Agronomy Research **23**(S2), 609–627, 2025
https://doi.org/10.15159/AR.24.103

Assessment of new citrus hybrid rootstocks to salinity at the early seedling stage under greenhouse conditions

M. Majji[1,2], O. Chetto[1], A. Talha[1], G. Bouaaza[1,2], L. Beniken[1], R. Benkirane[2] and H. Benyahia[1,*]

[1]Unit research of Plant Breeding and Germplasm Conservation, National Institute for Agricultural Research (INRA), Kenitra, Morocco
[2]Laboratory of Plant, Animal, and Agro-Industry Productions, Ibn Tofail University, Kenitra, Morocco
[*]Correspondence: hamidbenyahia2002@yahoo.fr

Received: July 16th, 2024; Accepted: October 29th, 2024; Published: December 25th, 2024

Abstract. The citrus industry in arid areas is largely constrained by the salinity of irrigation water and soil. This study was conducted to determine how six novel citrus hybrid rootstocks will respond to salinity at the seedling stage. Three different NaCl concentrations, 0, 2, and 5 g L^{-1}, were added to the half-concentrated Hoagland solution (corresponding to 1.3 (control), 4 and 9 dS m^{-1}, respectively). Three-month-old seedlings grown in greenhouse conditions and transplanted in plastic pots were used. After two months of stress, different responses from the rootstocks and salt levels were observed. The addition of NaCl to the irrigation solution considerably decreased the fresh and dry weight and leaf chlorophyll content. Additionally, the proline content, soluble sugar, and the leaf chloride content increase with the increase in salinity. Our findings demonstrated that the hybrid *Poncirus Trifoliata* × *Citrus reshni Hort. ex Tan. (V5)* H6 is salt-sensitive, accumulating a high leaf chloride level of 46.92 mg g^{-1} of dry matter and a low chlorophyll content of 1.12 mg g^{-1} of fresh matter associated with signs of leaf toxicity, leading to poor fresh and dry weight. Although hybrid *Poncirus Trifoliata* × *Citrus reshni Hort. ex Tan. (V1)* H2 is thought to be salt-tolerant, it accumulates 38.88 mg g^{-1} of dry-matter leaf chloride and 1.72 mg g^{-1} of fresh-matter chlorophyll content.

Key words: citrus, hybrid rootstock, chloride, salt stress, NaCl.

INTRODUCTION

Citrus is a fruit crop of great commercial importance in the Mediterranean basin, where it produces over 26 million metric tonnes and accounts for 13% of the world's citrus yield (FAOSTAT, 2021). The detrimental impacts of climate change in citrus-growing regions should not be overlooked, as they exacerbate the adverse effects of salinity and water stress. Around 1 billion hectares of soil worldwide are salinised (Ivushkin et al., 2019), it accounts for 33% of irrigated soils and 20% of all cultivated soils used for agriculture (Shrivastava & Kumar, 2015). The Mediterranean basin covers 27.3 million hectares of salt-affected areas (Aragüés et al., 2011). In many cases, additional irrigation is necessary to achieve optimal yield (Maas, 1993; Srivastava & Singh, 2009; Syvertsen & Garcia-Sanchez, 2014; Othman et al., 2023). Additionally, the aquifer irrigation water may have an excessive quantity of soluble salts, which might increase the electrical conductivity (Navarro et al., 2014; Ziogas et al., 2021; Othman et al., 2023). Salinity significantly reduces yield, plant growth, and fruit quality, particularly in dry and semiarid areas (Grattan et al., 2015; Shafieizargar et al., 2015; Sajyan et al., 2018; Issa et al., 2020; Martínez-Cuenca et al., 2021; Marathe et al., 2022; Zhang et al., 2023; Maaroufi-Dguimi et al., 2024). According to reports, citrus growth and yield are adversely affected by soil salinity levels below 2 dS m^{-1}, and fruit yield decreases by 13% for every 1 dS m^{-1} salinity increase above 1.4 dS m^{-1}, which is the electrical conductivity threshold for saturated soil extract (Maas, 1993; Murkute et al., 2005). Previous research revealed that the salinity effects were associated with chloride ion accumulation (Cole, 1985; Storey & Walker, 1998; López-Climent et al., 2008; Khalil et al., 2011; Hussain et al., 2012; Brito et al., 2015), which can be affected by passive forces and associated with water consumption (Moya et al., 1999; Moya et al., 2003). The chloride ion accumulation in leaves leads to a gradual decline in plant growth, accompanied by reduced rates of transpiration and photosynthesis and low productivity (Bañuls et al., 1997; Moya et al., 2002; Arbona et al., 2006; Ziogas et al., 2021). The chloride ion contributes to macromolecule disruption and enzyme inactivation (Kamran et al., 2020). Citrus species vary significantly in their ability to limit sodium and chloride ion uptake at the root level and their transfer to shoots (Storey & Walker, 1998; Hussain et al., 2012). Hence, the tolerance of certain citrus genotypes to salinity seems to be related to their ability to prevent or reduce the transfer of chloride ions into the leaves (Moya et al., 2003; Kamran et al., 2020). Several years of field studies have successfully identified some citrus species and rootstocks tolerant to salinity (Davies & Albrigo, 1994). These studies have revealed that Rangpur lime and Cleopatra mandarin are more resilient to high salinity levels. They also demonstrated that they can survive high chloride levels in leaves and exclude and restrict chloride ion transfer from roots to shoots (Douglas & Walker, 1983). This capacity is the result of physiological and biochemical changes at the cellular level that are regulated by gene expression in reaction to high salinity levels (Romero-Aranda et al., 1998; Storey & Walker, 1998). *Citrus aurantium* (sour orange), the main rootstock used in citrus orchards, was found sensible to the *citrus tristeza virus*, a devastating disease in the Mediterranean basin, and needs to be replaced. Currently used rootstocks such as *Citrus macrophylla* and *Citrus volkameriana* are considered susceptible to salinity, an environmental factor increasing with climate change. Under these circumstances, there is a need to find novel rootstock candidates tolerant to salinity to replace the susceptible ones used. Forner-Giner et al. (2009) and Forner-Giner et al. (2011) have developed, through directed hybridisation, two new rootstocks tolerant to salinity: Forner-Alcaide 5 and Forner-Alcaide 13. In various citrus rootstocks and cultivars, physiological and biochemical alterations in response to excessive salt have been examined (GuetaDahan et al., 1997; Benyahia et al., 2004; Fadli et al., 2014; Ait El Aouad et al., 2015). The objective of the current study is to evaluate the resistance of new citrus hybrid rootstocks, resulting from a hybridisation between parents with salt tolerance and the citrus tristeza virus characteristics, to salt stress using morphological, physiological, and biochemical indicators under greenhouse conditions.

