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Optimized in vitro micropropagation and microtuber production in potato (*Solanum tuberosum* L.) through apical buds using hormone regulation and tissue culture techniques

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Micropropagation

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ABSTRACT

Potato is an essential crop worldwide, and optimizing micropropagation techniques is important for enhancing germplasm conservation and large-scale production. This study focuses on the in vitro propagation of two potato varieties, *Agata* and *Fianna*, emphasizing optimizing sterilization protocols, shoot induction, rooting, and microtuber production. Apical buds from healthy, disease-free plants were selected as explants. These buds were surface-sterilized using 70% ethanol and sodium hypochlorite (NaOCl) with Tween-20. The explants were excised from tuber sprouts and cultured on Murashige and Skoog (MS) medium supplemented with various concentrations of plant growth regulators, including benzylaminopurine (BAP) at 0.10–0.40 mg/L, gibberellic acid (GA3) at 0.20–1.00 mg/L, and naphthalene acetic acid (NAA) at 0.01 and 0.04 mg/L to promote root development. The study also explored the effects of these hormonal treatments on shoot induction, proliferation, and rooting. Significant differences were observed between the two varieties regarding oxidation, contamination, and aseptic conditions, with *Fianna* demonstrating better resistance to oxidation and contamination than *Agata*. Shoot multiplication was most efficient with BAP concentrations of 0.40 mg/L for *Fianna* and 0.30 mg/L for *Agata*. Root induction was maximized with higher sucrose concentrations (110 g/L) and GA3 (1.00 mg/L) for *Agata*, while moderate concentrations of these compounds produced optimal results

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for *Fianna*. Microtuber formation was most successful with moderate sucrose (80–100 g/L) and GA3 (0.25–0.75 mg/L) concentrations. This study provides valuable insights into optimizing tissue culture practices for potato propagation, enhancing both microtuber production and the overall efficiency of potato production systems.

1 Introduction

Potato (*Solanum tuberosum* L.) is among the most important tuber crops worldwide, serving as a significant source of food and income for many regions, including Nuevo Leon, Mexico (Aksoy et al. 2021; SIAP 2024). The potato plant is highly valued for its nutritional content, which includes carbohydrates, vitamins, and minerals, as well as its adaptability to various climatic conditions and soil types (Dolničar 2021; Heuberger et al. 2022). Given potatoes' economic and nutritional importance, understanding the mechanisms of organogenesis, mainly through apical buds, can provide critical insights for improving propagation techniques and crop yields (Navarro et al. 2015; De Koeyer and Harding 2019). Plant organogenesis involves forming and developing organs such as roots, shoots, and leaves from undifferentiated cells (Smet and Beeckman 2019). This process is essential for plant regeneration and is of particular interest in the context of vegetative propagation, a common practice in potato cultivation (O'Brien and McCleary 2023). Apical buds, located at the tip of the stem, play a crucial role in the growth and development of new shoots, which are vital for clonal propagation (Vlahova et al. 2022).

Numerous studies have investigated the factors influencing organogenesis in potatoes. For instance, García-González and Quiroz (2018) examined the impact of plant growth regulators on shoot regeneration from potato explants, emphasizing the importance of hormonal balance for successful organogenesis. Similarly, Bustos and Carrillo (2017) explored the genetic and environmental factors that affect the differentiation of apical meristems into various organ systems in potato plants. These studies highlight the complexity of organogenesis and the need for a comprehensive understanding of intrinsic and extrinsic factors.

Hormonal regulation, particularly the roles of cytokinins and auxins, remains a primary focus in organogenesis research. Cytokinins, such as benzylaminopurine (BAP), have been found to stimulate shoot proliferation, while auxins like indole-3-acetic acid (IAA) are essential for root development (Šmeringai et al. 2023). Furthermore, gibberellic acid (GA_3) is often used to enhance elongation and reduce apical dominance, underscoring the interplay of growth regulators in tissue culture systems (Ritonga et al. 2023).

Environmental factors also significantly influence outcomes in organogenesis. Light quality, temperature, and photoperiod conditions have been shown to affect the efficiency of organogenesis in potato explants (Develi and Miler 2023). Studies indicate that red and blue light spectra positively influence microtuberization, while green light inhibits these processes (Abu-Zeid et al. 2022). Additionally, the composition of the growth medium, including macronutrients, micronutrients, and gelling agents, plays a crucial role in the success of organogenesis (Anjum and Ali 2004). For instance, adding activated charcoal has been reported to reduce oxidative stress and phenolic exudation, common challenges in potato tissue culture (Thomas 2008; Permadi et al. 2024).

