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Effects of plant growth regulators on *in vitro* propagation of *Solanum curtilobum*, a threatened bitter potato

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Abstract

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Solanum curtilobum Juz. & Bukasov is a bitter potato, scarcely cultivated and placed as a vulnerable species. The effects of plant growth regulators (PGR) were evaluated to approach its micropropagation using Murashige & Skoog medium supplemented with sucrose 3%, phytagel 0.3% adjusted pH 5.7, 16/8 h photoperiod and at 20°C. The shoots of greenhouse plants were disinfected with 0.50, 0.75, and 1.0% NaClO. PGRs applied for multiplication were benzylaminopurine (BAP) or gibberellic acid (GA_3) at 0.5, 1.0, 1.5, 2.0 mgL^{-1}, and 1.5 mgL^{-1} BAP + GA_3 at 0.5, 1.0, 1.5, 2.0 mgL^{-1}, whereas indoleacetic acid (IAA) at 0.5, 1.0, 1.5, 2.0 mgL^{-1} was used for rooting. In all experiments, PGR-free treatments were included as a control. There were no differences between the disinfection treatments and high explants survival percentage (> 80%), and reduced contamination (< 12%) were observed. In the multiplication, BAP (0.5–2.0 mgL^{-1}) and GA_3 (0.5 mgL^{-1}) induced a higher number of axillary buds per explant, but combinations of both PGR did not gain better results. However, BAP (0.5–2.0 mgL^{-1}) reduced areal part length, and GA_3 or GA_3 + BAP did not affect aerial plant length. For rooting, the indole-acetic acid (IAA) increased the number of roots achieving better results at 2.0 mgL^{-1}, also this IAA concentration did not negatively affect the aerial growth. This is the first report about BAP, GA_3, and IAA effects during *in vitro* growth and development of *S. curtilobum*. It could be useful to approach methodologies for its conservation by micropropagation and other future genetic research, where *in vitro* culture will be required.

Keywords: tissue culture; plant growth regulators; micropropagation; shoots; plants

Introduction

Potato is the common name of several species of *Solanum* genus, which have been cultivated in the Andean mountains since 7000 years ago (de Haan et al., 2006). Among diverse species, *S. tuberosum* has been extended around the world, and is considered the third crop of economic importance, further *S. tuberosum* subsp. *tuberosum* is cultivated in more than 130 countries, and is the fourth crop of world importance after corn, wheat, and rice (de Haan et al., 2006; Gavrilenko et al., 2013). Besides, some wild *S. tuberosum* subspecies from Peru, Bolivia, and Ecuador

grow under diverse habitats, including extreme conditions like low temperatures and drought, and exhibit resistance to diseases and plague, placing as attractive gene sources useful for potato genetic improvement by traditional or modern techniques (Hijmans et al., 2003). However, some cultivated Andean potatoes belong to other species, such as *S. goniocalyx*, *S. stenotomum*, *S. chaucha*, *S. phureja*, *S. curtilobum*, *S. jueepczukii*, and *S. ajanhuiri*, which were originated by hybridizations with wild or cultivated close species or subspecies (de Haan et al., 2006). These species constitute an interesting source of genetic biodiversity.

