Bionatura
Ibero American Journal of Biotechnology and Life Sciences

Article

Effect of different phytohormones on *in vitro* multiplication of *Solanum tuberosum* L. var. Cecilia

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RESUMEN

Las fitohormonas se utilizan ampliamente en plantas de *Solanum tuberosum* con la finalidad de acelerar el proceso de multiplicación *in vitro*. El objetivo principal de esta investigación fue evaluar el efecto de diferentes concentraciones de fitohormonas: auxinas (AIA, AIB, ANA), citoquininas (6-BAP, TDZ, Zeatina) y giberelinas (AG3) sobre características morfológicas de *Solanum tuberosum* L. var. Cecilia. Se utilizaron plantas establecidas *in vitro*, la multiplicación *in vitro* se realizó mediante segmentos nodales cultivados en MS + 20 g. L^{-1} de sacarosa + 7 g.L^{-1} de agar + fitohormonas y se mantuvieron bajo condiones de luz blanca fluorescente total. Se evaluó el número de nudos, número de hojas, altura de la planta y número de brotes. A los 21 días al utilizar AIB (0.05 mg.L^{-1} y 0.25 mg.L^{-1}) se incrementó el número de nudos y el número de hojas; mientras que, las concentraciones de Zeatina de 0.05 mg.L^{-1}, 0.1 mg.L^{-1} incrementaron el número de hojas, altura de la planta y número de brotes, adicionalmente la concentración de 0.15 mg.L^{-1} incrementó la altura de planta, con respecto al AG3 la concentración de 0.15 mg.L^{-1} incrementó el número de nudos de plantas de *S. tuberosum* var. Cecilia. En base a los resultados del presente trabajo se concluyó que el AIB, Zeatina y AG3 favorecieron la multiplicación *in vitro* de plantas de *S. tuberosum* var. Cecilia.

Palabras clave: cultivo de tejidos; papa; reguladores de crecimiento; yemas.

ABSTRACT
Phytohormones are widely used in *Solanum tuberosum* plants to accelerate the *in vitro* multiplication process. The main objective of this research was to evaluate the effect of different concentrations of phytohormones: auxins (IAA, IBA, NAA), cytokinins (6-BAP, TDZ, Zeatin), and gibberellins (GA3) on the morphological characteristics of *Solanum tuberosum* L. var. Cecilia. *In vitro* established plants were used, and the *in vitro*

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multiplication was performed using nodal segments cultured in MS medium supplemented with 20 $g.L^{-1}$ sucrose, 7 $g.L^{-1}$ agar, and phytohormones. The plants were maintained under total fluorescent white light conditions. The number of nodes, number of leaves, plant height, and number of shoots were evaluated. At 21 days, the use of IBA (0.05 mg. L^{-1} and 0.25 mg. L^{-1}) increased the number of nodes and the number of leaves. Meanwhile, Zeatin concentrations of 0.05 mg. L^{-1} and 0.1 mg. L^{-1} increased the number of leaves, plant height, and number of shoots. Additionally, the concentration of 0.15 mg. L^{-1} increased the plant height compared to GA3, and the concentration of 0.15 mg. L^{-1} increased the number of nodes in *S. tuberosum var*. Cecilia plants. Based on the results of this study, it was concluded that IBA, Zeatin, and GA3 promoted the in vitro multiplication of *S. tuberosum var.* Cecilia plants.

Keywords: buds, growth regulators, potato, tissue culture.

INTRODUCTION

Solanum tuberosum L., commonly known as potato and belonging to the Solanaceae family[1], stands out as the world's fourth most important food crop due to its high productivity and nutritional value for human consumption[2]. In 2017, global production reached 388 million tons cultivated over 19.5 million hectares[3].

The primary global challenge in potato cultivation lies in reducing yield losses caused by the degeneration of planting material associated with various diseases [4]. The most economically significant losses are caused by late blight (caused by *Phytophthora infestans* de Bary)[5-7]. This disease can devastate crops within two weeks under high humidity conditions [8]. Moreover, it can survive even in adverse conditions and quickly spread through soil, water, rain, and wind[9]. Similarly, viruses pose a significant threat to potato crops worldwide. Viral infections are concerning not only due to the noticeable decline in production but also because the disease often lacks visible symptoms[10].

The known susceptibility of this crop to disease, primarily viral infections, implies a scarcity of high-quality seed, significantly restricting potato production in several countries[12,13]. Acquiring certified seed can be difficult for farmers due to its high cost or limited availability[14]. However, enhancing seed quality through healthy and virus-free potato plantlets and microtubers15 is crucial. This improvement can lead to a yield increase of 15% to 20%[16].

Micropropagation technology represents an alternative to conventional propagation and has proven to be effective in systematically eliminating viral infection[19], ensuring the acquisition of high-quality and disease-free planting material[20-22]. This technology offers a solution to the challenge mentioned above in potato production[23]. It is based on cultivating plant cells, tissues, organs, seeds, protoplasts, or embryos in a sterile environment within a nutrient medium[24,25]. Factors such as temperature, photoperiod, humidity, light, and medium components must be meticulously controlled to provide an optimal growth environment[26,27].

The culture medium is one of the most critical aspects that directly influences the success of the micropropagation technique[28,29]. The addition of plant hormones in appropriate quantities is crucial for regulating plant growth and development[30-32]. Among the growth hormones, cytokinins and auxins are commonly employed[33,34]. Among cytokinins, 6-benzylaminopurine (6-BAP) and kinetin (KIN) stand out for their ability to stimulate cell division and shoot development. On the other hand, key auxins include 1-naphthaleneacetic acid (NAA), indole-3-butyric acid (IBA), and indole-3-acetic acid (IAA), which play a crucial role in the overall development of plants[35,36].