Genetic factors must also be considered, as different potato cultivars respond variably to the same growth conditions and hormonal treatments (Coleman et al. 1990). Advances in transcriptomics and proteomics have provided more profound insights into gene expression patterns during organogenesis, identifying key regulatory pathways and candidate genes responsible for shoot and root differentiation (Dobránszki et al. 2019). Collectively, these studies illustrate the multifaceted nature of organogenesis in potatoes and underscore the necessity of optimizing both biotic and abiotic conditions for successful micropropagation.

In Nuevo León, potato cultivation faces water scarcity, soil salinity, and temperature fluctuations. These environmental stressors significantly affect potato growth, yield, and quality, making adopting innovative strategies to enhance crop resilience essential. To tackle these challenges, improving propagation techniques, including the efficient use of apical buds, can bolster the resilience and productivity of potato crops in this region. Recent advancements in tissue culture and molecular biology offer new avenues for optimizing organogenesis in potatoes, providing potential solutions to overcome local agricultural constraints (Hasnain et al. 2022). However, despite significant progress in potato tissue culture research, a critical gap remains in developing region-specific protocols tailored to the unique environmental conditions of Nuevo León. Most existing studies focus on generalized propagation techniques without considering the specific physiological and environmental responses of potato varieties grown under local stress conditions.

The novelty of this research lies in its targeted approach to optimizing hormonal combinations and culture conditions specifically for potato varieties cultivated in arid and semi-arid environments like Nuevo León. This study aims to establish a robust and reproducible protocol for organogenesis and microtuber production by analyzing the interaction between growth regulators, environmental factors, and potato genotypes. Additionally, the

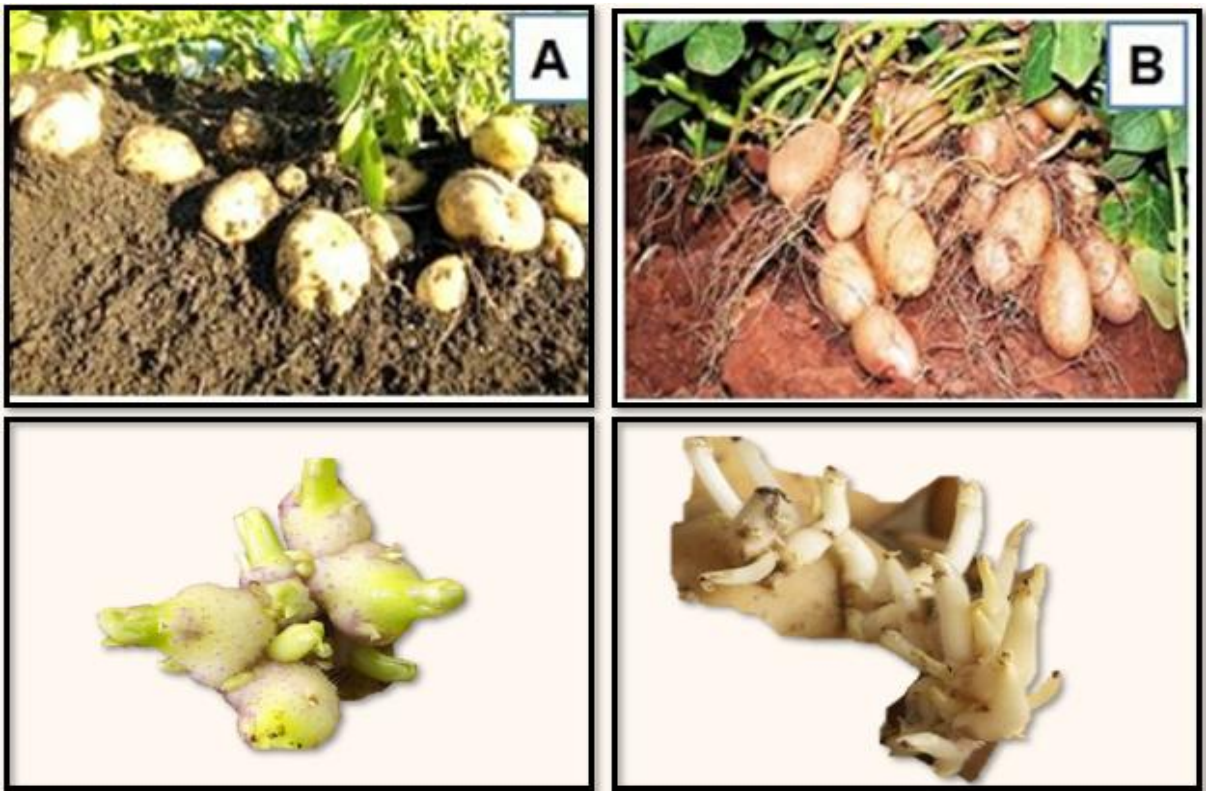

Figure 1 Potato tubers from three varieties of *S. tuberosum* (A) Agata: Smooth, oval-shaped tuber with light brown skin and shallow eyes, (B) Fianna: Round to oval-shaped tuber with a slightly rough texture and medium-depth.

