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Original Research

Genetic Variation for the Tolerance to NaCl Stress in Relation to Cultivars: Rooted vs Non-Rooted *In vitro* Studies

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Abstract

Micro-propagated plantlets were used to screen potatoes for salinity (NaCl) tolerance. Nodal cuttings were placed in media with NaCl. The ability of cuttings to induce roots, in part, determined the tolerance of a given clone. We compared the response of rooted versus non-rooted nodal cuttings to salinity stress. Rooted or non-rooted nodal cuttings of "Russet Burbank", "Dark Red Norland", "Snowden", "Atlantic", and "Superior" potato plants were propagated *in vitro* on a medium containing 60 mM NaCl with 3 mM $CaCl_2$. Rooted cuttings were produced by growing cuttings in normal MS medium and MS medium + 60 mM NaCl for 11 days. These rooted cuttings were then transferred to media containing salinity treatments. Thirty replicates were used per treatment. Observations were made up to 32 days after transfer. Results show: (i) Rooted cuttings displayed more tolerance to normal MS medium + 60 mM NaCl stress than non-rooted cuttings; (ii) Injury by NaCl does not appear to be due to osmotic stress. The primary cause of injury is likely ionic toxicity rather than osmotic stress, as evidenced by the protective effect of added $CaCl_2$, which mitigates the toxic effects of Na^+ ions, and the observed symptoms of necrosis and chlorosis. We suggest that rooted cuttings better simulate the response to saline water irrigation.

Keywords: *Solanum tuberosum*, micropropagation, salt stress

Introduction

Plants are constantly subjected to various biotic and abiotic stress factors from their environment. The most significant abiotic stress factors reported are light, temperature, water, and salinity. Salinization of soil through irrigation is an increasing risk factor in plant production. Studies suggest that about 67% of agricultural areas have the potential for transient salinity. This type of salinity is associated with groundwater-related salinity [1-3]. Salinity in soil often leads to other problems, such as soil sodicity and alkalinity. Thus, salinity is a major factor limiting sustainable agriculture [4, 5].

As well as the other ions, NaCl accumulates, the osmotic potential of the soil increases, and the uptake of water is blocked. Moreover, perturbations of plant metabolism occur when ions such as Na^+ or Cl^- move into the plant [6].

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Salinity considerably affects germination, vegetative growth, and reproductive development of plants. Salinity in soil forces the ion toxicity, osmotic stress, deficiency of N-Ca-K-P-Fe-Zn, and oxidative stress. Plants are classified as halophytes, which can grow and reproduce under high salinity (>400 mM NaCl), or glycophytes, which cannot survive high salinity in terms of their tolerance to salinity conditions [3, 7]. In addition, salinity causes water deficit even under well-watered conditions by reducing the osmotic potential of soil solutes, making it difficult for roots to extract water from their surrounding medium. Supplementing the medium with Ca improves growth [8].

Salt-tolerant plants differ in terms of maintaining a low rate of Na^+ and Cl^- transport to leaves and in their ability to sequester these ions in vacuoles to prevent their buildup in the cytoplasm or cell walls, thereby avoiding salt toxicity. "Salt exclusion" functions to reduce the rate at which salt accumulates in transpiring organs. The salts carried in the transpiration stream accumulate in leaves as the water evaporates, leading to salt buildup over time. Consequently, the salt concentration in older leaves is much higher than in younger leaves. Eventually, the concentration of salt becomes high enough to destroy the cells in the older leaves [9].

Many crops, such as cassava, potato, sugarcane, sweet potato, garlic, pineapple, banana, and plantain, are propagated from vegetative parts. Potato (*Solanum tuberosum*) is moderately sensitive to salinity. Variability in tolerance exists among varieties and wild relatives, but little effort has been made to improve tolerance in this species. A positive correlation between root fresh weight and salt stress tolerance was also observed for ten potato clones under salt stress *in vitro* and in the field. Moreover, the presence of a significant correlation between specific *in vitro* and in vivo physiological parameters is encouraging [10].

Tolerance to salinity was shown in the maintenance of vegetative growth, tuber yield, and reduced leaf necrosis [11]. The level of tolerance in plants can differ from genotype to genotype [12]. In the case of three genotypes presumably demonstrating diverse sensitivity to salt stress that were exposed at tuber to salinity levels ranging from 0 to 300 mM NaCl for 30 days, the 150 mM NaCl treatment led to differentiation between these levels of salt sensitivity. Higher salt concentrations (175, 200, or 300 mM) were reported to be lethal to the more sensitive clone [13]. A significant knowledge gap exists in this research area. Currently, no standardized *in vitro* screening method exists that can accurately simulate transplant conditions in saline soil.