Andean population diet and economy are based on potatoes addressing 80% of its agricultural land to culture *S. tuberosum* and native potatoes belonging to *S. curtilobum*, *S. juzepczukii*, and *S. ajanhuiri* (Brown, 1999; Fonseca et al., 2014). *Solanum curtilobum* and *S. juzepczukii* are called bitter species due to their high contents of glycoalkaloids (de Haan and Rodriguez, 2016), like demissine (Kozukue et al., 2008) that place them at a disadvantage by their unpleasant taste driving them to reduced culture and biodiversity lost. However, they are disease-resistant and cold-resistant potatoes from the Andes (Yoshikawa and Apaza, 2020). *Solanum curtilobum* is a pentaploid and high-Andean native potato that grows over 3400 to 4100 m above sea level (m.a.s.l.) from Ancash (Perú) to Cochabamba and Potosí (Bolivia) (de Haan and Rodriguez, 2016; Ochoa, 2001), and commonly named as "ancu" in Ancash. It is used for the preparation of traditional and ancestral foods (de Haan and Rodriguez, 2016), such as "tocosh" an anaerobic fermented food that is consumed as porridge or "mazamorra" (Jiménez et al., 2018). In South Peruvian Andean, it is known as *Ruki* (similar to *S. juzepczukii*), and used to prepare "chuño" by a freeze-dried technique that removes its poison glycoalkaloids making it edible and storable for long term (Brown, 1999; de Haan et al., 2010; Yoshikawa and Apaza, 2020).

Western South America is the center of potatoes' primary origin and diversity, so the genes of wild potatoes offer attractive prospecting options, pre-breeding, and niche market development (de Haan and Rodriguez, 2016). However, genetic resources from bitter potato species are lost due to limited traditional use and global warming that is changing their ecosystem conditions. Therefore, since the plant tissue culture is a frequent technique, used to obtain phytosanitary quality potatoes contributing to plant propagation and conservation (de Morais et al., 2018), this study aimed to evaluate the effect of some plant growth regulators (PGR) on *in vitro* culture of bitter potato *S. curtilobum* "ancu", as an alternative for its preservation as a genetic resource.

Materials and Methods

Plant material

Stems of *S. curtilobum* were collected from greenhouse plants. Vigorous and healthy samples were selected as donor of explants (nodal segments). They were placed into sterile box and brought to the laboratory for its immediate disinfection.

Basal culture medium and environment condition for in vitro culture

Basal culture medium (BCM) was prepared with Murashige & Skoog (MS) salts supplemented with sucrose 3% and phytagel 0.3%, adjusted at pH 5.67, and sterilized at 121°C for 15 min and 1.5 atm. Culture environment condition included 16 light/8 dark hours photoperiod and ambient temperature (20°C approx.). BCM was supplemented with PGR depending on the specific experiment: benzylaminopurine (BAP), gibberellic acid (GA_3), indole-acetic acid (IAA).

Disinfection and in vitro *culture establishment*

Samples (3–4 cm) were washed with running water and soap solution (1%) in continuous stirring (100 rpm) three times (10 min each). Disinfection was carried out in a sterile chamber, clean samples were immersed in alcohol (70%) for 30 s, followed by washing with sterile distilled water, submerging in sodium hypochlorite (NaClO) (0.50, 0.75, or 1.0%) for 10 min, and washing for three times with sterile distilled water by 3 min each. The sterile samples were cut to 1.0 cm approx., including an axillary bud (shoot). These explants were placed into vials with 2 ml of BCM and incubated for 28 days. Two blocks (50 units each) were performed for each treatment. The survival and contamination percentages were calculated. Seedlings without contamination were transferred to magentas with BCM for 60 days for their *in vitro* culture establishment.

Effect of BAP, GA_3, and BAP+GA_3 on shooting

Seedlings from *in vitro* culture establishment were cut 1.0 cm approx., including an axillary bud. Each piece was placed in 20 × 150 mm tubes with 5 ml BCM-PGR (0.5, 1.0, 1.5, or 2.0 mgL^{-1} BAP or GA_3). BCM was included as a PGR-free control. In total, there were 20 replicates for each treatment. Next, another experiment with selected BAP concentration was performed in combination with GA_3 (0.5, 1.0, 1.5, 2.0 mgL^{-1}). Explants were cultured for 28 days under conditions established before, and PGR effects were evaluated at 7, 14, 21, and 28 days considering the length of the aerial part and the number of axillary buds.