research explores the influence of different hormonal treatments on various organ development stages, addressing the knowledge gap concerning variety-specific responses to tissue culture conditions. The findings are expected to enhance the scientific understanding of potato organogenesis and provide practical solutions for sustainable potato production in regions facing environmental stressors.

2 Materials and Methods

2.1 Plant Material and Sterilization

Apical buds from two potato varieties, *'Agata'* and *'Fianna*,' were selected as explants for in vitro propagation. These explants were carefully chosen from healthy, disease-free plants grown in greenhouse conditions. To prepare the buds, they were pre-washed with running tap water for 30 minutes to remove surface contaminants. Next, they underwent surface sterilization by being immersed in a 70% ethanol solution for 1 minute, followed by treatment with a sodium hypochlorite solution (0.5%) containing two drops of Tween-20 for 15 minutes. Benomyl (0.1 g/L) was included in the sterilization process to reduce fungal contamination further. After sterilization, the explants were rinsed three times with sterile distilled water to eliminate residual sterilizing agents. The effectiveness of the sterilization process was evaluated by assessing contamination rates, and the samples were examined under a light microscope to confirm their cleanliness (Figure 1).

2.2 Culture Medium Preparation

Murashige and Skoog (MS) medium was used as the basal culture medium due to its well-documented efficacy in supporting potato tissue culture. The medium was supplemented with varying concentrations of benzylaminopurine (BAP) (0.10, 0.20, 0.30, and 0.40 mg/L) and gibberellic acid (GA3) (0.25, 0.50, 0.75, and 1.00 mg/L) to evaluate their effects on shoot induction, proliferation, and microtuber formation. The medium also contained 3% (w/v) sucrose as a carbon source and 0.8% (w/v) agar as a gelling agent. The pH was adjusted to 5.8 using 0.1 N NaOH or HCl before autoclaving at 121°C for 20 minutes.

2.3 Pre-disinfestation process of Plant Material

Before the disinfection process of the plant material, 2 centimeter shoots from the varieties *Ágata* and *Fianna* were cultivated for six weeks under controlled conditions, which included a 16-hour light cycle (54 µmol m^{-2} s^{-1}) and 8 hours of darkness at a temperature of 24 ± 2 °C. The cleaning procedure began with washing the shoots' apical meristem, followed by brushing them with liquid soap and rinsing them with potable water. A final rinse with purified water

was then conducted. The shoots were subsequently transferred to an antifungal solution containing benomyl at a concentration of 1.0 mg/L for 30 minutes. After this time, the explants were rinsed with purified water to remove any residue from the antifungal solution. Finally, the plant material underwent disinfection under aseptic conditions (Forest et al. 2023).

2.4 *In Vitro* Aseptic Establishment of Explants

The pre-disinfested explants were placed in a laminar flow hood, where the disinfection process took place under aseptic conditions. The explants were immersed in a sodium hypochlorite (NaOCl) solution (Cloralex® at 6% active ingredient) at concentrations of 15% and 20% (v/v), supplemented with 0.02% Tween-20, for 10 minutes. Following this, the explants underwent three rinses with sterile double-distilled water. Each experimental unit consisted of four explants placed in a Magenta™ box, and they were incubated under controlled conditions: a 16-hour light cycle (at 54 µmol m^{-2} s^{-1}) followed by 8 hours of darkness, maintaining a temperature of 24 ± 2 °C. The experimental design for this establishment phase utilized a completely randomized design with a 3x2 factorial arrangement. Factor A consisted of the potato varieties, specifically *Fianna* and *Ágata*, while Factor B represented the concentration of the disinfecting agent. This resulted in a total of five treatments with 10 replications each. Over eight weeks, the variables of oxidation, contamination, and asepsis of the explants were evaluated. The results were analyzed using non-parametric statistics, mainly through Contingency Tables, and the Chi-square test (X^2) was employed for evaluation.

