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Growth and physiological responses of potato plantlets (*Solanum tuberosum* L. cv. Cipanas) to NaCl-induced salinity stress under in vitro conditions

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Abstract

Potato (*Solanum tuberosum* L. cv. Cipanas) is a high-yielding cultivar; however, its cultivation is often limited by salinity stress in marginal lands, which adversely affects plant growth and development. This study aimed to evaluate the effects of different NaCl concentrations on the growth and physiological responses of in vitro-grown potato plantlets and to determine the maximum salinity level that could be tolerated. The experiment consisted of five NaCl concentrations (0, 0.25, 0.50, 0.75, and 1.00%) arranged in a completely randomized design. Quantitative data were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's honestly significant difference (HSD) test at the 5% significance level. Increasing NaCl concentrations significantly reduced plantlet height, leaf number, and stomatal index, and induced progressive changes in plantlet color from green to yellowish-green and brownish-green. Potato plantlets remained tolerant to NaCl concentrations of 0.25% based on plantlet height and leaf number, whereas tolerance up to 0.50% was observed for plantlet color and stomatal index, with no significant differences compared with the control. These findings indicate that *S. tuberosum* L. cv. Cipanas exhibits moderate tolerance to NaCl-induced salinity under in vitro conditions.

Keywords: Potato (*Solanum tuberosum* L. cv. Cipanas); NaCl; Salinity stress; In vitro culture; Stomatal index

1. Introduction

Potato (*Solanum tuberosum* L.) is an important horticultural crop cultivated in highland areas and serves as a major source of carbohydrates with high nutritional value. Among the commercially cultivated potato cultivars, cv. Cipanas is considered one of the promising cultivars because it was developed through a cross between 'Thung 1510' and 'Desiree'. This cultivar has a yield potential of up to 34 t ha^{-1}, a harvesting period of 95–105 days, and resistance to late blight disease, making it suitable for wider cultivation [1]. Increasing food demand and the continuous decline in fertile agricultural land have encouraged the utilization of marginal lands for crop production. However, soil salinity remains one of the major abiotic stresses limiting plant growth and agricultural productivity worldwide [2]. The accumulation of sodium chloride (NaCl) in the soil induces osmotic stress, ion toxicity, nutrient imbalance, and oxidative stress, thereby disrupting water uptake, photosynthesis, and various metabolic processes in plants [3]. Salinity stress also alters plant hormonal balance by increasing abscisic acid (ABA) levels while reducing auxin, cytokinin, and gibberellin concentrations, ultimately inhibiting cell division and cell elongation. Furthermore, plants respond to salinity stress by reducing stomatal opening to minimize water loss and maintain cellular water balance [4].

In vitro culture provides a reliable and controlled approach for evaluating plant responses to salinity stress by minimizing environmental variation. The addition of NaCl to the culture medium is widely used to simulate saline conditions and to investigate plant growth responses and tolerance mechanisms. In addition, tissue culture plays an important role in producing uniform, disease-free, and high-quality planting materials, particularly for vegetatively

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propagated crops [5]. Previous studies have demonstrated that salinity stress adversely affects plant growth under in vitro conditions. Nufus *et al.* [6] reported that NaCl concentrations above 2.5 g L^{-1} reduced leaf and shoot formation in *Desmanthus virgatus*. Similarly, Indriani *et al.* [7] found that increasing NaCl concentrations ranging from 0 to 1% significantly reduced plant height, leaf number, and root length in *Cattleya* sp. Likewise, Putri *et al.* [8] observed a progressive decline in the growth of banana (*Musa acuminata* Colla) plantlets with increasing NaCl concentrations. These findings indicate that NaCl concentrations ranging from 0 to 1% are effective for inducing different levels of salinity stress under in vitro culture conditions.

Despite the economic importance of potato, information regarding the growth and physiological responses of in vitro-grown potato cv. Cipanas under NaCl-induced salinity stress remains limited. In particular, information on its tolerance level based on growth performance, plantlet color, and stomatal characteristics is still scarce. Therefore, this study aimed to evaluate the effects of different NaCl concentrations on the growth and physiological responses of in vitro-grown potato (*Solanum tuberosum* L. cv. Cipanas) plantlets and to determine the maximum NaCl concentration that could still be tolerated under in vitro conditions.

