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In vitro propagation of sweet cucumber (*Solanum muricatum* Ait): Effects of auxins and cytokinins

Propagación *in vitro* de pepino dulce (*Solanum muricatum* Ait): Efectos de las auxinas y las citoquininas

UNIVERSIDAD NACIONAL DE COLOMBIA

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Cecilia Lazaro-Rodriguez[1], Lia Loana Bendezu-Granda[1], Julio A. Olivera-Soto[2], Alvaro Tumpe-Jaqquehua[1], Graciela Gomez-Fuentes[1], Maria Vicente-Vega[1] and Luz Sanchez-Quispe[1]*

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ABSTRACT

Sweet cucumber (*Solanum muricatum* Ait.) is a perennial shrub native to the Andean region and closely related to tomato and potato. Its fruit is valued for its high-water content and nutritional properties, including antioxidants, potassium, and vitamin C. However, its agricultural potential is constrained by low germination rates, high heterozygosity, and susceptibility to diseases. *In vitro* culture techniques offer a viable alternative for obtaining plant material with high genetic and phytosanitary quality. This study evaluated the *in vitro* regeneration of *S. muricatum* through organogenesis and shoot proliferation. During the establishment phase on semi-solid MS medium, supplementation with 1.5 mg L^{-1} indole-3-acetic acid (IAA) enhanced bud regeneration, increased shoot height (2.69 cm), and promoted root formation, whereas higher concentrations (2.0 mg L^{-1}) negatively affected regeneration. Shoot proliferation was stimulated by specific combinations of auxins and cytokinins, particularly IAA+BAP (1.0 + 0.2 mg L^{-1}), which produced taller explants (7.7 cm) with a greater number of leaves (11.9). These findings provide useful information for optimizing *in vitro* propagation conditions for *S. muricatum*, with potential applications in germplasm conservation, production of disease-free plants, and genetic improvement of this underutilized species.

Palabras clave:
Citocinina
Reguladores del crecimiento de las plantas
Cultivo de tejidos vegetales

RESUMEN

El pepino dulce (*Solanum muricatum* Ait.) es un arbusto perenne originario de la región andina y estrechamente relacionado con el tomate y la papa. Su fruto es valorado por su alto contenido de agua y propiedades nutricionales, incluidos antioxidantes, potasio y vitamina C. Sin embargo, su potencial agrícola se ve limitado por las bajas tasas de germinación, la alta heterocigosidad y la susceptibilidad a las enfermedades. Las técnicas de cultivo *in vitro* ofrecen una alternativa viable para la obtención de material vegetal de alta calidad genética y fitosanitaria. Este estudio evaluó la regeneración *in vitro* de *S. muricatum* a través de la organogénesis y la proliferación de brotes. Durante la fase de establecimiento en medio MS semisólido, la suplementación con 1,5 mg L^{-1} de ácido indol-3-acético (IAA) mejoró la regeneración de las yemas, aumentó la altura de los brotes (2,69 cm) y promovió la formación de raíces, mientras que las concentraciones más altas (2,0 mg L^{-1}) afectaron negativamente la regeneración. La proliferación de brotes fue estimulada por combinaciones específicas de auxinas y citoquininas, particularmente IAA+BAP (1,0+0,2 mg L^{-1}), que produjeron explantes más altos (7,7 cm) con un mayor número de hojas (11,9). Estos hallazgos proporcionan información útil para optimizar las condiciones de propagación *in vitro* de *S. muricatum*, con aplicaciones potenciales en la conservación de germoplasma, la producción de plantas libres de enfermedades y el mejoramiento genético de esta especie subutilizada.