Effect of IAA on rooting and aerial growth

Explants from *in vitro* culture establishment (1.0 cm aprox. with an axillary bud) were cultured in glass magenta (55 mm diameter x 90 mm height) containing BCM (20 mL), supplemented with IAA (0.5, 1.0, 1.5, and 2.0 mgL^{-1}). A treatment with PGR-free BCM and two blocks with ten plants per magenta for each treatment were performed. Explants were grown for 21 days. It was evaluated root number, primary root length, stem length, and number of buds at 7, 14, and 21 days.

Statistical analysis

The completely randomized design and block randomized design were used depending on the experiments. The analysis of variance (ANOVA) and Duncan's mean comparison test with $p < 0.05$ were performed. The log (y) for correction data was used for plant size, √y correction data for number of buds, log (y + 1) for length of roots, and √ (y + 1) for number of roots.

Results and Discussion

Disinfection of plant material

Disinfection is a necessary step for the introduction and establishment of *in vitro* tissue plant culture. Different methodologies and compounds are used according to the contamination degree of explants, where the disinfection and survival percentages were evaluated to find an optimum protocol. *S. curtilobum* was disinfected with ethanol 70%, followed by low NaClO (0.5–1.0%), due to be collected from a greenhouse, achieving disinfection and survival over 90 and 85%, respectively, in all treatments (Figure 1A-1B). Xhulaj & Gixhari (2018) found that HCl_2 (1.0%) was more effective disinfectants than NaClO, but they obtained 57% of survival in *S. tuberosum* explants. Similarly, Naheed et al. (2006) disinfected some cultivars of *S. tuberosum* with calcium hypochlorite (5%) for 15 min reaching 63–77% of explant regeneration. Comparing these results, survival percentages with HCl_2 and $Ca(ClO)_2$ were less than NaClO, used for the disinfection of *S. curtilobum*.

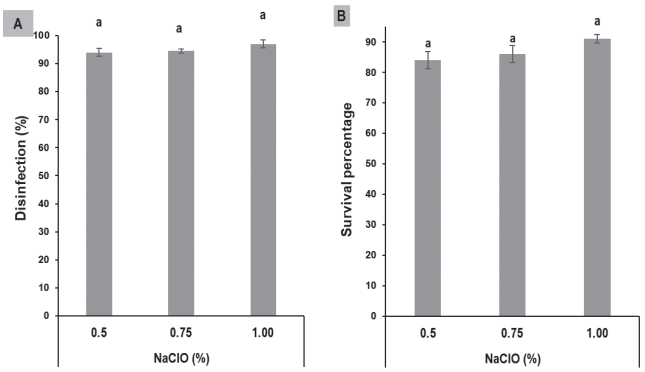

Fig. 1. Disinfection of *Solanum curtilobum* nodal explants with ethanol (70%), followed by NaClO: A: disinfection percentage; B: survival percentage. Values represent the mean of two replicates with 50 units, letters groups according to Duncan test ($p < 0.05$)

Effect of BAP, GA_3, and BAP+GA_3 on shooting

Increasing the number of shoots or axillary buds to obtain more micro-cuttings faster, is a priority of micropropagation. Consequently, effects on the shooting of BAP and GA_3 were evaluated. BAP is a cheaper cytokinin and consequentially widely used for shooting, but unfortunately could cause adverse effects, such as shoot necrosis, problems in greenhouse acclimatization or rooting inhibition (Plíhalová et al., 2016). In this research, *S. curtilobum* axillary buds per seedling were increased with BAP (1.0–1.5 mgL^{-1}) at 21 days, BAP (0.5–2.0 mgL^{-1}) at 28 days, and GA_3 0.5 mgL^{-1} at 28 days, without statistic differences among them (Table 1). However, aerial part length in BAP at 28 days was less than GA_3 and control treatments, where better size was achieved with GA_3 (1.0 mgL^{-1} at 14 days and 0.5–2.0 mgL^{-1} at 21–28 days), and control without PGR (Table 1, Figure 2A-B). Besides, BAP (0.5–2.0 mgL^{-1}) at 28 days showed slight effects on reducing the length of *S. curtilobum* and increasing the axillary bud number. Moreover, GA_3 is used in *in vitro* tissue culture of some *S. tuberosum* varieties (Ali et al., 2018; Xhulaj and Gixhari, 2018), breaks seed dormancy, improves plant growth and development (Ali et al., 2018), by stimulating the photosynthesis and nutrient uptake (Masood et al., 2016). Then, the axillary bud number of *S. curtilobum* was slightly increased with GA_3 at 0.5 mg L^{-1} up to 4 buds in 28 days of exposure, but GA_3 at higher concentrations (1–2 mgL^{-1}) reduced it. Ali et al. (2018) found similar results in *S. tuberosum*, where GA_3 1.0 mgL^{-1} induced shooting, but higher concentration of this phytohormone reduced the number of nodes, and increased the plant length.