In vitro culture practices are an effective way to avoid soil or environmental complexities when studying plant responses to an imposed stress factor [14-18]. Hence, results from the *in vitro* system provide useful evidence to explain plant reactions to stress in studies of plant stress differences [15]. Fast regeneration under controlled environmental conditions after exposure to a stress factor is considered the most important advantage of *in vitro* cultures [19].

Salinity stress primarily impacts the roots, and the effect of salinity on root system architecture (RSA) is crucial for regulating water extraction efficiency and ion exclusion. As shown by phenotyping studies, a reduction in main root elongation and a redistribution of root mass between the main root and lateral roots are key determinants of salt tolerance [20]. The natural variation in the dynamic response of the root system to salt and the four unique strategies further illustrate the role that root architecture plays in developing comprehensive salt tolerance screening approaches. This is especially relevant in agriculture, where plants are often established by transplanting seedlings rather than planting seeds directly into the soil. In these cases, the root system is already formed, and its response to salinity stress can be a critical factor in the plant's survival and productivity. However, most *in vitro* studies in potato have not clearly distinguished whether tolerance is expressed differently in cuttings that already possess roots compared to those that do not. This distinction is important because transplant establishment in saline soils begins from plantlets with functioning root systems, whereas non-rooted cuttings represent an earlier developmental stage with limited capacity to take up water and exclude toxic ions. Therefore, understanding whether the presence of roots alters the physiological response to salinity stress addresses a critical gap in salinity screening protocols.

The objectives of this research were: i) to compare the response of rooted vs. non-rooted nodal cuttings to salinity stress; ii) to screen potatoes for salinity (NaCl) tolerance using micro-propagated plantlets; and iii) to evaluate the ability of the cutting to determine the tolerance of a given clone to salinity stress.

Materials and Methods

Culture Procedure

Micropropagated *Solanum tuberosum* L. cv. "Russet Burbank", "Dark Red Norland", "Snowden", "Atlantic", and "Superior" potato plants were grown on Murashige and Skoog (MS) medium [21]. Single-node cuttings were taken from the second and third nodes of 1-month-old micropropagated potato plantlets. Each cutting consisted of a single node with a leaf and a lateral bud. The culture medium contained 3% sucrose and 0.56 mM myo-inositol. Single nodes were then transferred to MS medium containing either the control (normal MS medium) or 60 mM NaCl, with thirty replicates in each treatment. The pH was adjusted to 5.6±0.02. Agar was added (0.7%) before autoclaving at 132ºC for 15 min before use. Plants were cultured in 20×150 mm glass tubes containing 7.5 ml of medium. Culture tubes were placed under continuous light at 60 μmol m^{-2} s^{-1} photosynthetic photon flux density from cool white

fluorescent lamps [22]. The temperature was maintained at 22±2ºC.

Treatment Groups and Experimental Design

Our experiment was designed to compare the response of pre-rooted (rooted) and non-rooted cuttings to salinity stress. To achieve this, we established two main groups of cuttings:

1. Non-Rooted Cuttings: These cuttings were placed directly onto an MS medium containing either 0 mM NaCl (control) or 60 mM NaCl (salinity treatment). These cuttings were never exposed to a rooting period before the experiment.
2. Pre-Rooted Cuttings: These cuttings were first rooted for 11 days on a standard MS medium (0 mM NaCl) to allow for root formation. After 11 days, they were transferred to a fresh medium containing either 0 mM NaCl (control) to avoid the possibility of transfer shock or 60 mM NaCl (salinity treatment).

Both the non-rooted and pre-rooted cuttings were cultured for a total of 32 days from the start of their respective treatments. The transfer of the pre-rooted cuttings after 11 days was a critical step to ensure that both rooted and non-rooted groups were evaluated under their designated saline or non-saline conditions without the confounding factor of a pre-established root system in the non-rooted group. Each of the four treatment combinations (non-rooted control, non-rooted salinity, pre-rooted control, and pre-rooted salinity) had thirty replicates per cultivar.

Rationale for NaCl Concentration

Recent studies have revealed that optimal root responses to salinity stress involve cell expansion and carbon allocation, as these are necessary for plant survival in saline environments [23]. The ability to sustain root growth dynamics when exposed to salt stress is essential for a plant's capacity to adapt to saline environments; therefore, considering the responses of rooted versus non-rooted cuttings may be relevant to understanding the mechanisms behind potato tolerance to salinity.