[1]Escuela Profesional de Agronomía, Facultad de Ciencias Agrarias, Universidad Nacional de Cañete, San Luis, Cañete, Lima, Perú. lazarorosa0320@gmail.com, liabendezu3@gmail.com, alvarotumpej@gmail.com, gofugraci@gmail.com, Mariavicenteagro@gmail.com, Duquesanchez53@gmail.com
[2]Laboratorio de cultivo in vitro de tejidos vegetales, Centro Internacional de Investigación para la Sustentabilidad, Universidad Nacional de Cañete, Lunahuaná, Cañete, Lima, Perú. agols4407@gmail.com
*Corresponding author

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The sweet cucumber (*Solanum muricatum* Ait.) is a species native to the Andean region and is cultivated for its fruit, which contains 92% water and has a soft texture (Angulo 2024). It provides antioxidants, potassium, vitamin C, and dietary fiber (Campos et al. 2018) and has been associated with potential benefits such as weight management, immune system support, and relief of digestive discomfort (Cavusoglu and Sulusoglu-Durul 2013). The crop is grown in Peru, particularly in Arequipa, Ayacucho, Áncash, La Libertad, and Lima (Pickersgill 2007). Additionally, it has been introduced and adapted in countries including Spain, New Zealand, Turkey, Israel, the United States, and Japan (Torrent 2014).

Nevertheless, the cultivation of this species encounters several challenges, including low seed germination rates and high heterozygosity, which leads to progeny displaying significant variability in characteristics such as leaf type, fruit shape, color, and sensory attributes (González et al. 2015). Furthermore, production fields are impacted by diseases caused by bacteria, fungi, viruses, and mites pests (Kim et al. 2017). This issue is exacerbated when farmers propagate plants using cuttings from existing fields without implementing proper phytosanitary management, often resulting in reduced crop yields. To address these obstacles, the development and optimization of *in vitro* propagation protocols have emerged as a strategic approach to ensure genetic uniformity, quality, and plant health (Shahnawaz et al. 2021). This technique involves isolating cells, tissues, or organs under carefully controlled nutritional and environmental conditions, adhering to rigorous aseptic standards, in order to regenerate whole plants from tissue fragments (explants) (Sharry et al. 2015). Explants are cultivated in media such as Murashige and Skoog (MS) (Gonzales-Arteaga et al. 2023), supplemented with plant growth regulators, particularly auxins and cytokinins. Common auxin hormones used include indole-3-butyric acid (IBA) and indole-3-acetic acid (IAA).

Indole-3-butyric acid (IBA) functions as a hormone that remains active in the culture medium, promoting cell differentiation and elongation, which are necessary for root development. In contrast, indole-3-acetic acid (IAA) regulates cell division, elongation, and differentiation for both shoot and root formation; however, it is rapidly degraded in the culture medium. Cytokinins such as kinetin (KIN) stimulate shoot multiplication and formation, thereby enhancing organogenesis and plant regeneration. 6-Benzylaminopurine (BAP) promotes cell division and supports the robust development of shoots. The combination of IAA and BAP enables the regeneration of apical buds and shoot proliferation, whereas the combination of IBA and BAP creates a balance between shoot and root formation.

This study proposes the incorporation of auxins and cytokinins into an MS culture medium to achieve efficient establishment, multiplication, and rooting of *Solanum muricatum*.

MATERIALS AND METHODS

Plant material

The sweet cucumber cuttings (*S. muricatum* Ait.) were collected from mother plants of a local Lunahuaná ecotype, Cañete. Plants met phytosanitary and maturity standards. Cuttings were potted in peat and agricultural soil until new shoots formed. All activities took place at the International Research Center for Sustainability (CIIS) of the Universidad Nacional de Cañete, Peru.

In vitro establishment

The new shoots were defoliated and cleaned under running water using eucalyptus-based antibacterial soap from the Aval brand, then rinsed with distilled water. The plant material was placed in a laminar flow hood for disinfection: first with 70% alcohol for 1 min, followed by treatment with 1.5% sodium hypochlorite containing one drop of Tween 20 for 10 min. Finally, the material was rinsed three times with sterile distilled water. The treatments used the Murashige and Skoog basal medium (Gonzales-Arteaga et al. 2023) obtained from Caisson Lab, supplemented with sucrose, 8 g L^{-1} of agar, and 100 mL L^{-1} of myo-inositol at varying concentrations. Indole-3-butyric acid (IBA) (0.5, 1.0, 1.5, and 2.0) and indole-3-acetic acid (IAA) (0.5, 1.0, 1.5, and 2.0), both sourced from Caisson Lab, United States, were applied independently as treatments. The pH was adjusted to 5.7, and the medium was sterilized by autoclaving at 121 °C for 20 min under a pressure of 1.2 kg cm^{-2}. Treatments were incubated at a constant temperature of 24 °C and evaluated after 45 days.