2. Material and methods

2.1. Plant Materials and Experimental Design

Potato (*Solanum tuberosum* L. cv. Cipanas) explants were used as the plant material in this study. The culture medium consisted of Murashige and Skoog (MS) basal medium supplemented with sucrose, agar, and different concentrations of sodium chloride (NaCl). The chemicals used included analytical-grade NaCl, sucrose, agar, chloroform, 1% safranin, glycerin, distilled water, and 70% and 96% ethanol. The experiment was conducted using standard tissue culture equipment, including culture bottles, a laminar airflow (LAF) cabinet, an autoclave, an analytical balance, a pH meter, and a light microscope.

The experiment was arranged in a completely randomized design (CRD) with one factor, namely NaCl concentration, consisting of five treatment levels: 0, 0.25, 0.50, 0.75, and 1.00% (w/v). Each treatment was replicated five times, with one potato plantlet cultured in each experimental unit.

2.2. Culture Media Preparation and Plantlet Establishment

A NaCl stock solution (1 g mL^{-1}) was prepared by dissolving 100 g of NaCl in distilled water and adjusting the final volume to 100 mL. The culture medium consisted of Murashige and Skoog (MS) basal medium supplemented with 4.43 g L^{-1} MS basal salts, 30 g L^{-1} sucrose, and 7 g L^{-1} agar. The pH of the medium was adjusted to 5.7 using 1 N KOH or 1 N HCl, and the medium was heated with continuous stirring until all components were completely dissolved.

The prepared medium was divided into five portions of 200 mL each. Appropriate volumes of the NaCl stock solution (0, 0.5, 1.0, 1.5, and 2.0 mL) were added to obtain final NaCl concentrations of 0, 0.25, 0.50, 0.75, and 1.00% (w/v), respectively. The media were thoroughly mixed, dispensed into sterile culture bottles (20 mL per bottle), and sterilized in an autoclave at 121°C for 15 min. After sterilization, the media were incubated at room temperature (25 ± 2°C) for seven days to confirm the absence of contamination before use [9].

Uniform potato (*Solanum tuberosum* L. cv. Cipanas) plantlets were used as explants. Under aseptic conditions in a laminar airflow cabinet, one plantlet was transferred into each culture bottle containing the respective treatment medium according to the experimental design.

2.3. Observations

Observations were conducted two weeks after culture initiation. The parameters evaluated included the percentage of live plantlets, plantlet color, plantlet height, number of leaves, and stomatal index.

2.3.1. Percentage of Live Plantlets

The percentage of live plantlets was determined according to the method described by [10] using the following equation:

$$\text{Percentage} = \frac{\text{Number of live plantlets}}{\text{Total number of plantlets}} \times 100\%$$

2.3.2. Plantlet Color Visualization

Plantlet color was evaluated visually according to the method described by [11]. Color changes were classified into three categories, namely green, yellowish-green, and brownish-green, based on the visual appearance of the plantlet tissues.

2.3.3. Plantlet Height

Plantlet height was measured two weeks after culture initiation using a ruler. Measurements were taken from the base of the stem to the shoot apex according to the method described by [12].

2.3.4. Number of Leaves

The number of leaves was determined two weeks after culture initiation by counting the total number of fully developed leaves on each plantlet. The data obtained were recorded for subsequent statistical analysis.

2.3.5. Stomatal Index

The stomatal index was determined using the leaf clearing method according to Trisiswanti and Sugimin [13]. Approximately 10 mm of leaf tissue was fixed in 70% ethanol for 5 min and then immersed in chloral hydrate solution (chloral hydrate:distilled water, 5:2, w/v). The samples were heated for 15 min to obtain transparent leaf tissues. Subsequently, the cleared leaves were stained with 1% safranin for 1 min, rinsed with distilled water, and mounted on glass slides using glycerin as the mounting medium. The preparations were carefully covered with a coverslip to avoid air bubbles and observed under a light microscope at 100× magnification.