2.5 Shoot Induction and Proliferation

Sterilized explants were cultured in sterile glass containers containing MS medium with varying concentrations of BAP and GA3. Cultures were maintained in a growth chamber under controlled conditions: a photoperiod of 16 hours of light and 8 hours of darkness, a temperature of 24±2°C, and a light intensity of 50 µmol m^{-2} s^{-1} provided by cool-white, fluorescent tubes. Shoot induction was monitored weekly, and data were recorded for parameters such as shoot length, the number of shoots per explant, and oxidation levels. Oxidation severity was visually assessed, and strategies to minimize oxidation, such as frequent sub-culturing and antioxidant treatments, were applied when necessary.

2.6 Rooting Stage

Once shoots reached a 3-4 cm height, they were excised and transferred to MS medium supplemented with naphthalene acetic acid (NAA) at concentrations of 0.01 and 0.04 mg/L to promote root development (Table 1). Cultures were maintained under similar environmental conditions as the shoot induction stage. Root length, the number of roots per shoot, and overall root morphology were recorded after three weeks.

2.7 Microtuber Production

Shoots from well-rooted plants were transferred to MS medium containing 8% sucrose and varying concentrations of BAP and GA3 to promote microtuber formation. The cultures were kept under reduced light conditions, with 8 hours of light and 16 hours of darkness, at a temperature of 20±2°C to encourage tuberization (Table 2).

2.8 Data Analysis

All experiments were carried out using a completely randomized design, consisting of five treatments with ten replications for each treatment. Quantitative data was collected on shoot length, the number of shoots, rooting efficiency, oxidation levels, and microtuber yield. Statistical analysis was conducted using ANOVA, and mean comparisons were performed with Tukey's HSD test, set at a significance level of $p < 0.05$.

Table 1 Treatments applied during the multiplication stage for two potato varieties (Fianna and Ágata) of *S. tuberosum.*

Variety	Treatment[#]	NAA (mg/l)	GA_3 (mg/l)	BAP (mg/l)
Ágata	1	0.00	0.00	0.00
	2	0.01	0.25	0.10
	3	0.02	0.50	0.20
	4	0.03	0.75	0.30
	5	0.04	1.00	0.40
Fianna	1	0.00	0.00	0.00
	2	0.01	0.25	0.10
	3	0.02	0.50	0.20
	4	0.03	0.75	0.30
	5	0.04	1.00	0.40

Table 2 Treatments used during the microtuber production stage for two potato varieties (Fianna and Ágata) of *S. tuberosum*.

Variety	Treatment[#]	Sucrose (g/L)	GA_3 (mg/l)	BAP (mg/l)
Ágata	1	30	0.00	0.00
	2	80	0.25	0.10
	3	90	0.50	0.20
	4	100	0.75	0.30
	5	110	1.00	0.40
Fianna	1	30	0.00	0.00
	2	80	0.25	0.10
	3	90	0.50	0.20
	4	100	0.75	0.30
	5	110	1.00	0.40

3 Results and Discussion

3.1 Stage of Aseptic Establishment of Explants

3.1.1 Effect of Oxidation on Potato Varieties

The Chi-square test yielded a value of 8.128, which was higher than the tabulated value for p = 0.05. This led to the conclusion that the varieties displayed significant differences in oxidation levels. Notably, the Ágata variety exhibited the highest oxidation percentage at 63.63%, surpassing the Fianna variety with an oxidation percentage of 46.1% (Table 3). These results align with the findings of Smith et al. (2023) and García and López (2022), who noted that the in vitro establishment of potatoes (*S. tuberosum*) is typically characterized by a low percentage of aseptic explants and a high incidence of obscured explants. Furthermore, contamination and oxidation of donor tissue have been identified as significant challenges in the micropropagation of woody plants (Bettoni et al. 2024; Mohamed and Girgis 2023).

3.1.2 Effect of NaOCl on Oxidation of the Potato Explants

The results of the sodium hypochlorite (NaOCl) treatment on the oxidation of two potato varieties, Fianna and Ágata, reveal significant differences in their effects. This is supported by a calculated Chi-square value of 6.39, which exceeds the tabulated value at p = 0.05. This finding suggests that the doses of NaOCl significantly influence the degree of oxidation in the plants, indicating a dose-dependent response. The treatment with 20% NaOCl prevented oxidation, as 58.3% of the plants remained non-oxidized. In contrast, the 15% NaOCl treatment resulted in only 28.5% of the plants remaining non-oxidized. These results underscore the impact of NaOCl concentration on plant tissue and its ability to minimize oxidation, which is essential for preventing damage during processes such as sterilization or tissue culture propagation (Murashige and Skoog 1962). Previous studies have consistently shown that NaOCl concentration is crucial in determining its effectiveness. For instance, research has demonstrated that higher concentrations of NaOCl are generally more effective at sterilizing plant tissues. However, they can induce oxidative stress or damage if not carefully controlled (Yildiz et al. 2012).