In vitro multiplication

During this phase, plant material from the establishment stage was utilized, specifically handling axillary and apical buds. The MS medium culture included sucrose, 8 g L^{-1} of agar, and 100 mL L^{-1} of myo-inositol. Growth regulators applied were kinetin (KIN) and 6-benzylaminopurine (BAP), both individually and in combinations with indole-3-butyric acid (IBA) and indole-3-acetic acid (IAA). Treatments were maintained at a constant temperature of 24 °C in the incubation area and evaluated after 45 days.

Ex vitro acclimatization

The plantlets were removed from the culture medium, and their roots were washed with sterile distilled water to eliminate residual agar. A pre-acclimatization process was conducted in trays covered with transparent plastic to create a microclimate of elevated relative humidity, which was gradually decreased. Subsequently, the plantlets were transplanted into black low-density polyethylene (LDPE) bags containing UV additives, produced by the Peruvian company Maruplast. These bags were filled with a substrate composed of fine sand and agricultural soil at a 2:1 ratio and placed in a greenhouse to facilitate adaptation to external environmental conditions.

Experimental design and statistical analysis

The experiment was conducted using a completely randomized design (CRD), with each experimental unit consisting of a single cultured explant. For *in vitro* establishment, five explants per treatment were used, while 16 explants were used for multiplication. The Shapiro–Wilk test assessed the normality of sample distributions, and Bartlett's test verified homogeneity of variance. *In vitro* establishment data were analyzed by analysis of variance (ANOVA), and treatment means were compared using Tukey's test at $P \leq 0.05$ (Nanda et al. 2021). *In vitro* multiplication data that did not meet assumptions of normality and homogeneity were analyzed using the non-parametric Kruskal–Wallis test to determine significant differences among treatments. Dunn's post-hoc test was performed to identify group differences at a significance level of $P \leq 0.05$. All analyses were performed using R version 4.4.2 for Windows.

RESULTS AND DISCUSSION

In vitro establishment

In vitro establishment of sweet cucumber (*S. muricatum* Ait.), statistically significant differences were observed among treatments based on the type and concentration of growth regulator used. Regarding plantlet height, the treatment with 1.5 mg L^{-1} of indole-3-acetic acid (IAA) produced the highest mean value (2.69 cm), suggesting that this concentration effectively promotes plantlet height development. On the other hand, the treatment with 2.0 mg L^{-1} of IAA resulted in the lowest mean height (1.53 cm), indicating a potential inhibitory effect on growth caused by the higher concentration of the growth regulator.

In terms of leaf number, the combination of 1.0 mg L^{-1} (IBA) and 0.5 mg L^{-1} (IAA) yielded the highest mean value of 6.40 cm, suggesting that these concentrations are optimal for promoting foliar development. In contrast, the treatment with 2.0 mg L^{-1} (IBA) and 2.0 mg L^{-1} (IAA) resulted in the lowest leaf number, indicating a potential inhibitory effect associated with higher growth regulator concentrations.

For root length and root number, 1.5 mg L^{-1} (IAA) yielded the most favorable results, with mean values of 1.48 and 9.50 cm, respectively. Conversely, 2.0 mg L^{-1} (IBA) was the least effective, resulting in a mean root length of 0.11 cm and only 1.10 cm on average. Detailed data for all treatments are provided in Table 1.

Table 1. Effect of Growth Regulators (PGR) on *in vitro* establishment of sweet cucumber (*Solanum muricatum* Ait).