MATERIAL AND METHODS

Plant material and growing conditions

The experiment was conducted in the summer of 2022 in a greenhouse of the Regional Agricultural Research Centre (INRA), Kenitra, Morocco (Latitude 34.296722: Longitude -6.485867: Altitude 25 m), under the conditions of a 28 °C average temperature, 60% average relative humidity, and 14–15 h of daylight. The healthy and mature fruits of six rootstock hybrids (Table 1), belonging to the seed collection of INRA, were harvested at the experimental field of El Menzeh. Seeds were extracted, washed, and dried in the shade; the seeds were germinated in plastic basins filled with peat. The seedlings were irrigated regularly, twice a week. When the seedlings were three months old, uniform seedlings with 4 to 6 leaves and a height of about 10 cm were transplanted into 1 litre plastic pots (14 cm height, 13 cm top diameter, and 10 cm bottom diameter) in a mixture of 1:1 peat and sand. This substrate allows root aeration and solute leaching. The seedlings were routinely watered twice weekly with a half-concentrated Hoagland solution during a one-month acclimatisation period (Hoagland & Arnon, 1950).

Table 1. List of the new hybrid citrus rootstocks tested in the experiment

Rootstock	Code
Poncirus Trifoliata × *Citrus Volkameriana*	H1
Poncirus Trifoliata × *Citrus reshni Hort. ex Tan. (V1)*	H2
Poncirus Trifoliata × *Citrus reshni Hort. ex Tan. (V2)*	H3
Poncirus Trifoliata × *Citrus reshni Hort. ex Tan. (V3)*	H4
Poncirus Trifoliata × *Citrus reshni Hort. ex Tan. (V4)*	H5
Poncirus Trifoliata × *Citrus reshni Hort. ex Tan. (V5)*	H6
Citrus limonia Osbeck (Lime Rangpur)	LR
Poncirus Trifoliata	PT

Salt stress treatment

Salt stress treatment was performed for two months. Salt stress is applied after the period of acclimation to the new environment by adding NaCl to the half-concentrated Hoagland solution at two different levels: 2 g L^{-1} and 5 g L^{-1}, while the control was irrigated with the half-concentrated Hoagland solution, corresponding to 4, 9, and 1.3 dS m^{-1}. To avoid osmotic shock, the concentration of NaCl is increased gradually until the desired levels are reached. Irrigation is performed twice a week in addition to a water washout every two weeks to avoid salt ion accumulation.

Assessment of salt stress tolerance

Chlorosis symptoms

The response of seedlings to salt stress was determined by recording the appearance of leaf injury symptoms after two months. All seedlings were visually assessed, and a score from 0 to 5 was assigned to each plant according to the scale of Goell (1969). The score was given based on the severity of injury symptoms, such as chlorosis, wilting, and defoliation (Fadli et al., 2014; Ait El Aouad et al., 2015).

Determination of leaf chloride content

The chloride ion was extracted from 200 mg of dry leaf tissue by adding 50 mg of calcium hydroxide and a few drops of distilled water. The samples are then mixed and placed in an oven for high-temperature alkaline incineration at 550 °C for 90 min (Cotlove, 1964). After incineration, 50 mL of distilled water is added to the samples, and the mixture is brought to a boil. After filtration, 0.5 mL of the extract was taken to determine the content of the chloride ion, based on the chloride analyser Sherwood England manufacturer (Sherwood Scientific Ltd., Cambridge, UK), using a standard commercial solution of 200 mg L^{-1} of chloride for calibration (Vives-Peris et al., 2023). And the obtained values are expressed as mg g^{-1} of dry matter (DM) of leaves.

Estimation of total chlorophyll pigment

The chlorophyll content was determined using the Arnon (1949) method by measuring the optical density. For this analysis, fresh leaves weighing 0.1 g were mashed and transferred into screw-cap tubes containing 10 mL of 80% acetone. The tubes were then stored in darkness at a temperature of 4 °C. Subsequently, the mixture was filtered, and the resulting supernatant was collected. The optical density (DO) measurements were taken at both 645 nm and 663 nm for the collected supernatant. The obtained results were expressed as mg g^{-1} fresh matter (FM):

$$\text{Chlorophyll (mg g}^{-1}\text{FM)} = \frac{20.2\ (\text{DO645}) + 8.02\ (\text{DO663})] \times \text{V}}{1{,}000 \times \text{FM}}$$

where V = volume of acetone; FM = fresh leaf weight; DO645 = optical density at 645 nm, and DO663 = optical density at 663 nm.

Determination of leaf proline content

Leaf proline content is determined according to the methods of Bates (1973). In screw-cap tubes, 50 mg of dried and ground leaves is combined with 3 mL of 3% sulfosalicylic acid. The mixture is heated in a water bath at 80 °C for 1 hour. Afterward, 1 mL is taken and mixed with 1 mL of ninhydrin acid and 1 mL of glacial acetic acid. The resulting mixture is heated again in a water bath at 100 °C for 30 min. To each tube, 5 mL of toluene is added, and the contents are thoroughly mixed using a vortex mixer. Finally, the optical density of the mixture is measured at a wavelength of 520 nm using a spectrophotometer, and the obtained values are expressed in mg g^{-1} of dry matter (DM) using the equation of the standard curve prepared with L-proline.

Determination of leaf sugar content

The leaf sugar content was estimated according to the method of DuBois (1956). In a screw-cap tube, 3 mL of 80% ethanol and 50 mg of dried powdered leaves are combined. The mixture is kept in darkness for 48 hours. Afterward, 1 mL of the extract is mixed with 1 mL of 5% phenol and 5 mL of sulfuric acid. And the mixture is thoroughly mixed using a vortex. Finally, the reaction was stopped by placing the tubes in a water bath for 30 min, and then the optical density of the mixture was measured at a wavelength of 485 nm using a spectrophotometer. The values obtained were expressed in mg g^{-1} dry matter (DM) using the standard curve equation previously prepared with glucose.

Fresh and dry weights of stem, leaf and root

After harvesting, the plants were divided into leaves, stems, and roots. Each part of the plant was placed in a bag and weighed before and after drying at 80 °C for 72 h to determine their fresh and dry weights (Bañuls et al., 1997). Salinity tolerance was estimated by determining the relative percent reduction in fresh and dry weights (Fadli et al., 2014; Ait El Aouad et al., 2015).

Statistical analysis

The experiment was conducted using a split-plot design, with the salinity factor assigned to the main plot and the rootstock factor in the subplot. The data collected were imported into the Statistical Analysis System (SAS) software and analysed using a two-way analysis of variance (ANOVA) of the general linear models (GLM). The significant differences between means were determined using Duncan's multiple range test at a 95% confidence level.

RESULTS

Effect of salt stress on leaf injury symptoms

Chlorosis symptoms appear on the leaves 5 weeks after the beginning of the salt treatment. This phenomenon starts at the basal leaves and progresses to the apical leaves. The results presented in Table 2 show variability between rootstocks in relation to the stress levels applied. For the treatment control, the leaf injury was very low, with variability among the hybrids that varies between 0.33 and 0.67 depending on the hybrids. Indeed, at 2 g L^{-1} NaCl, the *Poncirus Trifoliata* × *Citrus Volkameriana* H1 rootstock showed the lowest toxicity symptoms (0.83); the *Poncirus Trifoliata* × *Citrus Reshni Hort. Ex Tan. (V1)* H2, *Poncirus Trifoliata* × *Citrus Reshni Hort. Ex Tan. (V2)* H3, And *Poncirus Trifoliata* × *Citrus Reshni Hort. Ex Tan. (V4)* H5 rootstocks showed an index of 1.17; whereas the *Poncirus Trifoliata* × *Citrus Reshni Hort. Ex Tan. (V5)* H6 rootstocks had an index of 1.50 according to the Goell scale (Figs 1, 2). At 5 g L^{-1}, all the rootstocks were affected by salt. The *Poncirus Trifoliata* ×*Citrus Volkameriana* H1 and *Poncirus Trifoliata* × *Citrus Reshni Hort. Ex Tan. (V2)* H3 rootstocks were the least affected and presented the lowest index of 1.50,