Besides, BAP and GA_3 in combination, are used to regenerate potato explants, i.e. Rawat et al. (2017) achieved direct regeneration of *S. tuberosum* "Kufri Jyoti" from internodes using BAP (3 mgL^{-1}) + GA_3 (1 mgL^{-1}). Then, the effects of BAP + GA_3 on *S. curtilobum* shooting were evaluated. BAP at 1.5 mg L^{-1} concentration was selected according to previous results, and was combined with GA_3 (0.5–2.0 mg L^{-1}), but they did not show different effects on axillary bud number and explant length at 28 days with results obtained for BAP 1.5 mg L^{-1} alone (Table 2, Figure 2C). Further none per-

Table 1. Length of aerial part and number of axillary buds of *Solanum curtilobum* seedlings treated with BAP or GA_3

Treatment, mg/L		Time, days			
		7	14	21	28
Number of axillary buds					
Control		1 ± 0.00^{k}	2 ± 0.68^{k}	$3 \pm 1.10^{e,f,g}$	$3 \pm 0.80^{c,d,e,f}$
BAP	0.5	1 ± 0.00^{k}	$2 \pm 0.52^{i,j,k}$	$3 \pm 0.64^{b,c,d,e,f}$	$3 \pm 1.35^{a,b,c,d,e}$
	1	1 ± 0.00^{k}	$2 \pm 0.98^{g,h,i}$	$3 \pm 1.00^{a,b,c,d}$	$4 \pm 1.07^{a,b}$
	1.5	1 ± 0.00^{k}	$2 \pm 0.94^{j,k}$	$3 \pm 0.99^{a,b,c,d}$	$4 \pm 0.92^{a,b}$
	2	1 ± 0.00^{k}	2 ± 0.50^{k}	$3 \pm 0.75^{b,c,d,e,f}$	$4 \pm 0.81^{a,b,c}$
GA_3	0.5	1 ± 0.44^{k}	$2 \pm 0.94^{i,j,k}$	$3 \pm 0.83^{d,e,f,g}$	4 ± 0.99^{a}
	1	1 ± 0.31^{k}	2 ± 0.50^{k}	$2 \pm 0.60^{h,i,j}$	$3 \pm 0.93^{f,g,h}$
	1.5	1 ± 0.00^{k}	1 ± 0.47^{k}	2 ± 0.46^{k}	$3 \pm 0.88^{g,h,i}$
	2	1 ± 0.22^{k}	2 ± 0.60^{k}	$2 \pm 0.64^{j,k}$	$2 \pm 1.10^{h,i,j}$
Length of aerial part, mm					
Control		$4.85 \pm 3.00^{n,o}$	$22.00 \pm 12.3^{j,k}$	$41.30 \pm 17.48^{d,e,f,g}$	$51.25 \pm 24.26^{a,b,c,d,e,f}$
BAP	0.5	6.50 ± 4.30^{n}	$29.35 \pm 10.39^{h,i}$	$41.80 \pm 10.00^{c,d,e,f}$	$44.15 \pm 10.71^{b,c,d,e,f}$
	1	4.70 ± 2.83^{o}	$22.50 \pm 7.48^{i,j}$	$33.95 \pm 6.35^{f,g,h}$	$37.55 \pm 4.36^{d,e,f,g}$
	1.5	$5.05 \pm 3.44^{n,o}$	$18.20 \pm 5.80^{j,k}$	$28.50 \pm 5.75^{g,h,i}$	$34.25 \pm 4.41^{f,g,h}$
	2	3.40 ± 1.47^{o}	$16.35 \pm 4.43^{k,l}$	$27.05 \pm 6.57^{h,i}$	$35.55 \pm 3.66^{e,f,g}$
GA_3	0.5	10.95 ± 6.91^{m}	$39.75 \pm 9.24^{c,d,e,f}$	$51.40 \pm 11.87^{a,b,c,d}$	$56.35 \pm 12.62^{a,b}$
	1	15.05 ± 10.51^{l}	$46.00 \pm 7.62^{a,b,c,d,e}$	$57.25 \pm 11.28^{a,b}$	64.05 ± 15.67^{a}
	1.5	7.25 ± 7.24^{n}	$38.70 \pm 8.78^{d,e,f,g}$	$49.60 \pm 9.48^{a,b,c,d,e}$	58.95 ± 13.78^{a}
	2	12.90 ± 8.63^{m}	$38.50 \pm 8.86^{d,e,f,g}$	$49.85 \pm 11.25^{a,b,c,d}$	$54.65 \pm 15.13^{a,b,c}$