The 60 mM NaCl concentration was chosen based on preliminary experiments and its relevance to field conditions for potato cultivation. Potatoes are a moderately sensitive crop to salinity, and concentrations above 100 mM are often lethal or cause severe yield loss in the field. A 60 mM concentration represents a significant level of transient salinity stress that is physiologically relevant and commonly encountered in irrigated agricultural lands, particularly in regions prone to groundwater-associated salinity. At this level, we anticipated a measurable yet non-lethal physiological response that would allow us to differentiate the tolerance levels among the five potato cultivars, as well as compare the distinct responses of rooted versus non-rooted cuttings. Using a higher concentration, while potentially causing a more drastic response, could obscure the subtle differences in tolerance that are critical for breeding and selection purposes.

Observations on the Plantlets and Calcium Contents

Observations for main shoot length (cm), axillary shoots (%), shoot tip injury, and presence of roots (%) were made six different times after culture. Axillary shoots were counted for each plantlet to indicate the percentage of axillary shoots. In addition, shoot tip injury was also counted per plantlet. For this purpose, shoot tips showing browning and necrotic lesions were counted as dead. The last observations were made 32 days after culture. Plantlets were destructively harvested after all observations were completed. The main shoot lengths were measured from the point of emergence of the lateral bud on the original single-node cutting or the point of emergence of the roots to the tip of the organ, respectively [24]. The selected parameters are established indicators of plant stress responses and salinity tolerance. For example, shoot length reflects general growth vigor while under stress, while axillary shoot emergence reflects loss of apical dominance as a result of stress-induced damage to the main meristem. Shoot tip injury (browning and necrotic lesions) reflects direct injury at the cellular level resulting from salt stress. Root development is an important factor influencing plant metabolism because it reflects the ability of the plant to continue to take up water when the growing environment is saline. A combination of these indicators provides the broadest picture of growth responses and physiological damage, both of which are important indicators in salinity tolerance screening protocols.

All of these observations were made on 30 separate plantlets from the rooted and non-rooted applications and five different potato cultivars. Relative salinity tolerance was determined based on the analysis of the growth parameters for each treatment. Every ten plants were assembled to form one group for calcium analysis. Three samples were obtained for each treatment. For this purpose, the samples were dried in an oven at 70ºC, weighed, and ashed (550ºC, 6 h). After that, the ash was dissolved in 2 N HCl and diluted with a lanthanum chloride ($LaCl_3 \times 7H_2O$) solution and distilled-deionized water to obtain samples in 0.2 N HCl and 2000 mg L^{-1}. Every sample was duplicated. Calcium concentration was determined by atomic absorption spectrometry (Varian model SpectrAA-20; Varian Associates, Inc., Sunnyvale, Calif.).

Statistical Analysis

The experiment was conducted using a completely randomized design. The treatments were applied to 30 replications for each of the five potato cultivars. Data for shoot tip injury, presence of roots, and axillary

shoots were collected as binomial (yes/no) outcomes. For example, a shoot tip was either "injured" or "not injured", and a plantlet either had roots or did not. Because these variables are categorical, they were analyzed using the PROC FREQ procedure in SAS (SAS version 9.4) with the binomial option to calculate 95% confidence intervals for the proportion of dead shoot tips, rooted plantlets, and those with axillary shoots. Shoot length was measured as a continuous variable. These data were analyzed using the PROC GLM (General Linear Model) procedure in SAS to perform an analysis of variance (ANOVA) [25]. The means of the treatments were compared using Duncan's multiple range test at a significance level of $P<0.05$ to determine pairwise differences.

A total of 30 plantlets were used as biological replicates for each treatment and cultivar combination. For the calcium content analysis, plantlets were destructively harvested and pooled into groups of 10 to form three technical replicates per treatment. This pooling strategy was implemented to obtain sufficient plant material for accurate chemical analysis while still maintaining a reasonable level of replication to capture the average effect of the treatments.

Results and Discussion

Relation of Growth Parameters with Rooted and Non-Rooted Plantlets

Reduction of the shoot length parameters was calculated for each cultivar as (rooted and non-rooted plantlets' shoot length)/(control plantlets' shoot length) on the 60 mM NaCl concentration medium. Overall, salinity significantly reduced shoot length in non-rooted plantlets for most cultivars, except cv. "Russet Burbank", which showed greater stability under stress (Fig. 1). In contrast, the axillary shoot percentage was increased by salinity for non-rooted cultured plantlets (Table 1).

In general, non-rooted plantlets were more severely affected in terms of shoot length reduction under salinity stress compared to rooted plantlets. The shoot length of rooted and non-rooted plantlets of cv. "Russet Burbank" did not show a significant difference during the 32 days after the first reading (Fig. 2). All genotypes' shoot length was inhibited by the 60 mM NaCl supply, but rooted cuttings consistently displayed greater tolerance to salinity than non-rooted cuttings. Results showed that *Solanum tuberosum* "Dark Red Norland" rooted plantlets were more tolerant to salinity than non-rooted plantlets (Fig. 1 and Fig. 2). These findings indicate that the presence of functional roots enhances salinity tolerance, likely by improving water uptake and ion regulation.