Although phytohormones significantly regulate the growth and development of in vitro-cultured plant tissues[37], their effects can vary considerably depending on factors such as plant species, genotype, and culture conditions. This variability can pose challenges in successfully reproducing plants through micropropagation [38,39]. Another critical aspect is the loss of tissue regeneration capacity over time. Moreover, excessive or inappropriate use of phytohormones can lead to undesired effects, including shoot deformations, hyperhydration (excessive tissue moisture)[41-44], necrosis, or explant oxidation[45].

Based on the issues mentioned earlier, the present study aimed to evaluate the effect of different concentrations of phytohormones, including auxins (IAA, IBA, NAA), cytokinins (6-BAP, TDZ, Zeatin), and gibberellins (GA3), on the number of nodes, number of leaves, number of shoots, and plant height of *Solanum tuberosum* L. var. Cecilia.

MATERIAL AND METHODS

The experiment was conducted at the Plant Biotechnology Laboratory of the Faculty of Agricultural Sciences at the Technical University of Ambato, located in the Cevallos canton, Tungurahua province, from April to September 2022.

Material vegetal

In vitro, established potato plants were utilized for this study. The in vitro multiplication stage involved culturing nodal segments on MS medium supplemented with 20 $g.L^{-1}$ sucrose, 7 $g.L^{-1}$ agar, and varying concentrations of phytohormones (auxins, cytokinins, and gibberellins). The cultures were maintained under full-spectrum fluorescent white light conditions.

Experiment 1: Influence of auxin concentrations (NAA, IBA, IAA) on in vitro multiplication of *Solanum tuberosum* L. var. Cecilia plants.

TREATMENTS	DESCRIPTION
T1	0.1 $mg.L^{-1}$ de ANA
T2	0.25 $mg.L^{-1}$ de ANA
T3	0.5 $mg.L^{-1}$ de ANA
T4	0.75 $mg.L^{-1}$ de ANA
T5	1 $mg.L^{-1}$ de ANA
CONTROL	Without ANA

Table 1. Treatments corresponding to different concentrations of naphthaleneacetic acid (NAA) for the *in vitro* multiplication stage.

TREATMENTS	DESCRIPTION
T1	0.05 $mg.L^{-1}$ de AIB
T2	0.25 $mg.L^{-1}$ de AIB
T3	0.5 $mg.L^{-1}$ de AIB
T4	0.75 $mg.L^{-1}$ de AIB
T5	1 $mg.L^{-1}$ de AIB

CONTROL	Without AIB

Table 2. Treatments corresponding to different concentrations of indole-3-butyric acid (IBA) for the *in vitro* multiplication stage.

TREATMENTS	**DESCRIPTION**
T1	0.5 $mg.L^{-1}$ de AIA
T2	1 $mg.L^{-1}$ de AIA
T3	1.5 $mg.L^{-1}$ de AIA
T4	2 $mg.L^{-1}$ de AIA
T5	2.5 $mg.L^{-1}$ de AIA
CONTROL	Without AIA

Table 3. Treatments corresponding to different concentrations of indole-3-acetic acid (IAA) for the *in vitro* multiplication stage.

Experiment 2: Influence of cytokinin concentrations (6-BAP, TDZ, Z) on the *in vitro* multiplication of *Solanum tuberosum* L. var. Cecilia plants.

TREATMENTS	**DESCRIPTION**
T1	0.3 $mg.L^{-1}$ de 6-BAP
T2	0.4 $mg.L^{-1}$ de 6-BAP
T3	0.5 $mg.L^{-1}$ de 6-BAP
T4	0.6 $mg.L^{-1}$ de 6-BAP
T5	0.7 $mg.L^{-1}$ de 6-BAP
CONTROL	Without 6-BAP

Table 4. Treatments corresponding to different concentrations of 6-benzylaminopurine (6-BAP) for the *in vitro* multiplication stage.

TREATMENTS	**DESCRIPTION**
T1	1 $mg.L^{-1}$ de TDZ
T2	2 $mg.L^{-1}$ de TDZ
T3	3 $mg.L^{-1}$ de TDZ
T4	4 $mg.L^{-1}$ de TDZ
T5	5 $mg.L^{-1}$ de TDZ
CONTROL	Without TDZ

Table 5. Treatments corresponding to different concentrations of thidiazuron (TDZ) for the *in vitro* multiplication stage.

TREATMENTS	**DESCRIPTION**
T1	0.05 $mg.L^{-1}$ de Z
T2	0.10 $mg.L^{-1}$ de Z
T3	0.15 $mg.L^{-1}$ de Z

T4	0.25 mg.L^{-1} de Z
CONTROL	Without Z

Tabla 6. Tratamientos correspondientes a diferentes concentraciones de Zeatina (Z) para la etapa de multiplicación *in vitro*.

Experiment 3: Influence of gibberellin concentrations (GA3) on the *in vitro* multiplication of *Solanum tuberosum* L. var. Cecilia plants.

TREATMENTS	DESCRIPTION
T1	0.15 mg.L^{-1} de AG3
T2	0.20 mg.L^{-1} de AG3
T3	0.25 mg.L^{-1} de AG3
T4	0.30 mg.L^{-1} de AG3
T5	0.35 mg.L^{-1} de AG3
CONTROL	Without AG3

Table 7. Treatments corresponding to different concentrations of gibberellic acid (GA3) for the *in vitro* multiplication stage.

Evaluations

The plant length (cm), number of leaves, number of nodes, and number of shoots per plant were determined at 21 days for each experiment.

Experimental design and statistic analysis

A completely randomized design was employed for each experiment. A control group (without phytohormone application) was included for each type of phytohormone. The data obtained for the variables, including the number of leaves, number of nodes, and number of shoots per plant, were recorded using SPSS version 26.0. Normal distribution was assessed using the Kolmogorov-Smirnov test, and homogeneity of variance was examined using Levene's test. For variables that did not meet these requirements, the Kruskal-Wallis test was used, followed by the Mann-Whitney U test. The significance level was set at 95%.