Table 2. Effect of salt stress on leaf injury symptoms according to the scale of Goell (1969)

Rootstock	Control	2 g L^{-1} NaCl	5 g L^{-1} NaCl
H1	0.33 ± 0.21^{b}	0.83 ± 0.31^{ab}	1.50 ± 0.22^{a}
H2	0.50 ± 0.22^{b}	1.17 ± 0.17^{a}	1.67 ± 0.21^{a}
H3	0.33 ± 0.21^{b}	1.17 ± 0.17^{a}	1.50 ± 0.22^{a}
H4	0.67 ± 0.21^{b}	1.33 ± 0.21^{ab}	2.00 ± 0.26^{a}
H5	0.50 ± 0.22^{b}	1.17 ± 0.17^{b}	2.50 ± 0.56^{a}
H6	0.33 ± 0.21^{c}	1.50 ± 0.22^{b}	3.33 ± 0.33^{a}
LR	0.17 ± 0.17^{b}	0.67 ± 0.21^{ab}	1.17 ± 0.17^{a}
PT	1.00 ± 0.26^{c}	2.67 ± 0.21^{b}	4.50 ± 0.22^{a}

For the same rootstock, stress levels with the same letter do not differ significantly; at $P \leq 0.05$ (*one-way ANOVA*, separated by *Duncan's test*), mean values ± standard error (n = 6). H1 = *Poncirus Trifoliata* × *Citrus Volkameriana* H1, H2 = *Poncirus Trifoliata* × *Citrus reshni Hort. ex Tan. (V1)* H2, H3 = *Poncirus Trifoliata* × *Citrus reshni Hort. ex Tan. (V2)* H3, H4 = *Poncirus Trifoliata* × *Citrus reshni Hort. ex Tan. (V3)* H4, H5 = *Poncirus Trifoliata* × *Citrus reshni Hort. ex Tan. (V4)* H5, H6 = *Poncirus Trifoliata* × *Citrus reshni Hort. ex Tan. (V5)* H6, LR = *Citrus limonia Osbeck (Lime Rangpur),* PT = *Poncirus Trifoliata.*

which is close to that of the resistant control LR (1.17). On the other hand, the rootstock *Poncirus Trifoliata* × *Citrus Reshni Hort. Ex Tan. (V5)* H6 showed the highest toxicity index of 3.33.

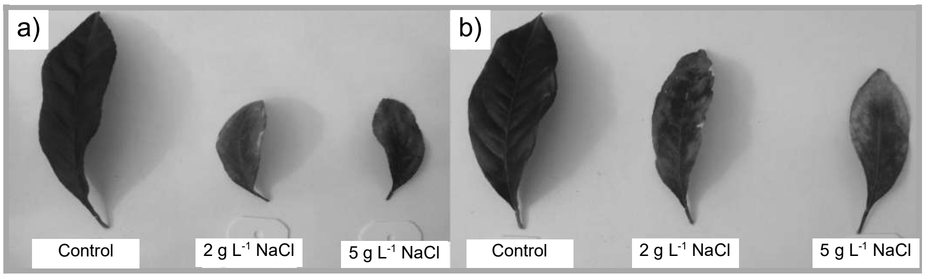

Figure 1. Effect of salinity levels on leaf injury of the rootstocks: (a) hybrid *Poncirus Trifoliata* × *Citrus reshni Hort. ex Tan. (V1)* H2 ; (b) hybrid *Poncirus Trifoliata* × *Citrus Reshni Hort. Ex Tan. (V5)* H6.

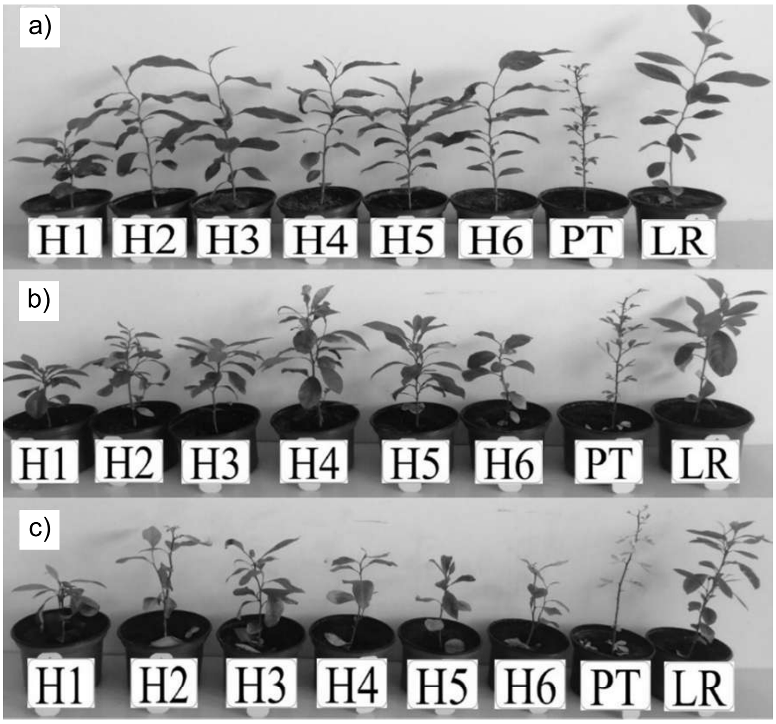

Figure 2. Effect of salt stress on the appearance of the rootstocks at the end of treatment: (a) the control; (b) 2 g L^{-1} NaCl; (c) 5 g L^{-1} NaCl.

H1 = *Poncirus Trifoliata* × *Citrus Volkameriana* H1, H2 = *Poncirus Trifoliata* × *Citrus reshni Hort. ex Tan. (V1)* H2, H3 = *Poncirus Trifoliata* × *Citrus reshni Hort. ex Tan. (V2)* H3, H4 = *Poncirus Trifoliata* × *Citrus reshni Hort. ex Tan. (V3)* H4, H5 = *Poncirus Trifoliata* × *Citrus reshni Hort. ex Tan. (V4)* H5, H6 = *Poncirus Trifoliata* × *Citrus reshni Hort. ex Tan. (V5)* H6, LR = *Citrus limonia Osbeck (Lime Rangpur)*, PT = *Poncirus Trifoliata.*

Effect of salt stress on the chloride ion content in leaves

Fig. 3 shows the chloride ion content in leaves as a function of the applied NaCl concentration. The results show that rootstocks accumulate chloride ions in the leaves in the presence of NaCl in the irrigation solution, and this accumulation varies with the concentration of salt in the irrigation solution and rootstock genotype. Chloride content ranged from 5.06 mg g^{-1} DM to 9.30 mg g^{-1} DM for control rootstocks. At 2 g L^{-1} NaCl, the chloride content was tripled or quadrupled depends on the rootstock; the lowest content of 19.34 mg g^{-1} DM was recorded in the rootstock *Poncirus Trifoliata* × *Citrus Reshni Hort. Ex Tan. (V1)* H2, while the highest content of 28.97 mg g^{-1} DM was recorded in the *Poncirus Trifoliata* × *Citrus Volkameriana* H1 rootstock. At a concentration of 5 g L^{-1} NaCl, the chloride ion content increased significantly, more than five times compared to the control. The highest content of 46.92 mg g^{-1} DM was recorded in rootstock *Poncirus Trifoliata* × *Citrus Reshni Hort. Ex Tan. (V5)* H6, followed by rootstock *Poncirus Trifoliata* × *Citrus Reshni Hort. Ex Tan. (V4)* H5 with a content of 45.50 mg g^{-1} DM. The lowest chloride contents of 38.88 and 38.90 were recorded in rootstocks *Poncirus Trifoliata* × *Citrus Reshni Hort. Ex Tan. (V1)* H2 and *Poncirus Trifoliata* × *Citrus Reshni Hort. Ex Tan. (V3)* H4, respectively.