The numbers of stomata and epidermal cells on the abaxial (lower) leaf surface were counted, and the stomatal index was calculated according to Mahesha [14] using the following equation:

$$\text{Stomatal Index} = \frac{\text{Number of Stomata}}{\text{Number of Stomata+Number of Epidermis}} \times 100\%$$

2.4. Data Analysis

The data obtained in this study consisted of qualitative and quantitative data. Qualitative data, including plantlet color changes, were described descriptively and supported by photographic documentation. Quantitative data, including the percentage of live plantlets, plantlet height, number of leaves, and stomatal index, were analyzed using one-way analysis of variance (ANOVA). When significant differences among treatments were detected, mean comparisons were performed using Tukey's Honestly Significant Difference (HSD) test at the 5% significance level ($\alpha = 0.05$).

3. Results and discussion

3.1. Percentage of Live Plantlets

The survival percentage of *Solanum tuberosum* L. cv. Cipanas plantlets cultured under different NaCl concentrations (0, 0.25, 0.50, 0.75, and 1.00%) for two weeks is presented in **Table 1**. All plantlets remained viable throughout the observation period, resulting in a survival percentage of 100% across all treatments.

Table 1 Survival Percentage of *Solanum tuberosum* L. cv. Cipanas Plantlets Cultured under Different NaCl Concentrations

NaCl concentration (%)	Survival percentage (%)	
	Week 1	Week 1
0.00	100	100
0.25	100	100
0.50	100	100
0.75	100	100
1.00	100	100

The results presented in **Table 1** show that all potato (*Solanum tuberosum* L. cv. Cipanas) plantlets remained alive throughout the two-week culture period, resulting in a survival percentage of 100% under all NaCl treatments. These findings indicate that NaCl concentrations of up to 1.00% did not induce plantlet mortality during the experimental period under in vitro conditions.

The absence of plantlet mortality suggests that in vitro-grown potato cv. Cipanas possesses sufficient tolerance to maintain viability during the early stages of exposure to salinity stress. However, plant survival does not necessarily indicate the absence of stress, as physiological and morphological disturbances may occur before visible symptoms of mortality appear. Junandi *et al.* [15] reported that plants exposed to salinity stress generally remain alive during the initial growth stage, although their growth is gradually inhibited due to physiological disturbances.

The inhibitory effects of salinity are primarily associated with osmotic stress caused by high salt concentrations in the culture medium, which reduce the water potential and consequently limit water uptake by plant tissues [16]. In addition, excessive accumulation of sodium (Na^+) and chloride (Cl^-) ions may induce ionic toxicity, disrupt nutrient balance, interfere with metabolic activities, and inhibit cell division and cell elongation [3]. As a result, although all plantlets survived during the experimental period, their growth and physiological performance were adversely affected by increasing NaCl concentrations.

Therefore, plantlet survival alone was not a sufficiently sensitive indicator for evaluating salinity tolerance under the experimental conditions. More sensitive parameters, including plantlet color, plantlet height, leaf number, and stomatal index, provided a better assessment of the physiological responses of potato cv. Cipanas to NaCl-induced salinity stress.

3.2. Plantlet Color Visualization

The effects of different NaCl concentrations on the visual appearance of *Solanum tuberosum* L. cv. Cipanas plantlets after two weeks of in vitro culture are presented in Table 2.

Table 2 Visual Appearance of *Solanum tuberosum* L. cv. Cipanas Plantlets Cultured under Different NaCl Concentrations

NaCl concentration (%)	**Week 1 (%)**			**Week 2 (%)**		
	G	YG	BG	G	YG	BG
0.00	100	–	–	100	–	–
0.25	100	–	–	80	20	–
0.50	80	20	–	60	40	–
0.75	60	40	–	40	40	20
1.00	60	40	–	40	40	20

Note: G = Green; YG = Yellowish-green; BG = Brownish-green.