RESULTS

Experiment 1. Influence of auxin concentrations (NAA, IBA, IAA) on the *in vitro* multiplication of *Solanum tuberosum* L. var. Cecilia plants.

At the 21-day evaluation, the naphthaleneacetic acid (NAA) at the utilized concentrations did not affect the number of nodes, leaves, shoots, and plant height of *S. tuberosum* (Table 8 and Figure 1).

TREATMENTS	NODE NUMBER		LEAVES NUMBER		SHOOTS NUMBER		PLANT HEIGHT (cm)	
	$\bar{X}$	MEAN RANK	$\bar{X}$	MEAN RANK	$\bar{X}$	MEAN RANK	$\bar{X}$	MEAN RANK

MS + 0.1 mg.L^{-1} de ANA	4,7	43,60	6,4	42,30	2,0	43,25	5,0	40,85
MS + 0.25 mg.L^{-1} de ANA	2,5	27,10	3,6	28,20	1,3	31,20	2,9	27,70
MS + 0.5 mg.L^{-1} de ANA	2,9	28,40	3,9	26,90	0,9	25,30	4,0	31,35
MS + 0.75 mg.L^{-1} de ANA	1,8	20,15	2,5	20,70	0,9	31,90	2,4	22,75
MS + 1 mg.L^{-1} de ANA	3,2	30,60	4,6	30,50	0,9	25,30	4,4	33,50
WITHOUT ANA	3,4	33,15	5,2	34,40	1,0	26,05	3,4	26,85

Table 8. Effect of naphthaleneacetic acid (NAA) concentrations on the number of nodes, leaves, shoots, and plant height in the in vitro multiplication of nodal segments of S. tuberosum var. Cecilia at 21 days.

Mean ranks that do not share common letters within the same column significantly differ according to the Kruskal-Wallis test, complemented by the Mann-Whitney U test at a significance level of $p<0.05$, with a sample size n=10

Figure 1. *In vitro* plants of *S. tuberosum* var. Cecilia at the multiplication stage on MS medium with NAA concentrations: 0.1 mg.L-1 (A), 0.25 mg.L-1 (B), Control (C) at 14 and 21 days.

At 21 days, when using 0.05 mg.L^{-1} and 0.25 mg.L-1 of IBA, there was an increase in the number of nodes, number of leaves, and number of shoots per plant, while the plant height variable did not show any differences (Table 9 and Figure 2).

TREATMENTS	NODE NUMBER		LEAVES NUMBER		SHOOTS NUMBER		PLANT HEIGHT (cm)	
	$\bar{X}$	MEAN RANK	$\bar{X}$	MEAN RANK	$\bar{X}$	MEAN RANK	$\bar{X}$	MEAN RANK
MS + 0.05 $mg.L^{-1}$ de AIB	5,4	40,95 a	7,9	42,25 a	2,5	45,45 a	4,9	32,95
MS + 0.25 $mg.L^{-1}$ de AIB	5,8	44,50 a	8,2	44,70 a	1,8	36,55 ab	3,2	25,70
MS + 0.5 $mg.L^{-1}$ de AIB	3,0	25,25 b	4,1	25,00 b	1,2	26,05 b	6,5	43,80
MS + 0.75 $mg.L^{-1}$ de AIB	3,1	23,95 b	4,2	22,40 b	0,9	21,50 bc	3,9	28,40
MS + 1 $mg.L^{-1}$ de AIB	2,7	21,10 b	3,9	21,50 b	1,3	28,35 b	3,7	27,00
WITHOUT AIB	3,7	27,25 b	5,3	27,15 b	1,1	25,10 bc	3,7	25,15

Table 9. Effect of indole-3-butyric acid (IBA) concentrations on the number of nodes, leaves, shoots, and plant height in the *in vitro* multiplication of nodal segments of *S. tuberosum* var. Cecilia at 21 days.

Mean ranks that do not share common letters within the same column significantly differ according to the Kruskal-Wallis test, complemented by the Mann-Whitney U test at a significance level of $p<0.05$, with a sample size n=10.