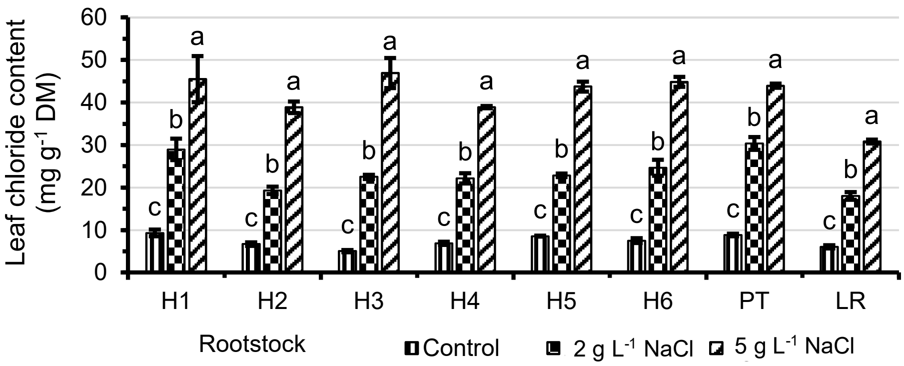

Figure 3. Effect of salt stress on leaf ion chloride content.

For the same rootstock, stress levels with the same letter do not differ significantly; at $P \leq 0.05$ (*one-way ANOVA*, separated by *Duncan's test*), vertical bars represent the standard error ($n = 6$). H1 = *Poncirus Trifoliata* × *Citrus Volkameriana* H1, H2 = *Poncirus Trifoliata* × *Citrus reshni Hort. ex Tan. (V1)* H2, H3 = *Poncirus Trifoliata* × *Citrus reshni Hort. ex Tan. (V2)* H3, H4 = *Poncirus Trifoliata* × *Citrus reshni Hort. ex Tan. (V3)* H4, H5 = *Poncirus Trifoliata* × *Citrus reshni Hort. ex Tan. (V4)* H5, H6 = *Poncirus Trifoliata* × *Citrus reshni Hort. ex Tan. (V5)* H6, LR = *Citrus limonia Osbeck (Lime Rangpur),* PT = *Poncirus Trifoliata.*

Effect of salt stress on leaf chlorophyll content

The results presented in Fig. 4 show that all the rootstocks tested respond negatively to salt stress. However, this response varies according to the rootstock and the intensity of the salt level. For the control, the chlorophyll content varies between 2.56 mg g^{-1} FM and 3.36 mg g^{-1} FM. At 2 g L^{-1} NaCl, chlorophyll content decreased in all rootstocks. The highest reduction percentage was recorded in rootstocks *Poncirus Trifoliata* × *Citrus Reshni Hort. Ex Tan. (V5)* H6 and *Poncirus Trifoliata* × *Citrus Reshni Hort. Ex Tan. (V1)* H2, and the lowest was recorded in rootstocks *Poncirus Trifoliata* × *Citrus Reshni*

Hort. Ex Tan. (V3) H4 and *Poncirus Trifoliata* × *Citrus Volkameriana* H1. Under 5 g L^{-1} NaCl, chlorophyll content decreased in all rootstocks. However, rootstock *Poncirus Trifoliata* × *Citrus Reshni Hort. Ex Tan. (V5)* H6 showed a significant reduction in chlorophyll content and recorded a percentage reduction of 64.89%. While rootstocks *Poncirus Trifoliata* × *Citrus Reshni Hort. Ex Tan. (V4)* H5 and *Poncirus Trifoliata* × *Citrus Reshni Hort. Ex Tan. (V3)* H4 seemed to be less affected by salinity, they recorded percentages of reduction of 37.02% and 37.63%, respectively.

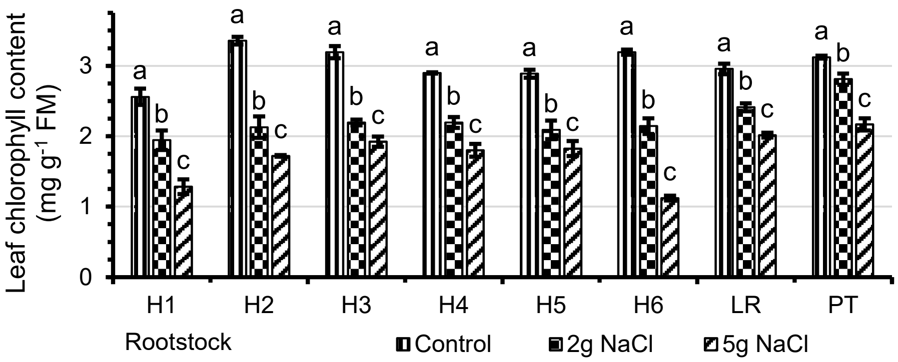

Figure 4. Effect of salt stress on leaf chlorophyll content.

For the same rootstock, stress levels with the same letter do not differ significantly; at $P \leq 0.05$ (*one-way ANOVA*, separated by *Duncan's test*), vertical bars represent the standard error ($n = 6$). H1 = *Poncirus Trifoliata* × *Citrus Volkameriana* H1, H2 = *Poncirus Trifoliata* × *Citrus reshni Hort. ex Tan. (V1)* H2, H3 = *Poncirus Trifoliata* × *Citrus reshni Hort. ex Tan. (V2)* H3, H4 = *Poncirus Trifoliata* × *Citrus reshni Hort. ex Tan. (V3)* H4, H5 = *Poncirus Trifoliata* × *Citrus reshni Hort. ex Tan. (V4)* H5, H6 = *Poncirus Trifoliata* × *Citrus reshni Hort. ex Tan. (V5)* H6, LR = *Citrus limonia Osbeck (Lime Rangpur),* PT = *Poncirus Trifoliata.*

Effect of salt stress on leaf proline content

Fig. 5 shows the results obtained from the proline content of the leaves of the rootstocks evaluated according to the levels of stress. According to these results, we can observe variability among rootstocks and stress levels. The proline content increased with increasing salt concentrations in the irrigation solution. Indeed, low levels were recorded in the control rootstocks, ranging from 48.97 µg g^{-1} DM recorded in the *Poncirus Trifoliata* × *Citrus Volkameriana* H1 rootstock to 61.10 µg g^{-1} shown by the *Poncirus Trifoliata* × *Citrus Reshni Hort. Ex Tan. (V2)* H3 rootstock. Under a concentration of 2 g L^{-1} NaCl, the proline content increased. Rootstock *Poncirus Trifoliata* × *Citrus Reshni Hort. Ex Tan. (V3)* H4 accumulated the lowest value of 71.59 µg g^{-1} DM, and rootstock *Poncirus Trifoliata* × *Citrus Reshni Hort. Ex Tan. (V2)* H3 accumulated the highest amount of 89.12 µg g^{-1} DM. When the rootstocks were irrigated with 5 g L^{-1} NaCl, the proline content increased significantly, and the rootstock *Poncirus Trifoliata* × *Citrus Reshni Hort. Ex Tan. (V1)* H2 recorded the lowest value of 95.82 µg g^{-1} DM, and the rootstock *Poncirus Trifoliata* × *Citrus Reshni Hort. Ex Tan. (V4)* H5 accumulated more proline, 117.89 µg g^{-1} DM.