Based on the observations presented in **Table 2**, increasing NaCl concentrations induced progressive changes in the visual appearance of *Solanum tuberosum* L. cv. Cipanas plantlets. In the control treatment (0% NaCl), all plantlets remained green throughout the two-week observation period. In contrast, NaCl-treated plantlets gradually exhibited yellowish-green coloration, accounting for 20% and 40% of the plantlets at NaCl concentrations of 0.25% and 0.50%, respectively, after two weeks of culture. At higher NaCl concentrations (0.75% and 1.00%), more severe symptoms were observed, with 20% of the plantlets exhibiting a brownish-green coloration. These findings indicate that increasing NaCl concentration progressively altered plantlet color from green to yellowish-green and eventually to brownish-green, reflecting the adverse effects of salinity stress on plantlet physiological condition. Representative images of the visual appearance of potato plantlets under different NaCl treatments are presented in **Figure 1**.

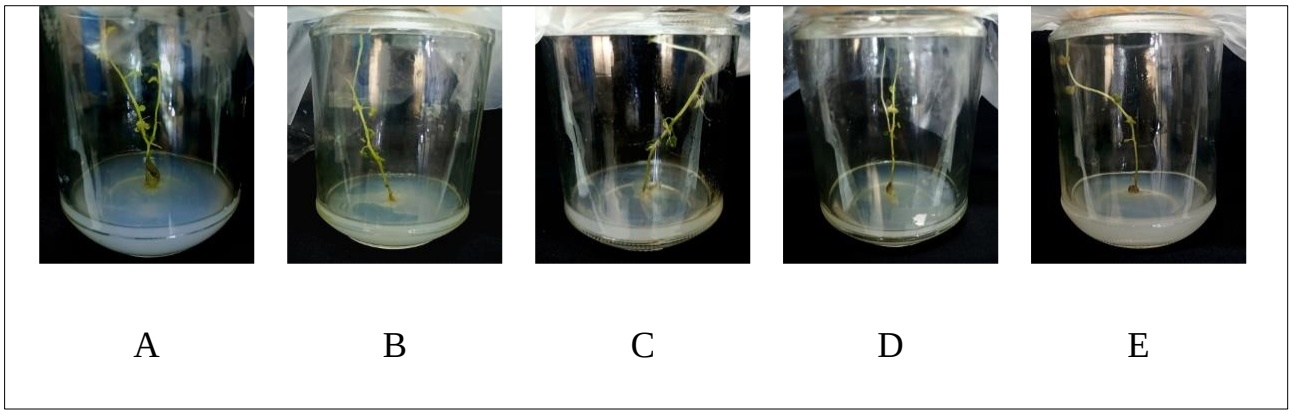

Figure 1 Visual appearance of *Solanum tuberosum* L. cv. Cipanas plantlets cultured under different NaCl concentrations after two weeks of in vitro culture: (A) 0.00%, (B) 0.25%, (C) 0.50%, (D) 0.75%, and (E) 1.00

Based on **Figure 1**, clear differences in the visual appearance of *Solanum tuberosum* L. cv. Cipanas plantlets were observed among the NaCl treatments. Plantlets cultured on the control medium (0.00% NaCl) remained fresh green throughout the observation period. In contrast, plantlets exposed to 0.25% and 0.50% NaCl gradually developed yellowish-green leaves, whereas those cultured at 0.75% and 1.00% NaCl exhibited more pronounced symptoms, characterized by brownish-green leaves and reduced visual quality compared with the control. These observations demonstrate that increasing NaCl concentration progressively affected the physiological condition of the plantlets during in vitro culture.

The deterioration in plantlet color is associated with the physiological effects of salinity stress. High NaCl concentrations reduce the water potential of the culture medium, thereby limiting water uptake by plant tissues and inducing osmotic stress. Under prolonged stress conditions, osmotic imbalance may lead to plasmolysis and damage to the cell protoplasm, resulting in impaired cellular functions, including chlorophyll degradation [17]. Consequently, the normal green color of the leaves gradually changed to yellowish-green and eventually brownish-green as the severity of salinity stress increased.

Leaf color is closely associated with chlorophyll content and photosynthetic activity, making it a useful indicator of plant physiological status. Under salinity stress, excessive accumulation of Na^+ and Cl^- ions disrupts chloroplast structure and function, accelerates chlorophyll degradation, and suppresses photosynthesis [18]. Continued exposure to high salt concentrations may further damage cellular structures, leading to tissue browning and, under severe stress, cell death [19]. Therefore, the progressive discoloration observed in the present study indicates that increasing NaCl concentrations adversely affected the physiological performance of in vitro-grown potato plantlets.