3.1.3 Contamination

The chi-square test results that compare contamination levels among the potato varieties (Fianna and Ágata) and different

Table 3 Comparison of oxidation, contamination, and asepsis rates between *Fianna* and *Ágata* potato varieties under different NaOCl concentrations.

Parameter	Fianna	Ágata	Statistical Results
Oxidation Percentage	46.1%	63.63%	Chi-square = 8.128, $p < 0.05$
Contamination Rate	40%	100%	Chi-square = 35.52, $p < 0.05$
Asepsis Percentage	60%	0%	Chi-square = 35.52, $p < 0.05$
Effect of NaOCl (15% concentration)	28.5% non-oxidized	28.5% non-oxidized	Chi-square = 6.39, $p < 0.05$
Effect of NaOCl (20% concentration)	58.3% non-oxidized	58.3% non-oxidized	Chi-square = 6.39, $p < 0.05$
NaOCl Concentration Effect on Contamination	1.63 (no significant effect)	1.63 (no significant effect)	Chi-square = 1.63, $p > 0.05$

concentrations of NaOCl provide significant insights into the sterilization process. The chi-square value of 35.52 for contamination among the varieties was much higher than the tabulated value of 9.21 (p=0.05), indicating that Fianna had a significantly lower contamination rate of 40% compared to Ágata, which had a contamination rate of 100% (Table 3). This finding aligns with previous research suggesting that different potato varieties may exhibit varying resistance to contamination due to genetic factors, such as surface properties or antimicrobial compounds (Nagy et al. 2023). Conversely, the chi-square test for NaOCl concentrations yielded a value of 1.63, lower than the tabulated value of 3.84 (p=0.05). This indicates that the NaOCl concentrations used were ineffective in preventing contamination, highlighting the need to optimize sterilization protocols. As Nagy et al. (2023) emphasized, sterilization treatments' effectiveness depends on factors such as concentration and exposure time, which must be carefully adjusted to improve outcomes in tissue culture practices.

3.1.4 Asepsis

The chi-square test to assess environmental asepsis among different potato varieties revealed a significant difference in contamination resistance. The variety Fianna exhibited 60% asepsis, while Ágata displayed 0%, indicating that Fianna has a higher resistance to contamination. The calculated chi-square value was 35.52, which surpassed the tabulated value of 9.21 (p=0.05), demonstrating Fianna's superior ability to maintain aseptic conditions. In contrast, no significant difference was found between the tested NaOCl concentrations. The chi-square value for these concentrations was 1.63, lower than the tabulated value of 3.84 (p=0.05). This suggests that the NaOCl concentrations used in the experiment were insufficient to control contamination (Table 3) effectively. These results underscore the need to optimize NaOCl concentrations to ensure effective sterilization in tissue culture. Nagy et al. (2023) recommended that future studies investigate higher NaOCl concentrations or consider alternative sterilization methods to enhance asepsis.

3.2 Shoot Induction, Proliferation, and Multiplication

Under various hormonal treatments, the study observed significant differences in shoot induction, proliferation, and multiplication between the potato varieties Ágata and Fianna. The effects of hormonal combinations consisting of NAA (naphthalene acetic acid), GA3 (gibberellic acid), and BAP (benzylaminopurine) were tested to evaluate their impact on the number of shoots, shoot length, and node number per shoot (Table 4). The number of shoots per explant varied significantly between the two varieties and the treatments applied. Ágata demonstrated the highest shoot number (5.237) under Treatment 4 (0.01 mg/L NAA, 0.20 mg/L GA3, and 0.50 mg/L BAP), while Fianna produced the highest shoot number (6.233) in Treatment 5 (0.01 mg/L NAA, 0.20 mg/L GA3, and 1.00 mg/L BAP). The higher concentration of BAP in Treatment 5 was particularly effective for Fianna, leading to a more significant number of shoots. This finding aligns with previous studies, such as Vázquez-Martínez et al. (2022), which highlighted that increased BAP concentrations in potato cultivars enhance shoot multiplication, indicating that BAP plays a crucial role in shoot proliferation. Regarding shoot length, Fianna exhibited the longest shoots in Treatment 5 (5.293 cm), significantly surpassing those in other treatments. In contrast, Ágata produced the longest shoots in Treatment 1 (control), with an average length of 5.551 cm. The longer shoot length observed in Fianna may be attributed to the hormonal combination in Treatment 5, particularly the higher concentration of BAP, which is known to promote shoot elongation (Srivastava et al., 2012; Hussain et al., 2023). Notably, Ágata did not show significant

Table 4 Shoot induction, proliferation, and length in *Agata* and *Fianna* potato varieties under different hormonal treatments.