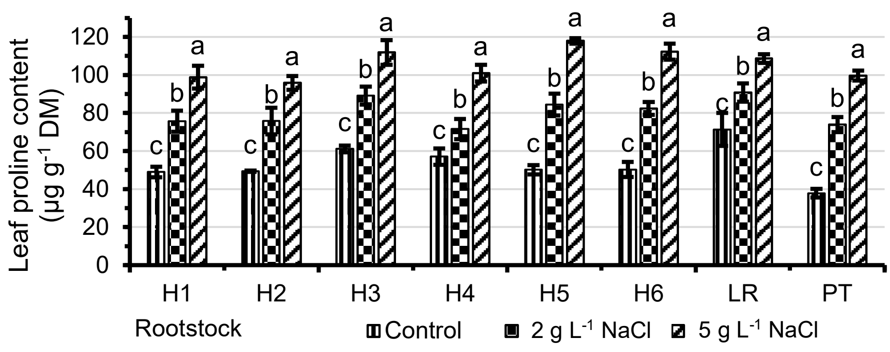

Figure 5. Effect of salt stress on leaf chlorophyll content.

For the same rootstock, stress levels with the same letter do not differ significantly; at $P \leq 0.05$ (*one-way ANOVA*, separated by *Duncan's test*), vertical bars represent the standard error ($n = 6$). H1 = *Poncirus Trifoliata* × *Citrus Volkameriana* H1, H2 = *Poncirus Trifoliata* × *Citrus reshni Hort. ex Tan. (V1)* H2, H3 = *Poncirus Trifoliata* × *Citrus reshni Hort. ex Tan. (V2)* H3, H4 = *Poncirus Trifoliata* × *Citrus reshni Hort. ex Tan. (V3)* H4, H5 = *Poncirus Trifoliata* × *Citrus reshni Hort. ex Tan. (V4)* H5, H6 = *Poncirus Trifoliata* × *Citrus reshni Hort. ex Tan. (V5)* H6, LR = *Citrus limonia Osbeck (Lime Rangpur),* PT = *Poncirus Trifoliata.*

Effect of salt stress on leaf sugar content

Fig. 6 shows the variation in soluble sugar content in the leaves of the citrus rootstocks as a function of irrigation solution salt concentration. It has been shown that the soluble sugar content of leaves increases when salt concentration increases.

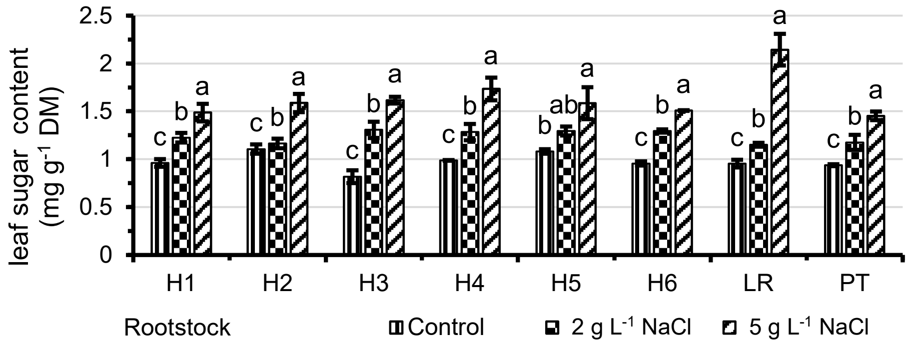

Figure 6. Effect of salt stress on leaf sugar content.

For the same rootstock, stress levels with the same letter do not differ significantly; at $P \leq 0.05$ (*one–way ANOVA*, separated by *Duncan's test*), vertical bars represent the standard error ($n = 6$). H1 = *Poncirus Trifoliata* × *Citrus Volkameriana* H1, H2 = *Poncirus Trifoliata* × *Citrus reshni Hort. ex Tan. (V1)* H2, H3 = *Poncirus Trifoliata* × *Citrus reshni Hort. ex Tan. (V2)* H3, H4 = *Poncirus Trifoliata* × *Citrus reshni Hort. ex Tan. (V3)* H4, H5 = *Poncirus Trifoliata* × *Citrus reshni Hort. ex Tan. (V4)* H5, H6 = *Poncirus Trifoliata* × *Citrus reshni Hort. ex Tan. (V5)* H6, LR = *Citrus limonia Osbeck (Lime Rangpur),* PT = *Poncirus Trifoliata.*

Under low stress (2 g L^{-1}), the soluble sugar content in the leaves of the different rootstocks varied between 1.15 mg g^{-1} DM and 1.31 mg g^{-1} DM. Under a concentration of 5 g L^{-1} NaCl, the content of soluble sugars in the leaves of different rootstocks increases significantly. The highest soluble sugar content was recorded in rootstock *Poncirus Trifoliata* × *Citrus Reshni Hort. Ex Tan. (V3)* H4, and the lowest was recorded in rootstock *Poncirus Trifoliata* × *Citrus Reshni Hort. Ex Tan. (V5)* H6 and *Poncirus Trifoliata* × *Citrus Volkameriana* H1. However, all rootstocks recorded lower soluble sugar content than the resistant control LR and higher than the susceptible PT.

Effect of salt stress on the variation of fresh weight of the rootstocks

The salt stress reduces the fresh weight of different plant organs, leaves, stems, and roots, as well as the whole plant. At whole plant level, the rootstock *Poncirus Trifoliata* × *Citrus reshni Hort. ex Tan. (V1)* H2 has a reduction of 8% when the plants are treated with 2 g L^{-1} NaCl, and the rootstock *Poncirus Trifoliata* × *Citrus Reshni Hort. Ex Tan. (V5)* H6 has a reduction of 30%. At a 5 g L^{-1} NaCl level, the fresh weight of rootstock *Poncirus Trifoliata* × *Citrus Reshni Hort. Ex Tan. (V1)* H2 has reduced by 29%, while a fresh weight reduction of 50% has been recorded for rootstock *Poncirus Trifoliata* × *Citrus Reshni Hort. Ex Tan. (V5)* H6.

Effect of salt stress on fresh weight of leaves. Table 3 shows that the fresh weight of leaves responds negatively with the increase in NaCl in irrigation solution. The fresh weight ranges between 10.35 g and 3.43 g for the control and between 5.13 g and 1.14 g for 5 g L^{-1} NaCl. The rootstock *Poncirus Trifoliata* × *Citrus reshni Hort. ex Tan. (V1)* H2 has the greatest weight of all treatments. While the rootstock *Poncirus Trifoliata* × *Citrus Reshni Hort. Ex Tan. (V5)* H6 has the lowest.