3.3. Plantlet Height

The average height of *Solanum tuberosum* L. cv. Cipanas plantlets cultured under different NaCl concentrations is presented in Table 3.

Table 3 Plantlet Height of *Solanum tuberosum* L. cv. Cipanas Cultured under Different NaCl Concentrations

NaCl Concentration (%)	Plantlet height (cm) (Mean ± SE)
0.00	15.20 ± 0.46^{b}
0.25	13.26 ± 0.43^{b}
0.50	10.40 ± 0.56^{a}
0.75	10.20 ± 0.67^{a}
1.00	9.60 ± 0.65^{a}

Note: Values are presented as mean ± standard error (SE). Means followed by the same letter are not significantly different according to Tukey's HSD test at $P < 0.05$.

Based on the results presented in **Table 3**, increasing NaCl concentrations significantly reduced the height of *Solanum tuberosum* L. cv. Cipanas plantlets. The tallest plantlets were observed in the control treatment (0.00% NaCl), whereas the shortest plantlets were recorded at 1.00% NaCl. Statistical analysis showed that plantlets cultured in media containing 0.50%, 0.75%, and 1.00% NaCl were significantly shorter than those grown in the control and 0.25% NaCl treatments. However, no significant differences were observed among the 0.50%, 0.75%, and 1.00% NaCl treatments, indicating that NaCl concentrations of 0.50% or higher produced a comparable inhibitory effect on plantlet height.

These findings are consistent with those reported by Pratiwi *et al.* [20], who found that salinity stress at 3,000 ppm (0.3% NaCl) significantly reduced the height of tomato plants. The similarity between the two studies suggests that increasing salinity generally suppresses shoot growth in different plant species under saline conditions.

The reduction in plantlet height under salinity stress is primarily associated with osmotic stress caused by the decrease in the water potential of the culture medium. Reduced water availability limits water uptake by plant tissues, decreases cell turgor pressure, and consequently inhibits cell elongation, resulting in reduced shoot growth [21]. In addition, excessive accumulation of sodium (Na^+) ions disrupts ionic homeostasis and interferes with the uptake of essential nutrients such as potassium (K^+) and calcium (Ca^{2+}), which are required for normal physiological processes. This ionic imbalance impairs metabolic activities, damages cell membranes, and ultimately suppresses plant growth [22].

3.4. Number of Leaves

The average number of leaves of *Solanum tuberosum* L. cv. Cipanas plantlets cultured under different NaCl concentrations is presented in **Table 4**.

Table 4 Effect of Different NaCl Concentrations on the Number of Leaves of *Solanum tuberosum* L. cv. Cipanas Plantlets

NaCl Concentration (%)	Number of leaves (Mean ± SE)
0.00	6.8 ± 0.58^{c}
0.25	6.2 ± 0.37^{bc}
0.50	4.8 ± 0.37^{ab}
0.75	4.2 ± 0.37^{a}
1.00	4.0 ± 0.44^{a}

Note: Values are presented as mean ± standard error (SE). Means followed by the same letter are not significantly different according to Tukey's HSD test at $P < 0.05$.

Based on the results presented in **Table 4**, increasing NaCl concentrations reduced the number of leaves produced by *Solanum tuberosum* L. cv. Cipanas plantlets. The highest mean number of leaves was recorded in the control treatment (0.00% NaCl), whereas the lowest was observed in plantlets cultured on medium containing 1.00% NaCl. Statistical analysis showed that the 0.75% and 1.00% NaCl treatments produced significantly fewer leaves than the control treatment. The intermediate treatments (0.25% and 0.50% NaCl) exhibited transitional responses, indicating a gradual decline in leaf production with increasing salinity. Furthermore, no significant differences were observed between the 0.75% and 1.00% NaCl treatments, suggesting that NaCl concentrations of 0.75% or higher exerted a comparable inhibitory effect on leaf formation.

These findings are consistent with those reported by Pratiwi *et al.* [20], who demonstrated that salinity stress above 3,000 ppm (0.3% NaCl) significantly reduced the number of leaves in tomato plants. The similarity between the two studies suggests that increasing salinity generally suppresses leaf development in different plant species under saline conditions.