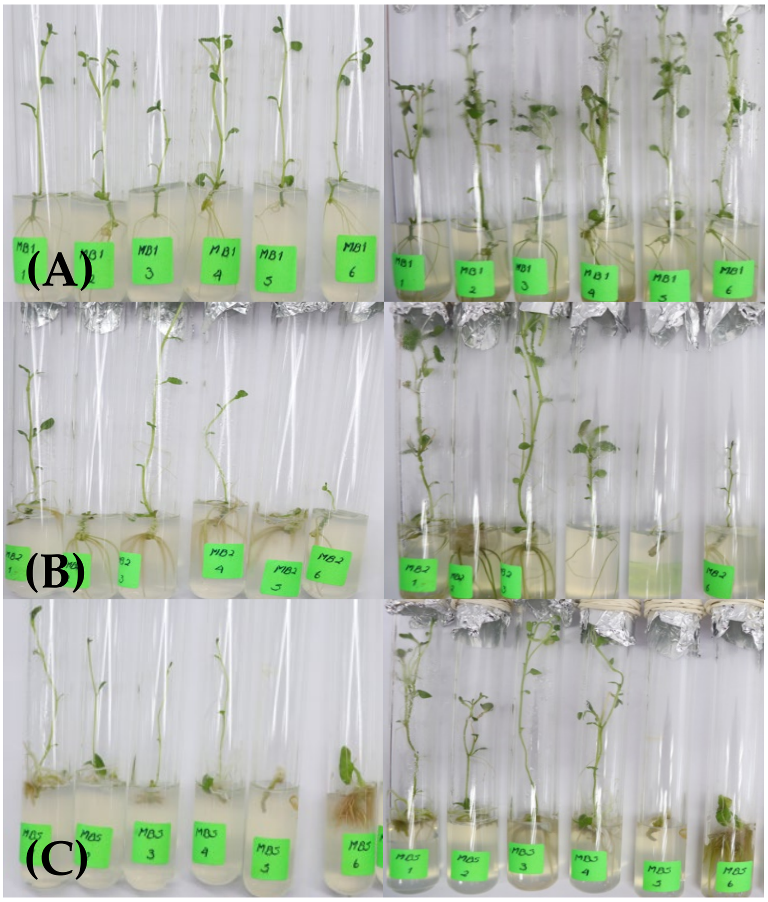

Figura 2. Plantas *in vitro* de *S. tuberosum* var. Cecilia en etapa de multiplicación en MS + concentraciones de AIB: 0.05 $mg.L^{-1}$ (A), 0.25 $mg.L^{-1}$ (B), Control (C) a los 14 y 21 días.

At the 21 day evaluation of the effect of IAA on the *in vitro* multiplication of *S. tuberosum* var. Cecilia, no statistically significant differences were observed among the treatments when evaluating the number of nodes, leaves, shoots, and plant height (Table 10 and Figure 3).

TREATMENTS	NODE NUMBER		LEAVES NUMBE		SHOOTS NUMBER		PLANT HEIGHT (cm)	
	$\bar{X}$	MEAN RANK	$\bar{X}$	MEAN RANK	$\bar{X}$	MEAN RANK	$\bar{X}$	MEAN RANK
MS + 0.5 mg.L^{-1} de AIA	2,9	25,70	4,8	28,45	1,5	29,20	3,2	25,65
MS + 1 mg.L^{-1} de AIA	3,6	28,00	4,7	26,60	1,1	23,80	3,7	25,45
MS + 1.5 mg.L^{-1} de AIA	4,5	32,85	6,3	33,05	1,6	33,30	6,0	37,70
MS + 2 mg.L^{-1} de AIA	4,4	31,95	6,0	29,90	1,5	30,25	5,7	32,80
MS + 2.5 mg.L^{-1} de AIA	4,9	35,35	7,2	34,70	2,1	35,60	5,1	32,35
WITHOUT AIA	3,8	29,15	5,6	30,30	1,5	30,85	4,4	29,05

Table 10. Effect of indole-3-acetic acid (IAA) concentrations on the number of nodes, leaves, shoots, and plant height in the *in vitro* multiplication of nodal segments of *S. tuberosum* var. Cecilia at 21 days.

Mean ranks that do not share common letters within the same column significantly differ according to the Kruskal-Wallis test, complemented by the Mann-Whitney U test at a significance level of $p<0.05$, with a sample size n=10.

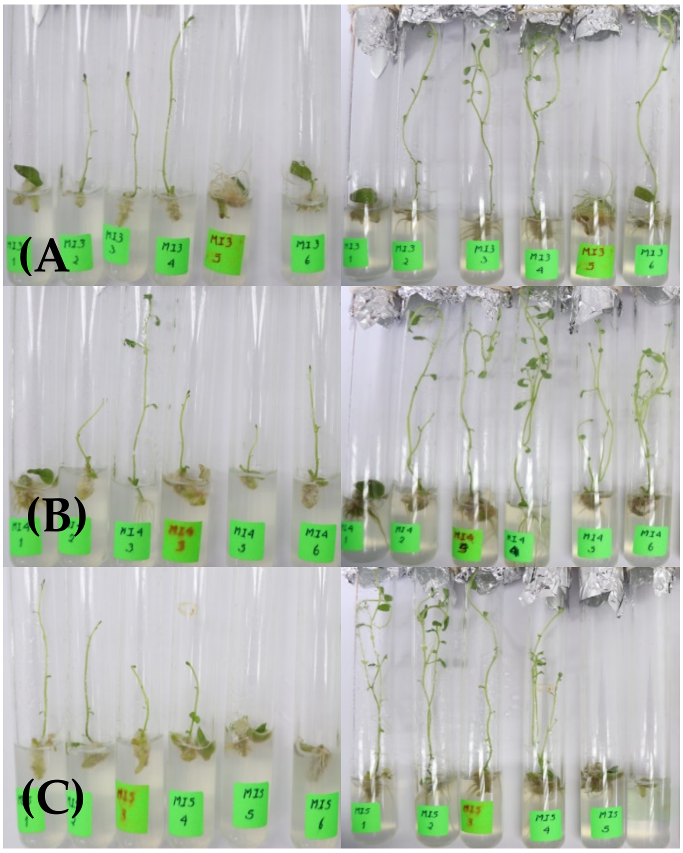

Figura 3. Plantas *in vitro* de *S. tuberosum* var. Cecilia en etapa de multiplicación en MS + concentraciones de AIA: 2 mg.L^{-1} (A) y 2.5 mg.L^{-1} (B), Control (C) a los 14 y 21 días.

Experiment 2: Influence of cytokinin concentrations (6-BAP, TDZ, Z) on the *in vitro* multiplication of *Solanum tuberosum* L. var. Cecilia plants.

At the 21-day evaluation of the effect of 6-BAP on the *in vitro* multiplication of nodal segments of *S. tuberosum* var. Cecilia, no statistically significant differences were observed among the treatments (Table 11 and Figure 4).