Values represent the mean ± DS of 20 replicates, letters groups according to Duncan test ($p < 0.05$). The data of number of nodes were corrected with and length of plant by log (y), methodology for their statistical analysis. BAP, benzylaminopurine. GA_3, gibberellic acid.

formed combination treatments showed better results than those found with BAP or GA_3. Contrary, (Rout et al., 2022; Xhulaj and Gixhari, 2018) found that BAP + GA_3 (0.25 mg L^{-1}) increased shooting of *S. tuberosum* "excuisita" and "bergerac", while combination of BAP with KIN improved bud development of *S. tuberosum* "kufri lima" (Rout et al., 2022).

The effect of BAP and GA_3 on rooting was not measured in this part, but it was observed that explants development roots in PGR-free and GA_3 treatments, contrary the presence of BAP inhibited rooting (Figure 2A-C) exhibiting a rooting negative effect (Plíhalová et al., 2016) as additional finding.

Effect of IAA on rooting

Auxins are the phytohormone that regulates root development, primary and lateral roots, and it is involved in other plant development processes like embryogenesis, organogenesis, tissue patterning, and tropisms (Gonin et al., 2019; Kondhare et al., 2021). In this research, IAA (2.0 mgL^{-1}) increased the development of lateral roots of *S. curtilobum* (Table 3, Figure 2D). Similarly, Zhang et al. (2005) found that increasing the concentration of IAA in the culture medium had direct effects on number and weight of *S. tuberosum* roots. Furthermore, rooting could be improved using a combination of auxins with other phytohormones or PGR, i.e. Kumlay (2014) obtained higher number of roots (27.00) in *S. tuberosum* cv. "caspar" using GA_3 (0.25 mgL^{-1})+IBA (1 mgL^{-1}).

However, the root length of *S. curtilobum* was better in free-PGR culture medium than medium with IAA (2.0 mgL^{-1}) (Table 3). Zhang et al. (2005) found that the addition of exogenous IAA has an inverse effect on root length, then the absence of IAA could have allowed more elongation of *S. curtilobum* roots.