Plant Growth Regulator	Concentration (mg L^{-1})	Shoot length (cm)	Number of leaves	Root length (cm)	Number of roots
Control	0	1.93^{bcd}	5.50^{ab}	1.36^{bc}	2.70^{cd}
IBA	0.5	1.93^{bcd}	6.20^{ab}	2.75^{a}	4.40^{bc}
	1.0	2.46^{ab}	6.40^{a}	1.26^{bc}	3.30^{cd}
	1.5	1.65^{cd}	5.00^{ab}	0.15^{d}	3.30^{cd}
	2.0	1.57^{d}	4.70^{b}	0.11^{d}	1.10^{d}

Table 1

Plant Growth Regulator	Concentration (mg L^{-1})	Shoot length (cm)	Number of leaves	Root length (cm)	Number of roots
IAA	0.5	2.21^{abc}	6.40^{a}	0.62^{cd}	1.60^{d}
	1.0	1.87^{cd}	6.30^{a}	0.43^{d}	2.10^{cd}
	1.5	2.69^{a}	6.00^{ab}	1.48^{b}	9.50^{a}
	2.0	1.53^{d}	6.20^{ab}	1.42^{b}	6.80^{a}

Values followed by different letters within each column are significantly different according to Tukey's test (**$P \leq 0.05$).

Moreover, IAA treatments were superior, resulting in increased plant height, leaf number, and root production (Figure 1B), in comparison with IBA treatments (Figure 1A) and the control.

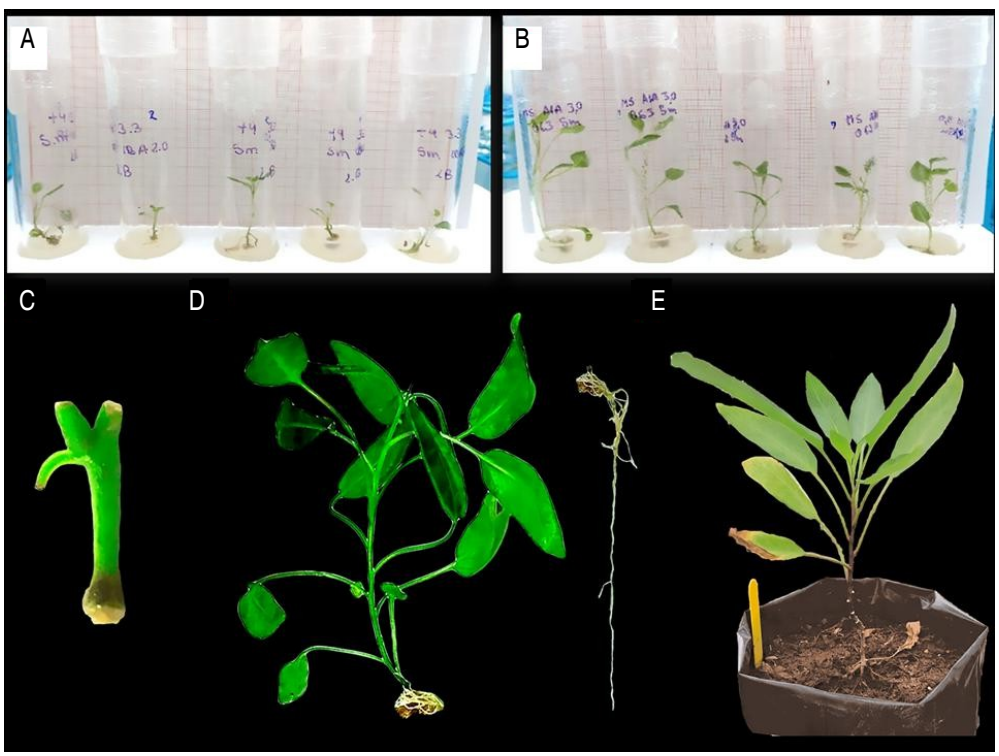

Figure 1. *In vitro* establishment and multiplication of sweet cucumber (*S. muricatum* Ait.). **A.** Treatment supplemented with IBA (2.0 mg L^{-1}) during *in vitro* establishment; **B**. Treatment supplemented with IAA (1.5 mg L^{-1}) during *in vitro* establishment; **C**. Shoots without roots in MS medium supplemented with BAP (1.0 mg L^{-1}); **D.** Plantlet with fully developed roots in medium enriched with IAA+BAP (1.0 + 0.1 mg L^{-1}); **E.** An *in vitro*-grown plant is fully acclimatized to *ex vitro* conditions.