TREATMENTS	NUMBER OF NODES		NUMBER OF LEAVES		NUMBER OF SHOOTS		HEIGHT (cm)	
	$\bar{X}$	MEAN RANK	$\bar{X}$	MEAN RANK	$\bar{X}$	MEAN RANK	$\bar{X}$	MEAN RANK
MS + 0.3 mg.L^{-1} de 6-BAP	4,2	35,70	5,7	32,70	2,5	39,60	3,2	33,90
MS + 0.4 mg.L^{-1} de 6-BAP	4,5	35,80	8,2	40,35	2,5	35,75	3,1	34,55
MS + 0.5 mg.L^{-1} de 6-BAP	2,6	21,00	4,2	23,60	1,6	27,95	1,5	18,85
MS + 0.6 mg.L^{-1} de 6-BAP	3,2	25,40	5,1	26,80	1,4	25,20	2,3	26,75
MS + 0.7 mg.L^{-1} de 6-BAP	3,7	31,05	5,8	31,30	1,8	31,15	2,5	28,60
WITHOUT 6-BAP	3,9	34,05	5,3	28,25	1,3	23,35	3,9	40,35

Table 11. Effect of 6-benzylaminopurine (6-BAP) concentrations on the number of nodes, leaves, shoots, and plant height in the *in vitro* multiplication of nodal segments of *S. tuberosum* var. Cecilia at 21 days.

Mean ranks that do not share common letters within the same column significantly differ according to the Kruskal-Wallis test, complemented by the Mann-Whitney U test at a significance level of $p<0.05$, with a sample size n=10.

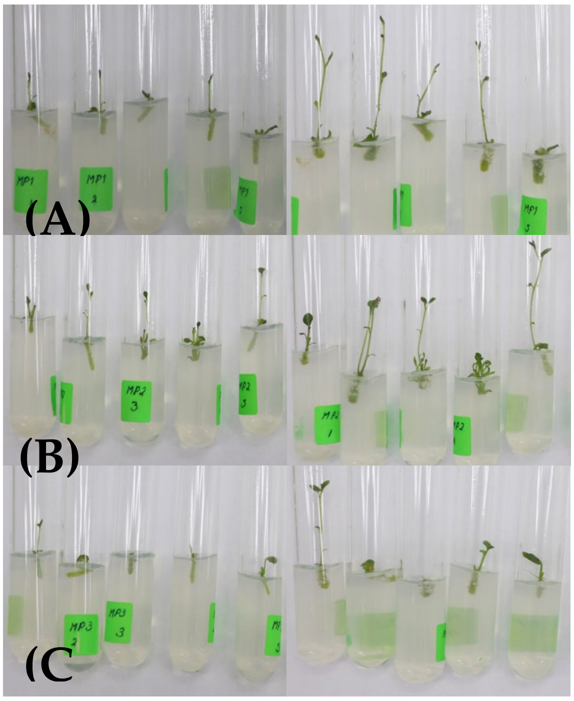

Figura 4. Plantas *in vitro* de *S. tuberosum* var. Cecilia en etapa de multiplicación en MS + concentraciones de 6-BAP: 0.3 mg.L^{-1} (A), 0.4 mg.L^{-1} (B), Control (C) a los 14 y 21 días.

At the 21-day evaluation of the effect of TDZ, it was determined that there were no statistically significant differences when evaluating the number of nodes per plant, number of leaves per plant, number of shoots, and plant height (Table 12 and Figure 5).

TREATMENTS	NUMBER OF NODES		NUMBER OF LEAVES		NUMBER OF SHOOTS		HEIGHT (cm)	
	$\bar{X}$	MEAN RANK	$\bar{X}$	MEAN RANK	$\bar{X}$	MEAN RANK	$\bar{X}$	MEAN RANK
MS + 1 mg.L^{-1} de TDZ	3,5	35,05	5,5	36,60	1,7	36,70	1,9	32,20
MS + 2 mg.L^{-1} de TDZ	3,3	33,50	4,7	31,50	1,5	32,30	2,0	32,00
MS + 3 mg.L^{-1} de TDZ	3,3	32,25	5,3	34,85	1,4	30,60	1,8	30,25
MS + 4 mg.L^{-1} de TDZ	3,2	29,40	4,8	29,45	1,2	28,20	2,1	30,40
MS + 5 mg.L^{-1} de TDZ	2,3	21,35	3,2	18,50	1,0	22,60	0,8	18,40
WITHOUT TDZ	3,3	31,45	5,2	32,10	1,4	32,60	3,4	39,75

Table 12. Effect of Thidiazuron (TDZ) concentrations on the number of nodes, leaves, shoots, and plant height in the *in vitro* multiplication of nodal segments of *S. tuberosum* var. Cecilia at 21 days.

Mean ranks that do not share common letters within the same column significantly differ according to the Kruskal-Wallis test, complemented by the Mann-Whitney U test at a significance level of $p<0.05$, with a sample size n=10.

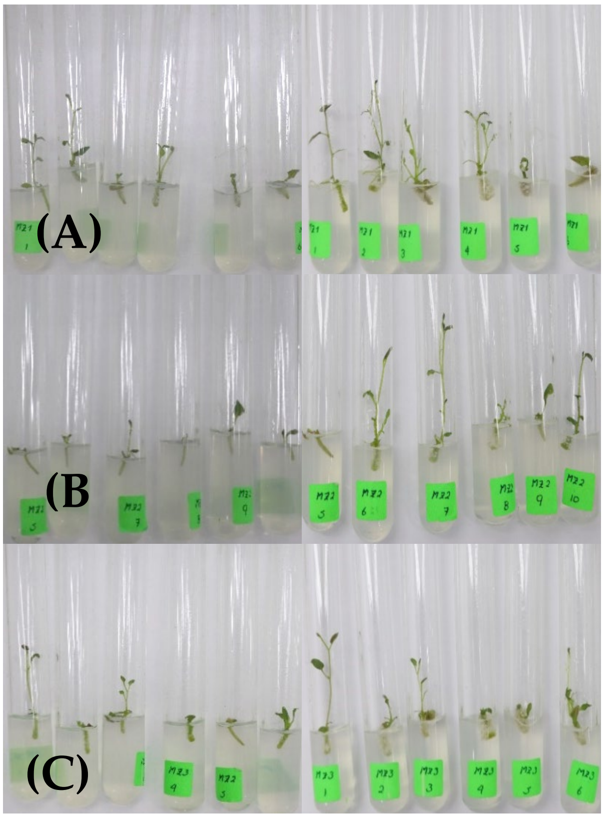

Figure 5. *In vitro* plants of *S. tuberosum* var. Cecilia at the multiplication stage on MS medium with Thidiazuron concentrations: 1 mg.L-1 (A), 2 mg.L-1 (B), Control (C) at 14 and 21 days.