About the aerial parts of *S. curtilobum*, higher shoot length was achieved with 2.0 mgL^{-1} of IAA at 21 days, whereas no difference was observed in the number of axillary buds among all treatments (Table 3). This could be due to IAA promoting the growth and development of stems and roots (Zhao, 2010). Ghaffoor et al. (2003) found that the addition of exogenous IAA in *in vitro* culture of *S. tuberosum* improved the length of the stem, and increased the number of nodes. In addition, combination of plant growth regulators could be used, i.e. Megrelishvili et al. (2016) found that BAP (1.0 mgL^{-1}) + IBA (0.05 mgL^{-1}) promoted better growth of *S. tuberosum* cv. "nevsky", "riviera", and "zefira" achieving 5, 6, 9 shoots per plant and 68.5, 83, 100 rooting percentages, respectively.

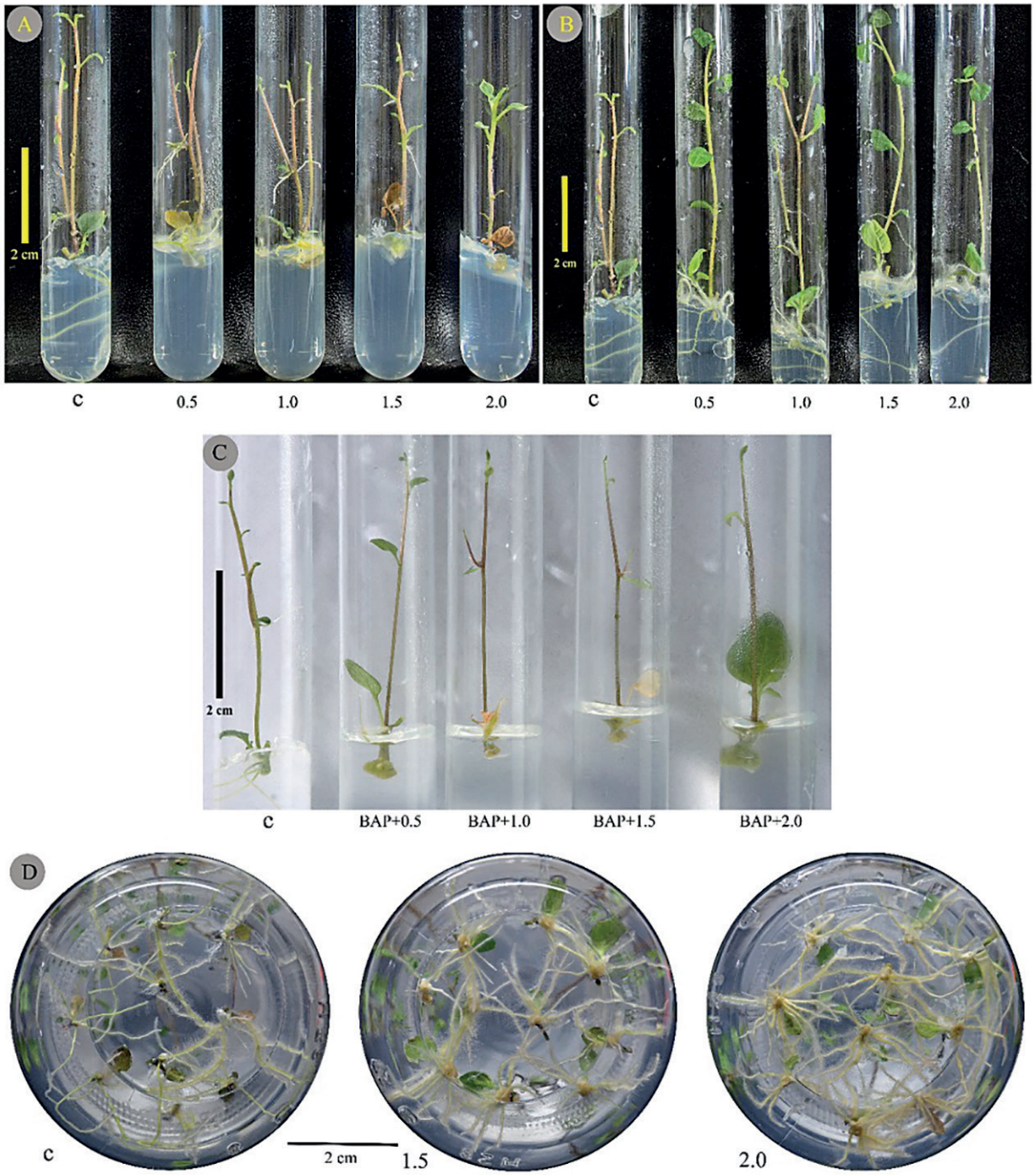

Fig. 2. Effect of plant growth regulator (PGR) during *in vitro* culture of *S. curtilobum:* A: seedlings at BAP (0 – 2.0 mgL^{-1}) by 28 days; B: seedlings at GA_3 (0 – 2.0 mg L^{-1}) by 28 days; C: seedlings at BAP (1.5 mgL^{-1}) + GA_3 (0 – 2.0 mgL^{-1}) by 28 days; D: roots seedlings at IAA (0 – 2.0 mgL^{-1}) by 28 days; c, culture without PGR. B, BAP