The synergistic effect of auxins has been shown to induce notable responses in solanaceous crops, significantly influencing leaf number, shoot height, root number, and root length. Studies such as Rehman et al. (2019) reported that the combination of BAP (1.2 mg L^{-1}) + IBA (2.0 mg L^{-1}) led to increased numbers of roots and shoots, achieving a hormonal balance. Similarly, Iftikhar et al. (2015) observed efficient rooting in *Solanum villosum* with 1.0 mg L^{-1} of IBA, which aligns with the present study on *S. muricatum*, as IBA stimulates the H^{+}-ATP_{ase} proton pump in the plasma membrane, acidifying the cell wall and activating proteins that facilitate cell elongation under turgor pressure. *Solanum muricatum*, phylogenetically related to major commercial crops such as *S. lycopersicum* and

S. tuberosum (Saldaña et al. 2022), has shown a similar response to auxin treatments. Higher concentrations of indole-3-acetic acid (IAA) have been observed to elicit better responses in these two crops (Agurto et al. 2024). In contrast, indole-3-butyric acid (IBA) tends to yield better results at lower concentrations. The application of IBA at 1.0 mg L^{-1} in *S. muricatum* resulted in a high shoot length (2.46 cm), as shown in Table 1, indicating a stimulating effect on shoot growth. This finding is consistent with Jabeen et al. (2021) in *Solanum tuberosum*, where moderate concentrations of IBA promoted optimal shoot elongation. Several studies have demonstrated that IBA has a greater capacity to promote adventitious root formation compared to IAA. Furthermore, IBA is more stable and less susceptible to enzymatic degradation, making it a more efficient source of free auxin. However, the present results for *S. muricatum* suggest that IAA may be more effective, as also reported by Cavusoglu and Sulusoglu-Durul (2013), highlighting the need for further exploration of optimal auxin conditions depending on the species and the developmental process under investigation.

In vitro multiplication

Explants of *S.muricatum* Ait. cultivated in MS medium supplemented with combinations of auxins and cytokinins (IAA+BAP and IBA+BAP) produced the highest number of shoots. The treatment with IBA+BAP (1.0 + 0.1 mg L^{-1}) yielded the best results, with an average of 7.5 shoots. However, this treatment did not show a statistically significant difference compared to treatments with 0.5 and 1.0 mg L^{-1} of KIN. On the other hand, treatments with 0.5, 1.0, 1.5, and 2.0 mg L^{-1} of BAP resulted in significantly lower averages, not exceeding 0.9 shoots, with minimal growth and callus formation at the cutting site (Figure 1C). This condition impeded root development and nutrient uptake during the initial stages, resulting in challenges to acclimatization due to unstructured morphogenesis, aberrant leaf formation, and hyperhydricity. Following 45 days of treatment, results demonstrated that combinations of auxins and cytokinins—specifically IAA + BAP and IBA + BAP—significantly improved plant size, vigor, firmness, and produced an intense, uniformly green coloration (Figure 1D).

After 45 days of cultivation, explants were evaluated based on the effects of different treatments. Combinations of auxins and cytokinins (IAA+BAP and IBA+BAP) produced significant variations in explant height. The treatment with 1.0 + 0.2 mg L^{-1} (IAA+BAP) yielded the best results in terms of height, with an average of 7.7 cm, ranging from 3.7 to 11.5 cm. This treatment was significantly superior compared to others, such as those containing only KIN or BAP, which resulted in lower average shoot lengths (Table 2).

Table 2. Influence of plant growth regulators on *in vitro* shoot multiplication of sweet cucumber (*Solanum muricatum* Ait.).