At 21 days, the concentrations of Zeatin at 0.05 mg.L^{-1} and 0.1 mg.L^{-1} increased the number of leaves and number of shoots per plant, except for the concentration of 0.25 mg.L^{-1}. The remaining treatments and the control increased the plant height (Table 13 and Figure 6).

TREATMENTS	NUMBER OF NODES		NUMBER OF LEAVES		NUMBER OF SHOOTS		HEIGHT (cm)	
	$\bar{X}$	MEAN RANK	$\bar{X}$	MEAN RANK	$\bar{X}$	MEAN RANK	$\bar{X}$	MEAN RANK
MS + 0.05 mg.L^{-1} de Z	5,3	31,55 a	7,9	33,70 a	2,2	32,80 a	4,9	25,25 a
MS + 0.10 mg.L^{-1} de Z	4,9	28,80 a	7,3	31,75 ab	2,4	34,75 a	5,3	29,45 a
MS + 0.15 mg.L^{-1} de Z	4,3	27,45 a	5,7	23,00 bc	1,2	19,90 b	4,9	28,85 a
MS + 0.25 mg.L^{-1} de Z	2,2	14,35 b	3,3	15,65 c	1,0	17,55 b	1,8	11,40 b
WITHOUT AIA	4,4	25,35 ab	6,2	23,40 bc	1,4	22,50 b	5,6	32,55 a

Tabla 13. Efecto de las concentraciones Zeatina (Z) sobre el número de nudos, número de hojas, número de brotes y altura de planta en la multiplicación *in vitro* de segmentos nodales de *S. tuberosum* var. Cecilia a los 21 días.

Mean ranks that do not share common letters within the same column significantly differ according to the Kruskal-Wallis test, complemented by the Mann-Whitney U test at a significance level of $p<0.05$, with a sample size n=10.

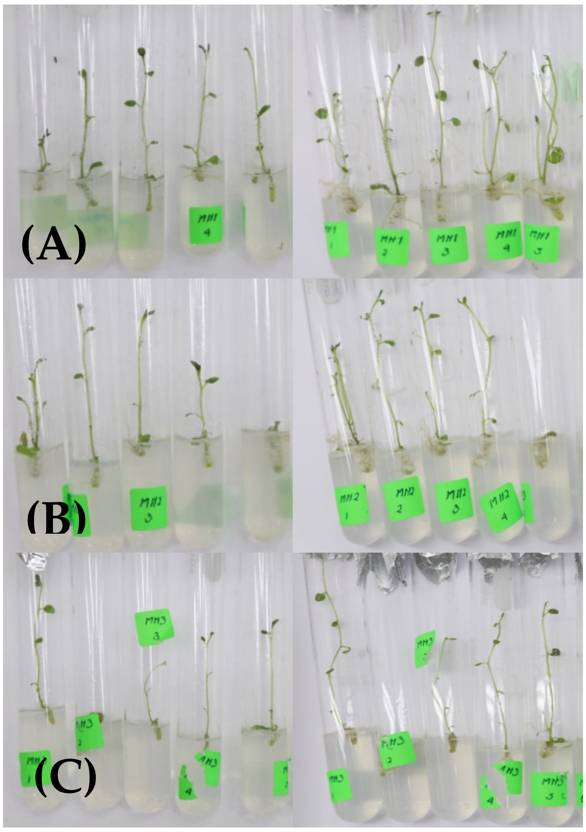

Figure 6. *In vitro* plants of *S. tuberosum* var. Cecilia at the multiplication stage on MS medium with Zeatin concentrations: 0.05 mg.L-1 (A), 0.1 mg.L-1 (B), 0.15 mg.L-1 (C), and Control (D) at 14 and 21 days.

Experiment 3: Influence of gibberellic acid (GA3) concentrations on the *in vitro* multiplication of *Solanum tuberosum* L. var. Cecilia plants.

At the 21-day evaluation of the effect of GA3, the concentration of 0.15 mg.L^{-1} increased the number of nodes per plant; however, no statistically significant differences were observed for the number of leaves, shoots, and plant height (Table 14 and Figure 7).

TREATMENTS	NUMBER OF NODES		NUMBER OF LEAVES		NUMBER OF SHOOTS		HEIGHT (cm)	
	$\bar{X}$	MEAN RANK	$\bar{X}$	MEAN RANK	$\bar{X}$	MEAN RANK	$\bar{X}$	MEAN RANK
MS + 0.15 mg.L^{-1} de AG3	5,7	45,00 a	7,9	43,60	2,0	41,35	6,2	43,50
MS + 0.20 mg.L^{-1} de AG3	3,1	27,35 b	4,4	26,55	1,0	25,75	3,9	31,10
MS + 0.25 mg.L^{-1} de AG3	3,4	31,40 b	4,9	32,75	1,4	33,00	3,7	30,90
MS + 0.30 mg.L^{-1} de AG3	2,8	25,70 b	4,4	27,65	1,1	26,80	3,8	28,75
MS + 0.35 mg.L^{-1} de AG3	2,5	21,50 b	3,7	22,25	0,9	23,35	1,9	21,15
WITHOUT AG3	3,7	32,05 ab	5,2	30,20	1,4	32,75	3,3	27,60

Table 14. Effect of gibberellic acid (GA3) concentrations on the number of nodes, leaves, shoots, and plant height in the *in vitro* multiplication of nodal segments of *S. tuberosum* var. Cecilia at 21 days.