Table 2. Length and number of axillary buds of *Solanum curtilobum* on BAP + GA_3

Treatment, mg/L		Time, days			
		7	14	21	28
Number of axillary buds					
BAP + GA_3	1.5 + 0.0	1 ± 0.00 ^{k}	2 ± 0.94 c	3 ± 0.99 ^{b}	4 ± 0.92 ^{a}
	1.5 + 0.5	1 ± 0.00 ^{c}	2 ± 0.51 ^{c}	2 ± 0.51 ^{c}	3 ± 0.60 ^{a}
	1.5 + 1.0	1 ± 0.00 ^{c}	1 ± 0.51 ^{c}	2 ± 0.72 ^{b}	3 ± 0.75 ^{a}
	1.5 + 1.5	1 ± 0.00 ^{c}	1 ± 0.44 ^{c}	2 ± 0.55 ^{c}	3 ± 0.69 ^{a,b}
	1.5 + 2.0	1 ± 0.00 ^{c}	2 ± 0.60 ^{c}	2 ± 0.60 ^{b}	3 ± 0.50 ^{a,b}
Length of areal part, mm					
BAP + GA_3	1.5 + 0.0	5.05 ± 3.44 ^{f}	18.20 ± 5.80 ^{e}	28.50 ± 5.75 ^{a,b,c}	34.25 ± 4.41 ^{a,b}
	1.5 + 0.5	5.95 ± 4.89 ^{f}	19.20 ± 5.27 ^{e}	26.60 ± 5.24 ^{b,c,d}	32.95 ± 4.82 ^{a,b}
	1.5 + 1.0	4.90 ± 2.20 ^{f}	17.25 ± 4.76 ^{e}	25.10 ± 5.67 ^{c,d}	31.30 ± 5.10 ^{a,b,c}
	1.5 + 1.5	4.10 ± 2.36 ^{f}	18.10 ± 5.96 ^{e}	27.45 ± 7.21 ^{b,c,d}	34.70 ± 7.71 ^{a,b}
	1.5 + 2.0	4.85 ± 4.70 ^{f}	22.90 ± 7.61 ^{d,e}	31.10 ± 4.09 ^{a,b,c}	36.75 ± 4.23 ^{a}

Values represent the mean ± DS of 20 replicates, letters groups according to Duncan test ($p < 0.05$). The data of number of nodes were corrected with and length of plant by log (y), methodology for their statistical analysis. BAP, benzylaminopurine. GA_3, gibberellic acid