Variety	Treatment #	NAA (mg/l)	GA_3 (mg/l)	BAP (mg/l)	Shoots number/ explant	Shoot length (cm)	Nodes number/shoot
Ágata	1	0.00	0.00	0.00	3.485^{b}	5.551^{a}	4.740^{a}
	2	0.01	0.25	0.10	4.342^{b}	4.688^{b}	4.829^{a}
	3	0.02	0.50	0.20	3.683^{b}	4.400^{b}	4.987^{a}
	4	0.03	0.75	0.30	5.237^{a}	4.077^{b}	4.775^{a}
	5	0.04	1.00	0.40	3.977^{b}	4.560^{b}	5.507^{a}
Fianna	1	0.00	0.00	0.00	3.844^{c}	4.928^{b}	2.962^{b}
	2	0.01	0.25	0.10	3.447^{c}	2.133^{d}	3.740^{b}
	3	0.02	0.50	0.20	4.173^{b}	3.267^{c}	2.015^{c}
	4	0.03	0.75	0.30	5.080^{a}	4.113^{b}	4.167^{a}
	5	0.04	1.00	0.40	6.233^{a}	5.293^{a}	4.693^{a}

†Averages with the same letter in each column show no significant difference, Tukey sig. $P \leq 0.05$.

differences in shoot length across the treatments, although Treatment 4 (0.01 mg/L NAA, 0.20 mg/L GA3, and 0.50 mg/L BAP) resulted in relatively longer shoots than the other treatments. Regarding node number, Ágata produced the highest number of nodes per shoot (5.507) in Treatment 5, although no significant differences were observed across the other treatments for node production. For Fianna, Treatment 5 also resulted in the highest node count (4.693). These results suggest that Treatment 5 promoted greater shoot multiplication and facilitated better node formation. The increase in nodes per shoot in both varieties under Treatment 5 supports the beneficial effect of BAP on node production, as reported in previous studies (Sota et al. 2020).

3.3 Rooting Efficiency

The effects of sucrose, GA3 (gibberellic acid), and BAP (benzylaminopurine) on root formation in the potato varieties Ágata and Fianna were evaluated, focusing on root number and root length (Table 5). For Ágata, Treatment 5 (sucrose 110 g/L, GA3 1.00 mg/L, BAP 0.40 mg/L) resulted in the highest root formation, with 7.100 roots and a root length of 5.260 cm, indicating that the combination of higher sucrose concentration and hormones was most effective in promoting root growth. Treatment 4 (sucrose 100 g/L, GA3 0.75 mg/L, BAP 0.30 mg/L) also supported significant root growth, producing 5.810 roots and a root length of 4.125 cm. In comparison, Treatment 3 (sucrose 90 g/L, GA3 0.50 mg/L, BAP 0.20 mg/L) resulted in fewer and shorter roots (3.956 roots and a length of 2.780 cm), suggesting that higher BAP concentrations may inhibit root formation, as found by Amghar et al. (2021). For Fianna, Treatment 4 (sucrose 100 g/L, GA3 0.75 mg/L, BAP 0.30 mg/L) resulted in the highest root number (6.254 roots) and root length (3.837 cm), demonstrating that moderate GA3 and BAP concentrations were optimal for root development in this variety. Treatment 2 (sucrose 80 g/L, GA3 0.25 mg/L, BAP 0.10 mg/L) also promoted good root growth (4.785 roots and a root length of 3.402 cm), though less effective than Treatment 4. Treatment 3 (sucrose 90 g/L, GA3 0.50 mg/L, BAP 0.20 mg/L) showed weaker root growth (5.134 roots and a length of 2.931 cm), further supporting the idea that excessive BAP may not always be beneficial for rooting, as indicated by Zhang et al. (2005). In summary, Treatment 5 consistently produced the best root formation in Ágata (7.100 roots, 5.260 cm). In comparison, Treatment 4 was optimal for Fianna (6.254 roots, 3.837 cm), highlighting the importance of balancing sucrose and plant hormone concentrations to promote efficient root growth. These results support the role of sucrose as an energy source and GA3 and BAP in regulating root development (Kumlay 2014; Pasternak and Steinmacher 2024).