Effect of salt stress on fresh weight of stems. Table 4 shows that the fresh weight of stem organs decreases with the elevation of NaCl concentration in the irrigation solution. The highest

Table 3. Effect of salt stress on fresh weight of leaves (g)

Rootstock	Control	2 g L^{-1} NaCl	5 g L^{-1} NaCl
H1	6.88 ± 0.50^{a}	5.66 ± 0.33^{a}	4.25 ± 0.59^{b}
H2	8.36 ± 0.13^{a}	7.25 ± 0.40^{b}	5.13 ± 0.24^{c}
H3	7.36 ± 0.60^{a}	5.66 ± 0.69^{ab}	4.29 ± 0.35^{b}
H4	7.99 ± 1.10^{a}	5.88 ± 1.46^{ab}	4.11 ± 0.77^{b}
H5	7.99 ± 0.97^{a}	7.73 ± 0.67^{a}	3.67 ± 1.05^{b}
H6	5.44 ± 0.31^{a}	3.88 ± 1.11^{ab}	2.36 ± 0.61^{b}
LR	10.35 ± 1.64^{a}	6.47 ± 0.32^{b}	4.76 ± 0.24^{b}
PT	3.43 ± 1.33^{a}	1.25 ± 0.20^{a}	1.14 ± 0.23^{a}

For the same rootstock, stress levels with the same letter do not differ significantly; at $P \leq 0.05$ (*one–way ANOVA*, separated by *Duncan's test*), mean values ± standard error ($n = 6$). H1 = *Poncirus Trifoliata* × *Citrus Volkameriana* H1, H2 = *Poncirus Trifoliata* × *Citrus reshni Hort. ex Tan. (V1)* H2, H3 = *Poncirus Trifoliata* × *Citrus reshni Hort. ex Tan. (V2)* H3, H4 = *Poncirus Trifoliata* × *Citrus reshni Hort. ex Tan. (V3)* H4, H5 = *Poncirus Trifoliata* × *Citrus reshni Hort. ex Tan. (V4)* H5, H6 = *Poncirus Trifoliata* × *Citrus reshni Hort. ex Tan. (V5)* H6, LR = *Citrus limonia Osbeck (Lime Rangpur),* PT = *Poncirus Trifoliata.*

Table 4. Effect of salt stress on fresh weight of stems (g)

Rootstock	Control	2 g L^{-1} NaCl	5 g L^{-1} NaCl
H1	2.62 ± 0.32^{a}	2.03 ± 0.05^{a}	2.01 ± 0.26^{a}
H2	4.68 ± 0.31^{a}	4.31 ± 0.19^{a}	3.65 ± 0.49^{a}
H3	3.49 ± 0.12^{a}	2.75 ± 0.04^{b}	2.13 ± 0.22^{c}
H4	3.81 ± 0.77^{a}	3.56 ± 0.45^{a}	2.43 ± 0.12^{a}
H5	2.40 ± 0.24^{a}	2.14 ± 0.22^{a}	1.58 ± 0.16^{a}
H6	2.83 ± 0.25^{a}	1.85 ± 0.27^{b}	1.33 ± 0.17^{b}
LR	5.02 ± 0.36^{a}	3.59 ± 0.22^{b}	2.87 ± 0.22^{b}
PT	3.77 ± 0.44^{a}	2.76 ± 0.15^{a}	2.77 ± 0.37^{a}

For the same rootstock, stress levels with the same letter do not differ significantly; at $P \leq 0.05$ (*one–way ANOVA*, separated by *Duncan's test*), mean values ± standard error ($n = 6$). H1 = *Poncirus Trifoliata* × *Citrus Volkameriana* H1, H2 = *Poncirus Trifoliata* × *Citrus reshni Hort. ex Tan. (V1)* H2, H3 = *Poncirus Trifoliata* × *Citrus reshni Hort. ex Tan. (V2)* H3, H4 = *Poncirus Trifoliata* × *Citrus reshni Hort. ex Tan. (V3)* H4, H5 = *Poncirus Trifoliata* × *Citrus reshni Hort. ex Tan. (V4)* H5, H6 = *Poncirus Trifoliata* × *Citrus reshni Hort. ex Tan. (V5)* H6, LR = *Citrus limonia Osbeck (Lime Rangpur),* PT = *Poncirus Trifoliata.*

fresh weights were observed for the control, and the lowest were observed in hybrids irrigated with 5 g L^{-1} NaCl. The rootstock *Poncirus Trifoliata* × *Citrus reshni Hort. Ex Tan. (V1)* H2 has the greatest weight of all treatments. While the rootstock *Poncirus Trifoliata* × *Citrus Reshni Hort. Ex Tan. (V5)* H6 has the lowest.

Effect of salt stress on fresh weight of roots. The fresh weight of roots (Table 5), decreased with the elevation of salt concentration in the solution. The rootstock *Poncirus Trifoliata* × *Citrus reshni Hort. ex Tan. (V1)* H2 has the greatest weight of all treatments and has the lowest reduction in root weight. While the rootstock *Poncirus Trifoliata* ×*Citrus Reshni Hort. Ex Tan. (V5)* H6 has the lowest fresh weight compared with other rootstocks.

Table 5. Effect of salt stress on fresh weight of roots (g)

Rootstock	Control	2 g L^{-1} NaCl	5 g L^{-1} NaCl
H1	5.68 ± 0.67^{a}	4.32 ± 0.11^{a}	4.29 ± 0.54^{a}
H2	6.54 ± 0.32^{a}	6.55 ± 0.66^{a}	5.08 ± 0.11^{b}
H3	5.70 ± 0.27^{a}	4.41 ± 0.13^{b}	3.41 ± 0.41^{b}
H4	5.98 ± 1.41^{a}	5.16 ± 0.44^{a}	3.12 ± 0.06^{a}
H5	5.70 ± 0.10^{a}	4.12 ± 0.41^{b}	3.11 ± 0.35^{a}
H6	4.74 ± 0.15^{a}	3.40 ± 0.34^{ab}	2.81 ± 0.51^{b}
LR	6.10 ± 0.74^{a}	4.20 ± 0.38^{ab}	3.35 ± 0.41^{b}
PT	5.57 ± 0.45^{a}	4.02 ± 0.37^{b}	3.52 ± 0.22^{b}

For the same rootstock, stress levels with the same letter do not differ significantly; at $P \leq 0.05$ (*one–way ANOVA*, separated by *Duncan's test*), mean values ± standard error ($n = 6$). H1 = *Poncirus Trifoliata* × *Citrus Volkameriana* H1, H2 = *Poncirus Trifoliata* × *Citrus reshni Hort. ex Tan. (V1)* H2, H3 = *Poncirus Trifoliata* × *Citrus reshni Hort. ex Tan. (V2)* H3, H4 = *Poncirus Trifoliata* × *Citrus reshni Hort. ex Tan. (V3)* H4, H5 = *Poncirus Trifoliata* × *Citrus reshni Hort. ex Tan. (V4)* H5, H6 = *Poncirus Trifoliata* × *Citrus reshni Hort. ex Tan. (V5)* H6, LR = *Citrus limonia Osbeck (Lime Rangpur),* PT = *Poncirus Trifoliata.*