The reduction in leaf number under salinity stress is primarily associated with osmotic stress and ionic toxicity, which impair water uptake and reduce photosynthetic efficiency. Zhao *et al.* [23] reported that salinity decreases the ability of plants to absorb water, thereby inhibiting vegetative growth, including the initiation and development of new leaves. In addition, reduced photosynthetic activity limits the availability of assimilates required for leaf formation, resulting in fewer leaves under saline conditions. Munns and Gilliham [24] further explained that plants respond to salinity stress by reducing leaf area to minimize transpiration and maintain cellular water balance. This adaptive response also limits the uptake and accumulation of toxic sodium (Na^+) and chloride (Cl^-) ions, thereby reducing the detrimental effects of salinity stress on plant growth.

3.5. Stomatal Index

The stomatal index of *Solanum tuberosum* L. cv. Cipanas plantlets cultured under different NaCl concentrations is presented in Table 5.

Table 5 Stomatal Index of *Solanum tuberosum* L. cv. Cipanas Plantlets Cultured under Different NaCl Concentrations

NaCl Concentration (%)	Stomatal index (Mean ± SE)
0.00	43.6 ± 0.50^{b}
0.25	35.6 ± 1.20^{ab}
0.50	34.0 ± 2.58^{ab}
0.75	33.0 ± 1.51^{a}
1.00	27.8 ± 4.42^{a}

Note: Values are presented as mean ± standard error (SE). Means followed by the same letter are not significantly different according to Tukey's HSD test at $P < 0.05$.

Based on the results presented in **Table 5**, the stomatal index of *Solanum tuberosum* L. cv. Cipanas plantlets decreased progressively with increasing NaCl concentration in the culture medium. The highest stomatal index was recorded in the control treatment (0.00% NaCl), whereas the lowest value was observed in the 1.00% NaCl treatment. Statistical analysis showed that plantlets cultured at 0.75% and 1.00% NaCl had significantly lower stomatal indices than those grown in the control treatment. The intermediate treatments (0.25% and 0.50% NaCl) exhibited intermediate responses and did not differ significantly from either the control or the higher NaCl concentrations. These results indicate that increasing salinity adversely affected stomatal development in potato plantlets.

These findings are consistent with those reported by Sobir *et al.* [25], who observed a reduction in stomatal number in eggplant grown under salinity levels of 8–10 mS cm^{-1} (approximately 0.5–0.64% NaCl). The similarity between the two studies suggests that elevated salinity commonly suppresses stomatal development in plants as part of their adaptive response to water-deficit conditions.

The reduction in stomatal index under salinity stress is closely associated with osmotic stress induced by high NaCl concentrations. Increased salinity lowers the water potential of the culture medium, restricting water uptake and reducing cell turgor pressure, thereby impairing stomatal differentiation and development [26]. In addition, excessive accumulation of sodium (Na^+) and chloride (Cl^-) ions disrupts cellular metabolism and normal leaf development, contributing to a decline in stomatal index [27]. A lower stomatal index represents an adaptive strategy that helps reduce transpirational water loss and maintain cellular water balance under saline conditions. However, this adaptation may also restrict CO_2 uptake, thereby reducing photosynthetic capacity and ultimately suppressing plant growth.

4. Conclusion

Different NaCl concentrations significantly affected the physiological responses of *Solanum tuberosum* L. cv. Cipanas plantlets cultured in vitro. Increasing NaCl concentrations reduced plantlet height, number of leaves, and stomatal index, while progressively altering plantlet color from green to yellowish-green and brownish-green, indicating increasing salinity stress. Based on the observed physiological responses, *S. tuberosum* L. cv. Cipanas plantlets tolerated NaCl concentrations of up to 0.25% for plantlet height and leaf number, and up to 0.50% for plantlet color and stomatal index, as these treatments did not differ significantly from the control and maintained relatively normal growth. Higher NaCl concentrations (≥0.75%) caused marked reductions in growth and physiological performance, indicating that they exceeded the tolerance threshold of the cultivar under the in vitro conditions used in this study.

Compliance with ethical standards

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Disclosure of conflict of interest

All authors have no conflicts of interest.

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