3.4 Optimizing Microtuber Formation: Influence of sucrose, GA_3, BAP, and culture medium on *Ágata* and *Fianna* varieties

The study examined how different concentrations of sucrose, gibberellic acid (GA3), and benzylaminopurine (BAP) affect microtuber production in the Ágata and Fianna varieties. Key factors evaluated included the average number of microtubers, microtuber diameter, and average microtuber weight. The data revealed significant variation in microtuber formation among the different treatments and varieties (Figure 2).

In the Ágata variety, Treatment 2 (sucrose 80 g/L, GA3 0.25 mg/L, BAP 0.10 mg/L) yielded the highest average number of microtubers (2.159), with the largest microtuber diameter (21.7 mm) and the highest average microtuber weight (1.45 g). This indicates that a moderate combination of sucrose and growth hormones significantly enhanced microtuber production in quantity and size. Treatment 4 (sucrose 100 g/L, GA3 0.75 mg/L, BAP 0.30 mg/L) also resulted in a relatively high production of microtubers

Table 5 Effect of different sucrose, GA3, and BAP concentrations on root formation in *Ágata* and *Fianna* varieties.

Variety	Treatment #	Sucrose (g/L)	GA_3 (mg/l)	BAP (mg/l)	Number of Roots	Root length (cm)
Ágata	1	30	0.00	0.00	4.501^{c}	2.325^{d}
	2	80	0.25	0.10	6.233^{b}	3.000^{c}
	3	90	0.50	0.20	3.956^{c}	2.780^{d}
	4	100	0.75	0.30	5.810^{b}	4.125^{b}
	5	110	1.00	0.40	7.100^{a}	5.260^{a}
Fianna	1	30	0.00	0.00	3.234^{d}	2.012^{d}
	2	80	0.25	0.10	4.785^{c}	3.402^{b}
	3	90	0.50	0.20	5.134^{b}	2.931^{d}
	4	100	0.75	0.30	6.254^{a}	3.837^{a}
	5	110	1.00	0.40	4.032^{c}	3.102^{c}

†Averages with the same letter in each column show no significant difference, Tukey sig. $P \leq 0.05$.

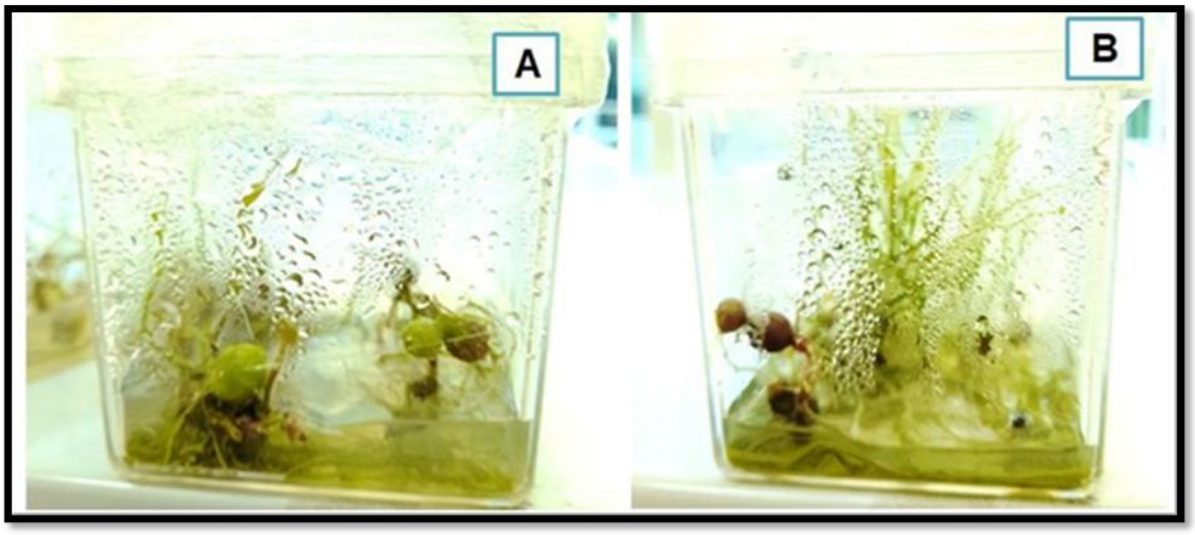

Figure 2 Effect of sucrose, GA_3, and BAP on microtuber number, diameter, and weight in *Ágata* and *Fianna* varieties.

Table 6 Effect of sucrose, GA_3, and BAP on microtuber number, diameter, and weight in *Ágata* and *Fianna* varieties.