Our study demonstrates the link between rooted plants and salinity tolerance. There have been several studies on salinity tolerance in potato; however, to the best of our knowledge, no previous study has explicitly compared rooted versus non-rooted potato plantlets under salinity stress *in vitro*. Our results showed the superiority of rooted cuttings over non-rooted ones for tolerance to salinity. The growth of cultured roots correlates well with whole plant responses [26].

Results from this study displayed remarkable differences between cultivars under salinity conditions. This genotype-dependent variability is consistent

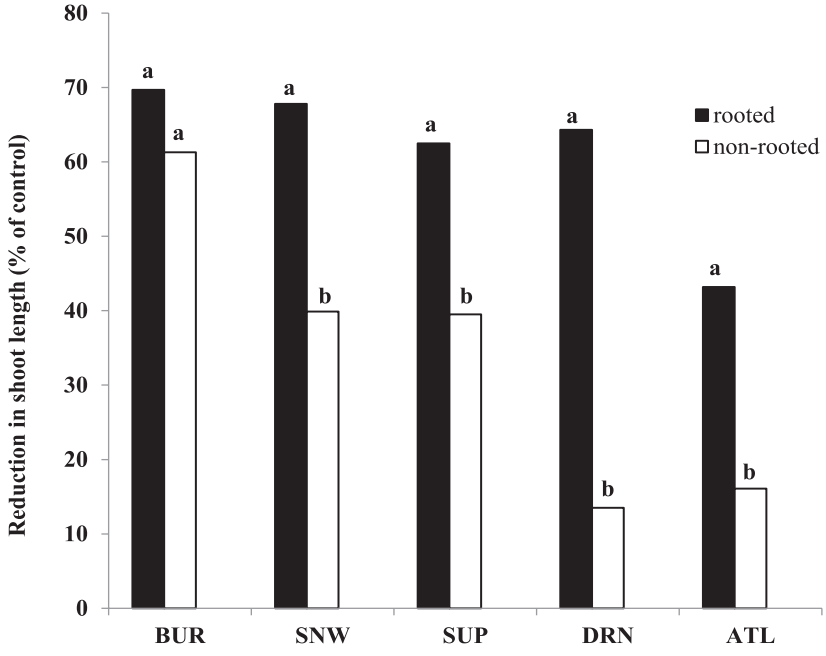

Fig. 1. Reduction of the shoot length parameters was calculated for each cultivar (ATL: Atlantic, BUR: Burbank, DRN: Dark Red Norland, SNW: Snowden, SUP: Superior); rooted or non-rooted plantlets' shoot length (cm/plant)/control plantlets' shoot length. Different letters represent significant differences of mean values according to the SAS General Linear Model procedure at $P\leq0.05$.

Table 1. Axillary Shoots (%)[z].

Plants with axillary shoots (%) Treatmeants			
Cultivar	Control[y]	Rooted	Non-rooted
Atlantic	0.00b	17.24a	30.77a
Dark Red Norland	0.00b	0.00b	83.33a
Burbank	0.00b	0.00b	10.00a
Snowden	0.00b	0.00b	16.67a
Superior	0.00b	0.00b	65.52a

[z] Relationship between rooted and non-rooted plantlets' axillary shoots (% plants) for each cultivar (ATL: Atlantic, BUR: Burbank, DRN: Dark Red Norland, SNW: Snowden, SUP: Superior) after 30 days of transfer. Main shoots showing axillary shoots were counted if they had axillary shoots. Rate values having different letters for axillary shoots are statistically different based on Fisher's exact test (P≤0.05). Each treatment had 30 plantlets (replications).
[y] Control treatment. It was included in the statistical analysis.

with previous studies. For example, [27] reported that *in vitro* culture of nodal segments indicated considerable variability for salt tolerance in Argentine Andean potatoes, which can be exploited for breeding or extending potato cultivation to marginal areas. Furthermore, [28] studied the *in vitro* screening of ten potato cultivars for salt tolerance at different concentrations of NaCl (0, 30, 60, 90, and 120 mM) to select the salt-tolerant and salt-sensitive cultivars. Physiological parameters such as shoot length, root length, shoot dry weight, root dry weight, leaf area, number of rooted shoots, and root dry weight/shoot