Mean ranks that do not share common letters within the same column significantly differ according to the Kruskal-Wallis test, complemented by the Mann-Whitney U test at a significance level of $p<0.05$, with a sample size n=10.

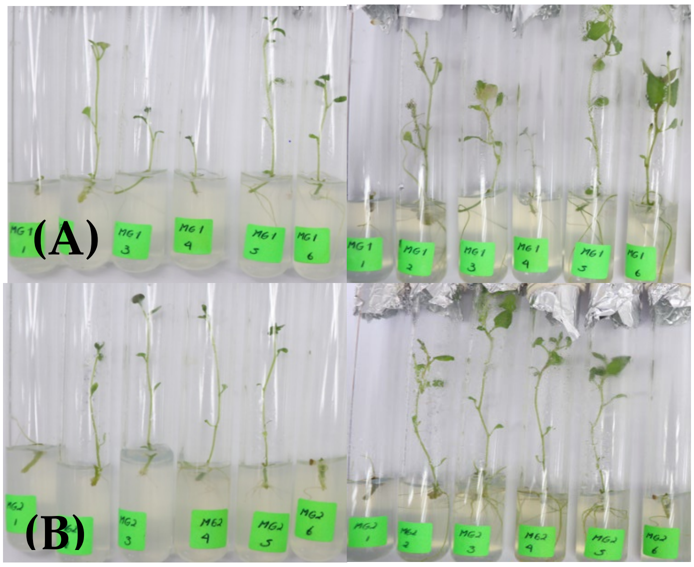

Figure 7. *In vitro* plants of *S. tuberosum* var. Cecilia is at the multiplication stage on an MS medium with GA3 concentrations of 0.15 mg.L^{-1} (A), Control (B) at 14 and 21 days.

DISCUSSION

Influencia de concentraciones de auxinas (ANA, AIB, AIA) sobre la multiplicación *in vitro* de plantas de papa var. Cecilia.

Auxins are plant hormones primarily known for their role in plant root development [46]. In the in vitro cultivation of *S. tuberosum*, adding auxins to the culture medium can enhance the formation of roots in the explants. The concentration and type of auxin used can influence the degree of stimulation of root formation [47]. For instance, naphthaleneacetic acid (NAA) is a commonly used auxin in plant micropropagation and has been shown to induce root formation in various plant species [48], including potatoes.

Auxins can regulate cellular growth by influencing gene expression in cell division and elongation [49]. It has been demonstrated that they can activate the expression of genes responsible for synthesizing structural cellular proteins and enzymes involved in cellular expansion and growth [50]. This regulation directly impacts the proper development of tissues during *in vitro* multiplication [51].

In addition to these direct effects, auxins can interact with other phytohormones, such as cytokinins and gibberellins, to coordinate tissue growth and in vitro culture development [52]. The relationship between different phytohormones and their balance in the culture medium are critical aspects to consider in optimizing the *in vitro* multiplication process of *S. tuberosum* [53].

The concentrations of naphthaleneacetic acid (NAA) and indole-3-acetic acid (IAA) used in the study may have been insufficient or excessive to promote an increase in the number of leaves, nodes, shoots, and plant height [54,55]. If the concentrations were not within the optimal range, it is possible that no effect on *in vitro* multiplication was observed [56,57]. Research has determined that NAA did not influence *in vitro* multiplication of *S. tuberosum* when shoot height and multiplication rate were evaluated [58]. Regarding the effect of indole-3-butyric acid (IBA), low concentrations positively impacted *in vitro* multiplication.

Influence of cytokinin concentrations (6-BAP, TDZ, Z) on the in vitro multiplication of Solanum tuberosum L. var. Cecilia plants.

Cytokinins are phytohormones that play a crucial role in plant growth and development [59], including in vitro multiplication of crops such as *S. tuberosum*. They are commonly used in plant micropropagation, where plant tissue is cultured under controlled conditions [60].

These phytohormones promote cell division and tissue expansion, significantly contributing to *in vitro* multiplication by enabling rapid cell proliferation [61]. They can induce shoot formation and can develop into complete plants when properly cultivated [62]. They are responsible for promoting meristem activity, which are regions of active plant growth, stimulating the production of new buds and shoots [63].

In addition to stimulating vegetative growth and development, cytokinins can delay cellular aging and promote longevity of plant cells [64], which is beneficial for maintaining the health and viability of *in vitro* cultured tissues over extended periods. Research indicates that supplementation of the medium with concentrations of 6-benzylaminopurine (6-BAP) ranging from 0.5 to 1 $mg.L^{-1}$ did not show an apparent effect on *in vitro* multiplication when evaluating the number of nodes and plant height in *S. tuberosum* group *Phureja* [65].

Some authors have noted that *S. tuberosum* cv. Almera and Diamante explants measuring 1 to 2 cm with 2 nodes, cultured in MS medium supplemented with TDZ and auxin concentrations showed positive effects by exhibiting more shoots per explant when using 3.0 $mg.L^{-1}$ of TDZ combined with 0.1 $mg. L^{-1}$ of NAA [67].

However, in this study, high concentrations of Zeatin had no effect when used on the Cecilia variety. This could be attributed to the fact that each variety responds differently to a specific phytohormone.