Table 3. Effects of IAA on develop of aerial part and rooting of *S. curtilobum*

Treatment, mg/L		Time, days					
		Length of root*, mm			Number of root/plant		
		7	14	21	7	14	21
Control	0.0	5.44 ± 1.63 ^{h}	15.60 ± 2.01 ^{d}	26.00 ± 2.75 ^{a}	1.0 ± 0.59 ^{h}	4.0 ± 0.98 ^{g}	7.0 ± 1.36 ^{c,d}
IAA	0.5	0.00 ± 0.00 ^{i}	7.75 ± 1.86 ^{f}	18.35 ± 4.17 ^{c}	0.0 ± 0.00 ^{i}	5.0 ± 1.33 ^{f}	8.0 ± 1.07 ^{b,c}
	1.0	0.00 ± 0.00 ^{i}	6.65 ± 2.43 ^{g}	19.30 ± 2.49 ^{c}	0.0 ± 0.00 ^{i}	6.0 ± 2.01 ^{e,f}	8.0 ± 1.10 ^{b,c}
	1.5	0.00 ± 0.00 ^{i}	7.95 ± 2.24 ^{e,f}	19.60 ± 1.93 ^{c}	0.0 ± 0.00 ^{i}	6.0 ± 1.88 ^{e}	8.0 ± 1.73 ^{b}
	2.0	0.00 ± 0.00 ^{i}	8.75 ± 1.71 ^{e}	23.15 ± 2.60 ^{b}	0.0 ± 0.00 ^{i}	7.0 ± 1.89 ^{d}	10.0 ± 2.05 ^{a}
		Length of aerial plant, mm			Number of nodes/plant		
Control	0.0	9.71 ± 2.30 ^{f}	28.05 ± 5.50 ^{d}	40.95 ± 7.98 ^{b,c}	1 ± 0.00 ^{d}	2 ± 0.22 ^{d}	3 ± 0.49 ^{a}
IAA	0.5	7.28 ± 2.90 ^{i}	28.62 ± 6.62 ^{e}	44.76 ± 7.58 ^{b,c}	1 ± 0.00 ^{d}	2 ± 0.51 ^{d}	4 ± 0.68 ^{a}
	1.0	8.29 ± 2.06 ^{h,i}	36.90 ± 4.57 ^{d}	50.48 ± 6.89 ^{b,c}	1 ± 0.00 ^{d}	2 ± 0.31 ^{d}	4 ± 0.47 ^{a}
	1.5	9.62 ± 2.05 ^{g,h}	42.43 ± 6.45 ^{d}	58.05 ± 8.07 ^{b}	1 ± 0.00 ^{d}	2 ± 0.57 ^{c}	4 ± 0.51 ^{a}
	2.0	10.62 ± 1.92 ^{f,g}	54.57 ± 6.28 ^{c}	72.57 ± 10.57 ^{a}	1 ± 0.00 ^{d}	3 ± 0.62 ^{b}	4 ± 0.88 ^{a}

Values represent the mean ± DS of 02 replicates with 10 experimental units each one, letters groups according to Duncan test ($p < 0.05$). The data of number of nodes were corrected with and length of plant by log (y), methodology for their statistical analysis. IAA, indole acetic acid

Conclusion

Solanum curtilobum explants collected from a greenhouse could be disinfected efficiently using ethanol (70% for 30 s) followed by NaClO (0.5–1.0%, 10 min). The longer aerial part was achieved with GA_3 (1.0-1.5 mgL^{-1}, 28 days), IAA (2 mgL^{-1}, 21 days), and without PGR (28 days). However, GA_3 (0.5 mgL^{-1}, 28 days) and IAA (2 mgL^{-1}, 21 days) induced more axillary buds. IAA increased the development of adventitious roots of *S. curtilobum*, but longer primary roots were found without PGR addition. BAP and combination of BAP + GA_3 reduced the shoot length, and inhibited the rooting on *in vitro* culture. Finally, this study found that GA_3 could be useful for *in vitro* multiplication of *S. curtilobum,* whereas IAA not only promotes lateral rooting, but also improves its growth and develop attaining more vigorous explants, which are features required for the greenhouse acclimatization stage.

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