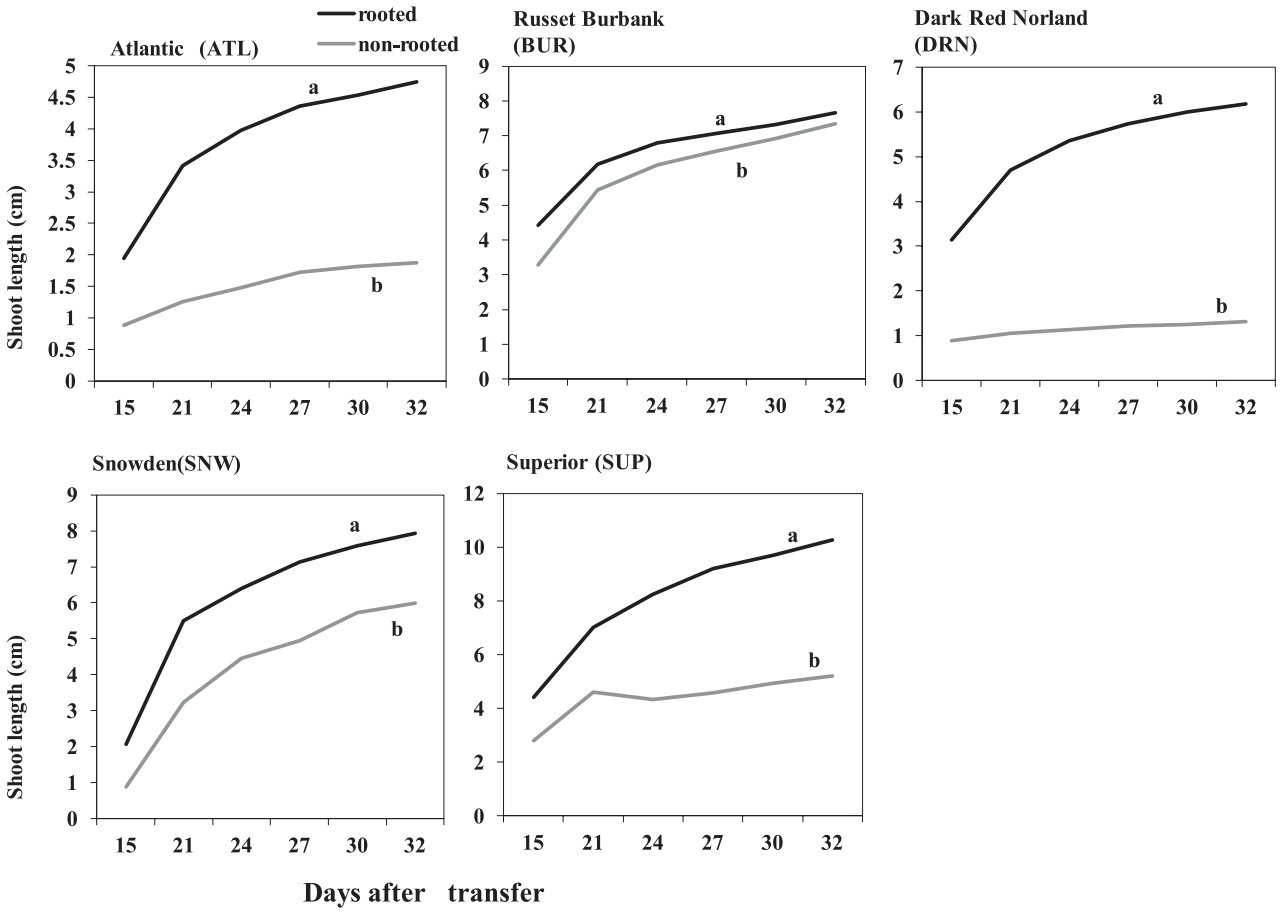

Fig. 2. Relationship between rooted and non-rooted plantlets' main shoot length (cm) for each cultivar (ATL: Atlantic, BUR: Burbank, DRN: Dark Red Norland, SNW: Snowden, SUP: Superior) after 32 days of transfer. Different letters represent significant differences of mean values according to the SAS General Linear Model procedure at P≤0.05.

dry weight were measured. This study suggested that the salt tolerance and salt sensitivity of some potato cultivars are due to genotypic variation and possibly not to epigenetic adaptation under stress conditions. Salinity effects observed in different plant species, as shown in an *in vitro* study conducted in Cairo, Egypt, showed a decrease in growth parameters of citrus plants with increasing NaCl levels. These findings are in agreement with our study [29-31]. The idea of comparing rooted versus non-rooted responses could be applied to crops like sweet potato, cassava, sugarcane, banana, and a variety of ornamental plants that are propagated through cuttings or tissue culture. It would be valuable for crops that are planted by transplanting in agricultural practices because it better mimics the field situation when plants experience salinity stress after their roots have established.