Growth Regulator	Concentration (mg L^{-1})	Number of shoots	Shoot length (cm)	Root length (cm)	Number of leaves
KIN	0.5	3.6^{ab}	3.1^{abc}	4.7^{ab}	9^{ab}
	1.0	2.9^{abc}	2.9^{abc}	4.5^{ab}	7.9^{abc}
	1.5	1.9^{bcd}	2.1^{bcd}	4.1^{ab}	6.2^{acd}
	2.0	1.6^{bcd}	1.6^{bd}	2.8^{bc}	5^{cde}
BAP	0.5	0.9^{cd}	1.0^{bd}	0^{c}	4.1^{cde}
	1.0	0.8^{d}	0.8^{d}	0.1^{c}	2.9^{de}
	1.5	0.7^{d}	0.7^{d}	0^{c}	0.6^{e}
	2.0	0.8^{d}	0.7^{d}	0^{c}	0.4^{e}
IAA+BAP	1.0+0.1	7.2^{a}	6.5^{ac}	7.2^{ab}	10.6^{ab}
	1.0+0.2	6.7^{a}	7.7^{a}	10.8^{a}	11.9^{b}
IBA+BAP	1.0+0.1	7.5^{a}	7.2^{a}	7.6^{a}	9.4^{ab}
	1.0+0.2	6.9^{a}	7.3^{a}	8.5^{a}	10.5^{ab}

Values followed by different letters within each column are significantly different according to Tukey's test (**$P \leq 0.05$).

In vitro multiplication of *S. muricatum* from explants in MS medium enriched with kinetin (KIN), a cytokinin, has proven effective for shoot formation and shoot elongation, particularly at concentrations of 0.5 and 1.0 mg L^{-1}. In this regard, Toma et al. (2021) reported that during the multiplication phase, treatments with KIN at 1.0, 2.0, and 3.0 mg L^{-1} produced averages of 1.6, 1.6, and 2.3 shoots, respectively, with shoot lengths of 2.16, 2.83, and 3.6 cm. In the present study, concentrations of 0.5 and 1.0 mg L^{-1} resulted in average values of 2.9±0.7 and 1.6±0.5 shoots, and shoot lengths of 2.9±0.7 and 1.6±0.7 cm. Previous studies on *S. muricatum* have also shown that a combination of 2 + 1 mg L^{-1} (BAP+KIN) yields the best response in shoot number, while 1 + 1 mg L^{-1} (BAP+KIN) is optimal for shoot length (Aghdaei et al. 2019).

As for BAP-based treatments, they were the least effective compared to other approaches. However, among BAP-only treatments, concentrations of 0.5 and 1.0 mg L^{-1} showed the best performance, with averages of 0.9±0.3 and 0.8±0.4 shoots and shoot lengths of 1.0±0.4 and 0.8±0.4 cm, respectively. These findings contrast with those reported by Cavusoglu and Sulusoglu-Durul (2013), who found that optimal BAP concentrations of 1, 2, and 3 mg L^{-1} resulted in shoot numbers of 3.33, 4.58, and 4.35, and shoot lengths of 1.49, 1.21, and 0.88 cm, respectively. Interestingly, the control treatment without any growth regulator outperformed all BAP treatments in shoot number. This discrepancy may be attributed to possible inhibition caused by the culture medium, which contains additional vitamins. While BAP is known to play a role in plant growth and development during organogenesis (Khatoon et al. 2022), the specific medium conditions in this study may have negatively affected its efficacy. Notably, in other solanaceous crops such as *Solanum tuberosum* (Nezami et al. 2018), *S. lycopersicum* (Kumari et al. 2024), and *S. melongena* (Foo et al. 2018), BAP has shown positive responses in shoot induction. These findings suggest that BAP effectiveness can vary considerably depending on the medium and the plant species used in the experiment. As noted by García and Javier (2016), morphological responses are determined by genotype and medium composition. Furthermore, optimal organogenesis is achieved through a specific balance between auxins and cytokinins, and their concentrations—used individually or in combination— depend on the developmental process being targeted (Alcantara-Cortes et al. 2019).

Treatments with auxins and cytokinins (IAA+BAP and IBA+BAP) resulted in the highest numbers of leaves. The treatment with 1.0 + 0.2 mg L^{-1} (IAA+BAP) produced the best results, with an average of 11.9 leaves, ranging from 8 to 26. This treatment outperformed others, including 1.0+0.1 mg L^{-1} (IAA+BAP), which had an average of 10.6 leaves. However, IBA+BAP treatments were also effective, with averages of 9.4 and 10.5 leaves. In contrast, KIN and BAP used individually showed significantly lower performance in leaf production, especially at higher BAP concentrations, which caused developmental issues such as thinner stems, reduced vigor, lower resistance, and pale coloration.