Effect of salt stress on the variation of the dry weight of the rootstocks. The dry weight of leaves, stems, and roots, as well as the whole plant, was affected negatively by the salt stress compared with the control; the variation of the dry weight is fairly the same as the fresh weight. At whole plant level, the rootstock *Poncirus Trifoliata* × *Citrus Reshni Hort. Ex Tan. (V1)* H2 has a reduction of 9% when the plants are treated with 2 g L^{-1} NaCl, and the rootstock *Poncirus Trifoliata* × *Citrus Reshni Hort. Ex Tan. (V5)* H6 has a reduction of 27%. At a 5 g L^{-1} NaCl level, the fresh weight of rootstock

Table 6. Effect of salt stress on dry weight of leaves (g)

Rootstock	Control	2 g L^{-1} NaCl	5 g L^{-1} NaCl
H1	1.82 ± 0.11^{a}	1.77 ± 0.19^{a}	1.47 ± 0.10^{a}
H2	2.55 ± 0.03^{a}	2.21 ± 0.06^{a}	1.61 ± 0.19^{b}
H3	2.08 ± 0.20^{a}	1.69 ± 0.18^{a}	1.57 ± 0.14^{a}
H4	2.27 ± 0.26^{a}	1.74 ± 0.31^{b}	1.37 ± 0.23^{b}
H5	2.40 ± 0.21^{a}	2.31 ± 0.23^{a}	1.34 ± 0.32^{b}
H6	1.78 ± 0.18^{a}	1.36 ± 0.18^{ab}	0.74 ± 0.20^{b}
LR	3.37 ± 0.51^{a}	2.11 ± 0.14^{ab}	1.61 ± 0.15^{b}
PT	0.86 ± 0.11^{a}	0.69 ± 0.08^{a}	0.61 ± 0.06^{a}

For the same rootstock, stress levels with the same letter do not differ significantly; at $P \leq 0.05$ (*one–way ANOVA*, separated by *Duncan's test*), mean values ± standard error ($n = 6$). H1 = *Poncirus Trifoliata* × *Citrus Volkameriana* H1, H2 = *Poncirus Trifoliata* × *Citrus reshni Hort. ex Tan. (V1)* H2, H3 = *Poncirus Trifoliata* × *Citrus reshni Hort. ex Tan. (V2)* H3, H4 = *Poncirus Trifoliata* × *Citrus reshni Hort. ex Tan. (V3)* H4, H5 = *Poncirus Trifoliata* × *Citrus reshni Hort. ex Tan. (V4)* H5, H6 = *Poncirus Trifoliata* × *Citrus reshni Hort. ex Tan. (V5)* H6, LR = *Citrus limonia Osbeck (Lime Rangpur),* PT = *Poncirus Trifoliata.*

Poncirus Trifoliata × *Citrus Reshni Hort. Ex Tan. (V1)* H2 has been reduced by 30%, while a fresh weight reduction of 50% has been recorded for rootstock *Poncirus Trifoliata* × *Citrus Reshni Hort. Ex Tan. (V5)* H6.

Effect of salt stress on dry weight of leaves. Table 6 shows the variation of dry weight of leaves. The salinity applied has a negative effect on the leaf dry weight; the rootstock *Poncirus Trifoliata* × *Citrus Reshni Hort. Ex Tan. (V1)* H2 has the greatest weight of all treatments. While rootstock *Poncirus Trifoliata* × *Citrus Reshni Hort. Ex Tan. (V5)* H6 has the lowest.

Effect of salt stress on dry weight of stems. The results of Table 7 show that the salinity has reduced the stems dry weight. The rootstock *Poncirus Trifoliata* × *Citrus Reshni Hort. Ex Tan. (V1)* H2 has the greatest weight of all treatments. While rootstock *Poncirus Trifoliata* × *Citrus Reshni Hort. Ex Tan. (V5)* H6 has the lowest.

Effect of salt stress on dry weight of roots. Table 8 shows that the salinity has reduced the roots dry weight. The rootstock *Poncirus Trifoliata* × *Citrus Reshni Hort. Ex Tan. (V1)* H2 has the greatest weight of all treatments. While rootstock *Poncirus Trifoliata* × *Citrus Reshni Hort. Ex Tan. (V5)* H6 has the lowest.

Table 7. Effect of salt stress on dry weight of stems (g)

Rootstock	Control	2 g L^{-1} NaCl	5 g L^{-1} NaCl
H1	0.93 ± 0.54^{a}	0.83 ± 0.48^{ab}	0.64 ± 0.37^{b}
H2	1.76 ± 1.01^{a}	1.53 ± 0.88^{a}	1.34 ± 0.78^{a}
H3	1.23 ± 0.71^{a}	0.97 ± 0.56^{ab}	0.79 ± 0.45^{b}
H4	1.47 ± 0.85^{a}	1.14 ± 0.66^{ab}	0.89 ± 0.51^{b}
H5	0.89 ± 0.51^{a}	0.73 ± 0.42^{ab}	0.62 ± 0.36^{b}
H6	0.99 ± 0.57^{a}	0.65 ± 0.38^{b}	0.51 ± 0.30^{b}
LR	2.10 ± 1.21^{a}	1.34 ± 0.78^{b}	1.08 ± 0.62^{b}
PT	1.62 ± 0.93^{a}	1.33 ± 0.77^{b}	1.21 ± 0.70^{b}

For the same rootstock, stress levels with the same letter do not differ significantly; at $P \leq 0.05$ (*one–way ANOVA*, separated by *Duncan's test*), mean values ± standard error (*n* = 6). H1 = *Poncirus Trifoliata* × *Citrus Volkameriana* H1, H2 = *Poncirus Trifoliata* × *Citrus reshni Hort. ex Tan. (V1)* H2, H3 = *Poncirus Trifoliata* × *Citrus reshni Hort. ex Tan. (V2)* H3, H4 = *Poncirus Trifoliata* × *Citrus reshni Hort. ex Tan. (V3)* H4, H5 = *Poncirus Trifoliata* × *Citrus reshni Hort. ex Tan. (V4)* H5, H6 = *Poncirus Trifoliata* × *Citrus reshni Hort. ex Tan. (V5)* H6, LR = *Citrus limonia Osbeck (Lime Rangpur),* PT = *Poncirus Trifoliata.*

Table 8. Effect of salt stress on dry weight of roots (g)

Rootstock	Control	2 g L^{-1} NaCl	5 g L^{-1} NaCl
H1	1.29 ± 0.74^{a}	1.12 ± 0.65^{ab}	0.82 ± 0.47^{ab}
H2	1.49 ± 0.86^{a}	1.51 ± 0.87^{a}	1.13 ± 0.65^{a}
H3	1.23 ± 0.71^{a}	1.09 ± 0.63^{ab}	0.80 ± 0.46^{b}
H4	1.55 ± 0.89^{a}	1.09 ± 0.63^{ab}	0.78 ± 0.45^{b}
H5	1.05 ± 0.61^{a}	0.86 ± 0.50^{ab}	0.65 ± 0.37^{b}
H6	1.00 ± 0.58^{a}	0.72 ± 0.42^{b}	0.65 ± 0.37^{b}
LR	1.41 ± 0.82^{a}	1.02 ± 0.59^{ab}	0.78 ± 0.45^{b}
PT	1.26 ± 0.73^{a}	0.99 ± 0.57^{a}	0.91 ± 0.52^{a}