Axillary Shoot Number Analysis

Axillary shoot numbers were negatively affected by NaCl. Significant differences were obtained between rooted and non-rooted plantlets for all cultivars after 30 and 32 days. However, cv. "Atlantic" rooted plantlets had slightly more axillary shoots than other cultivar plantlets after 30 and 32 days (Table 1 and Fig. 3). This suggests that some cultivars may partially compensate for apical meristem injury by promoting lateral shoot formation. Our growth medium contained 3 mM $CaCl_2$ besides the 60 mM NaCl treatment [32], indicating that the death of the shoot apical meristem, as well as the loss of apical dominance, were secondary injuries that followed the collapse of cells in the subapical region. As can be seen in Fig. 3, axillary shoot numbers increased because of the dead shoot apical meristem. Thus, axillary shoot formation in non-rooted cuttings appears to be a stress response rather than an indicator of vigor.

Shoot Tip Injury Analysis

No shoot tip injury was noted on any of the plantlets in cv. "Snowden", either for rooted or non-rooted plantlets. Rooted plantlets had no shoot tip injury after 32 days of culture, while the shoot tips of cv. "Atlantic" tended to have more injury. A statistically significant increase in the shoot tip injury proportion was observed between rooted and non-rooted plantlets for cv. "Dark Red Norland" and "Superior" (Table 2). These results