Explants treated with 1.0+0.2 mg L^{-1} IAA+BAP showed the best root length results, averaging 10.8 cm (range: 4.0–19.5 cm). IBA+BAP treatments also showed effectiveness, with mean root lengths of 7.6 and 8.5 cm. Figure 1D shows a plant with well-formed green leaves and an adequate root system capable of nutrient uptake, supporting development and acclimatization to *ex vitro* conditions. Auxins like IAA and IBA play a key role in the early stages of root formation. However, KIN and BAP alone resulted in significantly lower root lengths, particularly at higher BAP concentrations, as observed in Figure 1C, where no roots or leaves were visible.

In vitro multiplication of *S. muricatum* showed the best responses in terms of number of leaves and root length when treated with auxin + cytokinin combinations (IAA+BAP or IBA+BAP). It has been reported that 1.0 mg L^{-1} BAP and 3.0 mg L^{-1} KIN are individually effective for leaf formation (Toma et al. 2021). Particularly, good root lengths were observed with 0.2 and 0.3 mg L^{-1} IAA and 0.1 mg L^{-1} IBA, averaging over 6.33 cm, especially in media enriched with 3.0 mg L^{-1} KIN. These findings support the effectiveness of auxin-cytokinin combinations in promoting vegetative structure formation in this species. Nonetheless, the combined treatments (IAA+BAP and IBA+BAP) were superior across all parameters evaluated, partially aligning with the findings by Toma et al. (2021). Furthermore, BAP treatments were not as effective for root induction, as also documented by Cavusoglu and Sulusoglu-Durul (2013).

Statistical analysis confirmed that IAA+BAP and IBA+BAP combinations were significantly more effective for multiplication compared to KIN or BAP alone. Specifically, the combination 1.0+0.2 mg L^{-1} (IAA+BAP) achieved a

shoot length average of 1.08 cm, while 1.0+0.2 mg L^{-1} (IBA+BAP) reached 8.5 cm. Additionally, IBA has proven effective for root formation in *S. lycopersicum* (Alatar et al. 2017) and for root elongation in *S. tuberosum* (Jabeen et al. 2021). Similarly, IAA has shown high efficiency in both root formation and elongation (Bentes et al. 2022), supporting its inclusion in media aimed at root development.

Ex vitro acclimatization

After *in vitro* rooting, plantlets were pre-acclimatized for five days under high humidity, which was then reduced to harden the cuticle and minimize water loss. A 2:1 fine sand to agricultural soil mix achieved 95% survival after 45 days, with no pathogen issues. Figure 1E shows a vigorous plant with normal growth and no abnormal morphological features. Cavusoglu and Sulusoglu-Durul (2013) reported an 80–100% adaptation rate for this species. Similarly, Toma et al. (2021) achieved a 100% survival rate by acclimatizing plantlets in a greenhouse. In *S. tuberosum*, a 100% survival rate was reached after 28 days using a peat substrate, and a 90% acclimatization rate was achieved using a 2:1 peat-to-sand ratio (Aleaga 2023). Tacoronte et al. (2017) reported that the use of cellophane bags mitigated stress during acclimatization, while high humidity conditions enabled previously malformed *in vitro* roots to become fully functional. The success of this stage is contingent upon effective *in vitro* root development and the plantlets' progressive adaptation to their environment, given that *in vitro*-derived plantlets depend on external carbon sources from the culture medium rather than photosynthesis. Furthermore, these plantlets are maintained under low-light and high-humidity conditions, which are markedly different from those encountered in their natural habitats.

CONCLUSION

In vitro plant regeneration and shoot proliferation are key processes in plant micropropagation systems. The study indicates that indole-3-acetic acid (IAA) influences the *in vitro* regeneration of sweet cucumber through organogenesis and assists root induction. Additionally, when higher concentrations of auxin (IAA) are combined with lower concentrations of cytokinin (BAP) in MS medium, increased shoot proliferation from explants is observed. These findings suggest that the auxin-to-cytokinin ratio in the culture medium influences morphogenetic responses in *S. muricatum*. The present study provides preliminary data that can contribute to optimizing the use of plant growth regulators in the micropropagation of this species and guide future protocol adjustments.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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