Research conducted to evaluate the effect of Zeatin on the *in vitro* multiplication of *S. tuberosum* indicated that a medium supplemented with 0.1 mg.L^{-1} of Zeatin + 0.5 mg.L^{-1} of 2,3,5-triiodobenzoic acid (TIBA) promoted an increase in the number of shoots [68]. Furthermore, they noted that higher concentrations inhibited shoot development. The obtained results are consistent with the findings reported by the authors.

Influence of gibberellin concentrations (GA3) on the *in vitro* multiplication of *Solanum tuberosum* L. var. Cecilia plants.

Gibberellic acid (GA_3) can stimulate both cell elongation and division, resulting in increased growth of *in vitro* cultured plant tissues [69]. It is a crucial factor in plant micropropagation, as greater cell growth leads to more shoots and, consequently, a higher multiplication rate [70]. The use of GA_3 in the micropropagation of *S. tuberosum* activates the expression of genes involved in the initiation and development of shoots, leading to increased shoot formation on *in vitro* multiplied plants [71].

GA_3 can influence plant tissue morphogenesis, encompassing the formation of roots, stems, nodes, and leaves. This aspect is crucial for producing healthy seedlings that have a higher chance of surviving during acclimatization [72]. Additionally, GA_3 can affect various metabolic processes such as protein synthesis, photosynthesis, and nutrient uptake, directly contributing to a higher propagation rate under *in vitro* conditions by providing optimal conditions for the growth and development of plant cells [73].

Research conducted for the micropropagation of *S. tuberosum* using a liquid culture medium with different concentrations of phytohormones indicates that MS medium supplemented with 0.25 mg. L^{-1} of GA3, 5 mg. L^{-1} of pantothenic acid, 1 mg. L^{-1} of thiamine and 20 g.L^{-1} of sucrose, under constant agitation, yielded better results at *in vitro* multiplication stage [58]. Similarly, some authors mentioned that a concentration of 0.1 mg. L^{-1} of GA_3 significantly increases plant height and the number of nodes and leaves. It was also observed that high concentrations of GA_3 lead to a decrease in leaves number and number of nodes per explant [74]. These findings are consistent with the results obtained in the present study.

CONCLUSIONS

The present study concluded that specific phytohormone treatments positively affected the *in vitro* multiplication of *Solanum tuberosum* L. var. Cecilia. The auxin IBA at concentrations of 0.05 mg.L^{-1} and 0.25 mg.L^{-1} increased the number of nodes and leaves. The cytokinin Zeatin at concentrations of 0.05 mg.L^{-1} and 0.1 mg.L^{-1} significantly improved leaf number, plant height, and shoot proliferation, while 0.15 mg.L^{-1} Zeatin promoted increased plant height. The gibberellin GA3 at a concentration of 0.15 mg.L^{-1} notably increased the number of nodes. These findings offer valuable insights for optimizing *in vitro* multiplication protocols for *Solanum tuberosum* L. var. Cecilia contributes to the efficient production of disease-free potato plantlets and promotes sustainable agricultural practices.

Supplementary Materials: The following are available online at www.revistabionatura.com/xxx/s1, Figure S1: title, Table S1: title, Video S1: title.

Author Contributions: Conceptualization, Michel Leiva Mora, Justo Antonio Rojas Rojas and Catherine Lizzeth Silva Agurto; methodology, Catherine Lizzeth Silva Agurto, Yosbel Lazo Roger and Michel Leiva Mora; software, Catherine Lizzeth Silva Agurto and Freddy Santiago Córdova Frías; validation, Catherine Lizzeth Silva Agurto, Michel Leiva Mora, and Willian Ernesto Tipán Chinachi; formal analysis, Catherine Lizzeth Silva Agurto and Willian Ernesto Tipán Chinachi; investigation, Catherine Lizzeth Silva Agurto, Alfredo Jiménez González, Miguel Angel Osejos Merino, Rene Nazareno Ortiz and Michel Leiva Mora; resources, Catherine Lizzeth Silva Agurto, Miguel Angel Osejos Merino and Michel Leiva Mora; data curation, Freddy Santiago Córdova Frías, Justo Antonio Rojas Rojas and Tannia Elizabeth Gómez Pinto; writing – original draft preparation, Catherine Lizzeth Silva Agurto and Michel Leiva Mora; writing – review and editing, Catherine Lizzeth Silva Agurto and Michel Leiva Mora; visualization, Willian Ernesto Tipán Chinachi, Rene Nazareno Ortiz and Freddy Santiago Córdova Frías; supervision, Catherine Lizzeth Silva Agurto; project administration, Michel Leiva Mora; funding acquisition, Catherine Lizzeth Silva Agurto and Michel Leiva Mora. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Acknowledgments. Se thank the support provided by the research coordinator, Deysi Alexandra Guevara Freire, and the authorities of the Faculty of Agricultural Sciences and Research and Development Directorate (DIDE-FCAGP) at the Technical University of Ambato. We also acknowledge the recognizing and supporting the efforts made in this project, which was self-funded by Engineer Catherine Silva Agurto and Doctor Michel Leiva Mora.

Conflicts of Interest: The authors declare no conflicts of interest.

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Received: April 14, 2024 / **Accepted**: July 18, 2024 / **Published:** 15 September 2024

Citation: Silva Agurto C L, Leiva Mora M, Córdova Frías F S, Tipán Chinachi W E, Gómez Pinto T E, Jiménez González A, Osejos Merino M A, Nazareno Ortiz R, Lazo Roger Y, Rojas Rojas J A. Effect of different phytohormones on in vitro multiplication of Solanum tuberosum L. var. Cecilia.

Bionatura journal. 2024;1(3):22. doi: 10.70099/BJ/2024.01.03.22

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Peer review information. Bionatura thanks anonymous reviewer(s) for their contribution to the peer review of this work using https://reviewerlocator.webofscience.com/

ISSN.3020-7886