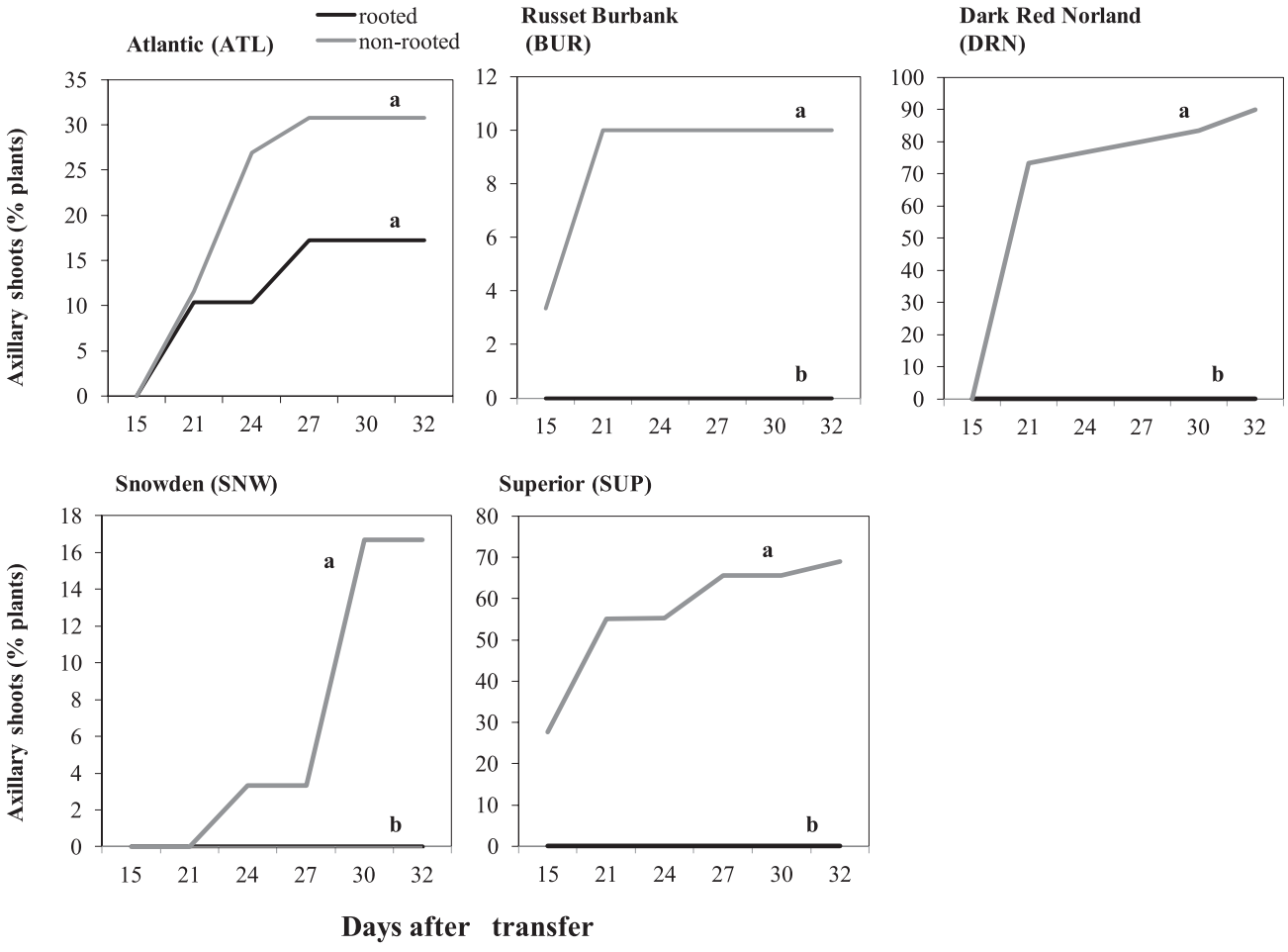

Fig. 3. Relationship between rooted and non-rooted plantlets' axillary shoots percentage (%) for each cultivar (ATL: Atlantic, BUR: Burbank, DRN: Dark Red Norland, SNW: Snowden, SUP: Superior) after 32 days of transfer.

Table 2. Rate of shoot tip injury[z].

Plants with dead shoot tips (%) Treatmeants			
Cultivar	Control[y]	Rooted	Non-rooted
Atlantic	0	13.33a	3.33a
Dark Red Norland	0	0b	26.67a
Burbank	0	0a	6.67a
Snowden	0	0a	0a
Superior	0	0b	16.67a

[z] Relationship between rooted and non-rooted plantlets' shoot tip injury (% plants) for each cultivar (ATL: Atlantic, BUR: Burbank, DRN: Dark Red Norland, SNW: Snowden, SUP: Superior) in media containing 3 mM calcium after 32 days of transfer. Shoot tips showing browning and necrotic lesions were counted as dead. Any two means within a row not followed by the same letter are significantly different according to Fisher's exact test ($P \leq 0.05$). Each treatment had 30 plantlets (replications).
[y] Control treatment. It was not included in the statistical analysis.

demonstrate that roots play a protective role in reducing apical meristem injury under salinity. Rooted plantlets had fewer axillary shoots but higher survival rates compared to non-rooted plantlets. Ca deficiency appears to significantly influence plant growth [32]. Our results are in agreement with this, as salinity controlled by Na salts reduces Ca^{2+} availability and mobility, leading to compromised growth of vegetative and reproductive organs. Salinity affects nutrient uptake, such as Na^+ reducing K^+ uptake or Cl^- reducing NO_3^- uptake, and causes a combination of complex interactions that affect plant metabolism, vulnerability to injury, or internal nutrient requirements [33]. Our interpretation is that the primary cause of injury is likely ionic toxicity rather than osmotic stress, as evidenced by the protective effect of added $CaCl_2$, which mitigates the toxic effects of Na^+ ions and the observed symptoms of necrosis and chlorosis that are characteristic of specific ion toxicity. While osmotic stress is a component of salinity, the alleviation of symptoms by $CaCl_2$ suggests a predominant role for ionic toxicity in this experimental system. Non-rooted single-node cuttings were transferred to media that contained normal + 60 mM NaCl. Thus, rooted plantlets had fewer axillary shoots and a lower shoot tip injury percentage (Fig. 3). Also, all cultivars showed a reduction in shoot length, root system development, and shoot tip injury under salinity [34-37]. Our observations suggest that growth reduction differed between rooted and non-rooted plantlets of the same cultivars (Fig. 1 and Fig. 2).

Root Development and Salinity Response

The presence of roots was inhibited by salinity in non-rooted plantlets. Direct transfer of non-rooted cuttings to salinity conditions resulted in significant growth inhibition, whereas pre-rooted cuttings transferred to salinity showed less damage, confirming that established roots confer a degree of tolerance (Table 1). Similar findings were reported by Zhang (1997), who showed root tip growth inhibition at 80 and 120 mM NaCl in all genotypes [24, 38].

Tissue Calcium Content Analysis

Tissue calcium content for cv. "Superior", "Russet Burbank", and "Snowden" showed a similar pattern across treatments (Fig. 4). The tissue calcium of the control in these cultivars was significantly higher than that of the rooted and non-rooted treatments. Likewise, the tissue calcium content of cv. "Atlantic" and "Dark Red Norland" across treatments had a comparable pattern. However, the means of tissue calcium content were significantly different among treatments (Fig. 4). Calcium is vital for plants to regulate their growth and development. Because of Ca's adaptability and specificity properties, it plays major structural and efficient roles in plants. Its roles range from physiological tasks to specific signaling mechanisms. Plants depend on the unique properties of Ca^{2+} for their structural, enzymatic, and signaling functions. Ca^{2+} strengthens cell walls and induces stress tolerance (biotic and abiotic). It stabilizes cell membranes by connecting various proteins and lipids at membrane surfaces [39]. Cultivars "Atlantic" and "Dark Red Norland" had lower tissue calcium content, with significant differences among control, rooted, and non-rooted treatments. These findings suggest that salinity reduces calcium uptake, leading to injury; however, rooted plantlets were able to maintain relatively higher Ca levels, which may contribute to their superior tolerance.

