 2023). Similarly, in gerbera (*Gerbera jamesonii* Bolus ex Hook.f.), a concentration of 1.5 mg L^{-1} of mT generated approximately 14 shoots per explant, surpassing results obtained with BAP, kinetin, thidiazuron, and zeatin (Mahanta *et al.*, 2023). In this study, using strawberry (*Fragaria*×*ananassa* Duch), 4.72 shoots per explant were obtained with 1.00 mg L^{-1} of meta-Topolin. Comparatively, Dhukate *et al.* (2021) reported in strawberry (*Fragaria*×*ananassa*) cvs. 'Sweet Charlie' and 'Winter Dawn' the production of 4-5 shoots per explant using 0.5 mg L^{-1} of BAP and 1.0 mg L^{-1} of kinetin. Similarly, Neri *et al.* (2022) reported in strawberry (*Fragaria*×*ananassa* Duch.) cv. 'Aroma' the production of 4.20 shoots per explant with the addition of 1.0 mg L^{-1} of zeatin. These results highlight the efficacy of mT compared to other plant growth regulators.

Acclimatization is one of the most challenging steps in the plant micropropagation process (De Stefano *et al.*, 2022). This process involves the gradual adaptation of *in vitro* plants to *ex vitro* conditions, which is essential for achieving high survival rates (Mahendra *et al.*, 2020). In this study, an 85% survival rate was achieved in strawberry (*Fragaria*×

ananassa Duch) plants obtained through plant tissue culture. In comparison, Dhukate *et al.* (2021) reported survival rates of 95% and 90% in the strawberry varieties 'Sweet Charlie' and 'Winter Dawn,' respectively. Similarly, Neri *et al.* (2022) achieved a 100% survival rate in the strawberry variety 'Aroma.' These results reflect the variability in plant response during acclimatization, depending on the variety and the specific conditions of the process.

CONCLUSIONS

The use of meta-Topolin resulted in a higher number of shoots per explant compared to the use of BAP. The concentration of 1.0 mg L^{-1} of mT produced the highest number of shoots per explant, while 1.5 mg L^{-1} generated the longest shoots. Additionally, an 85% survival rate was achieved during the gradual acclimatization process. These results represent a significant advancement in the propagation of strawberry (*Fragaria×ananassa* Duch) contributing to the production of propagules for the establishment of commercial plantations.

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