Variety	Treatment #	Sucrose (g/L)	GA3 (mg/l)	BAP (mg/l)	Average microtuber number	Microtuber Diameter (mm)	Average microtuber weight (g)
Ágata	1	30	0.00	0.00	1.173^{c}	18.5^{b}	1.23^{ab}
	2	80	0.25	0.10	2.159^{a}	21.7^{a}	1.45^{a}
	3	90	0.50	0.20	1.692^{b}	16.9^{c}	0.98^{c}
	4	100	0.75	0.30	1.404^{c}	20.3^{a}	1.35^{a}
	5	110	1.00	0.40	1.303^{c}	19.1^{ab}	1.10^{b}
Fianna	1	30	0.00	0.00	1.201^{c}	15.2^{d}	0.85^{d}
	2	80	0.25	0.10	2.284^{b}	18.7^{c}	1.10^{c}
	3	90	0.50	0.20	2.692^{a}	22.3^{b}	1.65^{b}
	4	100	0.75	0.30	1.392^{b}	25.0^{a}	2.02^{a}
	5	110	1.00	0.40	1.311^{b}	19.6^{c}	1.35^{c}

†Averages with the same letter in each column show no significant difference, Tukey sig. $P \leq 0.05$.

(1.404) and a microtuber diameter of 20.3 mm, with an average weight of 1.35 g. These results align with previous studies suggesting that increased sucrose concentration, combined with GA3 and BAP, promotes microtuber growth (Mohamed and Girgis 2023). In contrast, Treatment 3 (sucrose 90 g/L, GA3 0.50 mg/L, BAP 0.20 mg/L) produced a significantly lower number of microtubers (1.692) and a smaller diameter (16.9 mm), with a lower average weight (0.98 g). This finding suggests that higher levels of BAP may inhibit microtuber formation, consistent with the observed cytokinin-induced inhibition of tuberization (Kumlay 2014; García-García et al. 2019).

For the Fianna variety, Treatment 3 (sucrose 90 g/L, GA3 0.50 mg/L, BAP 0.20 mg/L) produced the highest number of microtubers (2.692) and the largest microtuber diameter (22.3 mm), along with an average weight of 1.65 g. This indicates that a lower sucrose concentration and a higher BAP concentration effectively promoted tuberization in the Fianna variety. Treatment 4 (sucrose 100 g/L, GA3 0.75 mg/L, BAP 0.30 mg/L) yielded a large microtuber diameter of 25.0 mm and an average weight of 2.02 g, although the average number of microtubers was lower (1.392) compared to Treatment 3. This suggests that higher concentrations of sucrose and GA3 promote fewer microtubers, resulting in larger sizes. In contrast, Treatment 1 (sucrose 30 g/L, GA3 0.00 mg/L, BAP 0.00 mg/L) produced the lowest number of microtubers (1.201) and the smallest diameter (15.2 mm), with the lowest average weight (0.85 g). This outcome shows that low sucrose levels and the absence of growth regulators are not conducive to microtuber development (Hossain et al. 2017; Gautam et al. 2021).

3.5 Varietal Differences

The study found significant differences between the Ágata and Fianna potato varieties. Ágata exhibited higher oxidation rates (63.63%) than Fianna (46.1%) and was more susceptible to contamination, with a 100% contamination rate in contrast to Fianna's 40%. Additionally, Fianna demonstrated better aseptic performance, achieving 60% asepsis, while Ágata recorded 0%. Regarding shoot induction, Fianna responded more effectively to higher concentrations of BAP, whereas Ágata performed better with more balanced hormone treatments. Ágata also showed superior root formation when exposed to higher sucrose treatments, while Fianna thrived with moderate hormone concentrations.

Conclusions

In conclusion, *in vitro* propagation of the potato varieties *Ágata* and *Fianna* demonstrated significant differences in response to various sterilization, hormonal treatments, and culture conditions. The pre-disinfestation and sterilization treatments, particularly with sodium hypochlorite (NaOCl), revealed that *Fianna* exhibited lower oxidation and contamination rates than *Ágata*, which showed higher susceptibility to oxidation and contamination. Fianna responded better to higher BAP concentrations for shoot induction and proliferation, particularly in moderate GA_3 levels, producing more shoots and longer shoots than *Ágata*. Rooting efficiency was also optimized in both varieties with higher sucrose and hormone concentrations, with *Ágata* showing the best results at a higher sucrose concentration of 110 g/L. Microtuber production was most successful in both varieties with moderate sucrose and BAP concentrations, with *Fianna* producing the highest number of microtubers under specific treatment conditions. The results underscore the importance of optimizing sterilization protocols and hormonal treatments to improve the efficiency of *in vitro* propagation and microtuber production for both potato varieties, contributing valuable insights to the micropropagation field.

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Ethical Approval

Not applicable to this study.

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