Cultivar-Specific Salinity Tolerance

The relationship between salinity tolerance and 30 European and North American potato cultivars was evaluated *in vitro*. A modified single-node cutting bioassay was used in which cultivars were exposed to a range of NaCl levels (0, 40, 80, and 120 mM) in a Murashige and Skoog-based medium for one month.

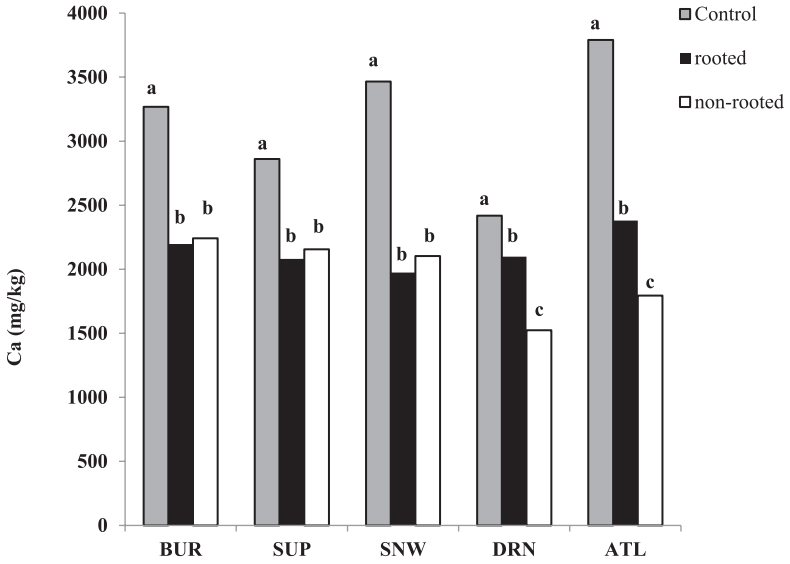

Fig. 4. Relationship between rooted and non-rooted plantlets' tissue calcium (mg/kg dry weight) for each cultivar (ATL: Atlantic, BUR: Burbank, DRN: Dark Red Norland, SNW: Snowden, SUP: Superior) after 32 days of transfer. Different letters represent significant differences of mean values according to the SAS General Linear Model procedure at P≤0.05 (analysis of variance and Duncan's multiple range test).

They suggested that *Solanum tuberosum* cvs. "Atlantic", "Russet Burbank", "Dark Red Norland", and "Snowden" are moderately salt-sensitive, while cv. "Superior" is more tolerant to 80 mM NaCl levels [40]. Our findings extend this work by showing that rooting status modifies this cultivar-specific response, suggesting that traditional screening may underestimate tolerance if rooted cuttings are not considered.

Similar varietal differences have been observed in other Solanaceae crops. For example, tomato (*Solanum lycopersicum* L.) varieties cultivated in Senegal showed significant variation in germination and seedling growth under increasing NaCl concentrations [41-43]. Findings from our study indicate that this variability can be further modulated by rooting status, highlighting the importance of considering plant developmental stage in screening assays. Our results are consistent with recent findings that revealed root dynamic growth strategies in response to salinity are highly variable among genotypes, indicating fundamental strategies of salt adaptation [44] (Zou et al., 2023). The favorable performance of rooted plantlets in our study illustrates the importance of having established root architecture to help retain plant functionality in response to salt. Root system architecture responses to salinity involve complex developmental programs that include changes in cell cycle activity, cell expansion, and resource allocation, which were better developed in rooted plantlets compared to cuttings that were tasked to establish roots and respond to salt stress.

Comparable observations were made in potato cultivars "Desiree" and "Unica", which maintained higher photosynthetic rates under stress compared to "Agria" and "Russet Burbank" [45]. Together, these results emphasize that both genetic background and the presence of functional roots play crucial roles in salinity tolerance, and integrating these factors can improve the reliability of *in vitro* screening protocols.

Conclusions

All genotypes were inhibited in shoot growth by the application of NaCl, but differences in NaCl tolerance were apparent between the rooted and non-rooted transferred plantlets. It could be concluded that rooted cuttings may better simulate plant response to water irrigation and could be used for screening for salinity. This is because the presence of an established root system allows for a more accurate assessment of how the plant manages both osmotic stress and, more critically, ionic toxicity from Na^+ and Cl^- ions. The improved tolerance observed in rooted cuttings suggests that the ability to regulate ion uptake and transport is a key determinant of salinity tolerance.

Conflict of Interest

The authors declare no conflict of interest.

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