For the same rootstock, stress levels with the same letter do not differ significantly; at $P \leq 0.05$ (*one–way ANOVA*, separated by *Duncan's test*), mean values ± standard error (*n* = 6). H1 = *Poncirus Trifoliata* × *Citrus Volkameriana* H1, H2 = *Poncirus Trifoliata* × *Citrus reshni Hort. ex Tan. (V1)* H2, H3 = *Poncirus Trifoliata* × *Citrus reshni Hort. ex Tan. (V2)* H3, H4 = *Poncirus Trifoliata* × *Citrus reshni Hort. ex Tan. (V3)* H4, H5 = *Poncirus Trifoliata* × *Citrus reshni Hort. ex Tan. (V4)* H5, H6 = *Poncirus Trifoliata* × *Citrus reshni Hort. ex Tan. (V5)* H6, LR = *Citrus limonia Osbeck (Lime Rangpur),* PT = *Poncirus Trifoliata.*

DISCUSSION

Salt stress is one of the environmental factors affecting plant growth and physiological and biochemical traits. In this study, we evaluated the effect of salt stress on six citrus hybrid rootstocks using morphological, physiological, and biochemical parameters as indicators. The results show that symptom severity varies according to rootstock and increases with salt concentration. Among the hybrids studied, rootstocks *Poncirus Trifoliata* × *Citrus Volkameriana* H1 and *Poncirus Trifoliata* × *Citrus Reshni Hort. Ex Tan. (V2)* H3 were less affected by salinity, while rootstocks *Poncirus Trifoliata* × *Citrus Reshni Hort. Ex Tan. (V4)* H5 and *Poncirus Trifoliata* × *Citrus Reshni Hort. Ex Tan. (V5)* H6 showed symptoms of chlorosis. These chlorosis symptoms might be a result of the accumulation of toxic ions such as chloride and sodium. Our results show that the presence of NaCl in the irrigation solution causes an increase in leaf chloride content, and the rate of accumulation varies according to rootstock. The rate is less pronounced on rootstocks *Poncirus Trifoliata* × *Citrus Volkameriana* H1 and *Poncirus Trifoliata* × *Citrus Reshni Hort. Ex Tan. (V2)* H3, and higher on rootstocks *Poncirus Trifoliata* × *Citrus Reshni Hort. Ex Tan. (V4) H5* and *Poncirus Trifoliata* × *Citrus Reshni Hort. Ex Tan. (V5)* H6. This accumulation of chlorides in the leaves could be the cause of the pronounced cellular toxicity noted in some rootstocks. Indeed, we found that rootstocks *Poncirus Trifoliata* × *Citrus Reshni Hort. Ex Tan. (V4)* H5 and *Poncirus Trifoliata* × *Citrus Reshni Hort. Ex Tan. (V5)* H6, which recorded high chloride levels, also exhibited pronounced cellular toxicity. Similar results were reported by Fadli et al. (2014); they stipulated the existence of a link between these two parameters and found that chloride is the cause of leaf chlorosis, and the resistant rootstock accumulates low chloride with fewer toxicity symptoms. Alam et al. (2020) also found that the amount of chloride was significantly increased in the leaves with an increase of NaCl. Previous studies found that excess ion accumulation of chloride and sodium in citrus tissues can cause specific ion toxicity (Maas, 1993; Alam et al., 2020). The accumulation of chloride ions in leaves reduces transpiration rate and photosynthesis, which leads to a decline in plant growth (Bañuls et al., 1997; Moya et al., 2002; Arbona et al., 2006; Ziogas et al., 2021). The chloride ion disrupts macromolecules and inactivates enzymes (Kamran et al., 2020). In citrus, significant changes in chlorophyll pigment content are generally observed when irrigated with a saline solution, whatever its concentration. Our results show that chlorophyll content decreases with increasing salt stress intensity and that this decrease varies according to rootstock. The lowest percentage reduction was noted in rootstock *Poncirus Trifoliata* × *Citrus Reshni Hort. Ex Tan. (V2)* H3, and the highest in rootstock *Poncirus Trifoliata* × *Citrus Reshni Hort. Ex Tan. (V5)* H6. The high chlorophyll contents recorded in some of the rootstocks we tested could be related to the degree of resistance of the chlorophyll pigments in these rootstocks to salinity. These results agreed with the findings of Alam et al. (2020) and Khoshbakht et al. (2015), who found a decrease in chlorophyll content at the highest NaCl concentration compared with the control. Recent studies have found that the salt treatment reduces the chlorophyll content of leaves (Issa et al., 2020; Sassine et al., 2022; Maaroufi-Dguimi et al., 2024). This reduction in chlorophyll content might be the result of salt ion accumulation in the chloroplast, which has a direct impact on photosynthetic activities and yield (Abbas et al., 2013). Chlorophyll decrease could result from the inhibition of enzymes necessary for the synthesis of photosynthetic pigments (Murkute et al., 2006, 2009) or from the negative effect of sodium on magnesium absorption, which is necessary for the biosynthesis of chlorophyll (Navarro et al., 2014). The results obtained show an accumulation of proline in all rootstocks in the presence of salt, with the intensity of accumulation differing according to the rootstock studied. Proline accumulation is among the main mechanisms to cope with salinity in citrus (Vives-Peris et al., 2018;

Ziogas et al., 2021). In this context, maximum values were recorded on rootstock *Poncirus Trifoliata* × *Citrus Reshni Hort. Ex Tan. (V4)* H5 and minimum values on rootstock *Poncirus Trifoliata* × *Citrus Reshni Hort. Ex Tan. (V1)* H2. Our findings agree with those of Huang et al. (2013), Shafieizargar et al. (2015), Alam et al. (2020), Goharrizi et al. (2020), and Sohby et al. (2023), who revealed that an increased level of salt stress led to an increase in proline content, and the tolerant genotype had a greater increase in proline concentration. Anjum (2008) and Balal et al. (2011) found that Cleopatra mandarin and Rangpur lime seedlings accumulated more proline as the salinity level increased. Maaroufi-Dguimi et al. (2024) reported that salt treatment induced proline accumulation in the tissues of *Lycopersicon esculentum*. Proline plays a key role in reducing the cell osmotic potential and stabilising proteins and cellular structures under salt stress (Yang & Guo, 2018). Proline acts as a compatible osmolyte and is considered a mechanism for carbon and nitrogen storage (Shafieizargar et al., 2015) and maintains the enzymatic activities in the presence of toxic ions (Xue et al., 2009). Soluble sugar content also increased under salt stress. The highest soluble sugar content was observed in rootstocks *Poncirus Trifoliata* × *Citrus Reshni Hort. Ex Tan. (V1)* H2 and *Poncirus Trifoliata* × *Citrus Reshni Hort. Ex Tan. (V2)* H3, while the lowest was recorded in rootstock *Poncirus Trifoliata* × *Citrus Reshni Hort. Ex Tan. (V5)* H6. Dong & Beckles (2019) suggest salinity might induce the accumulation of sugar as an osmotic response. Denaxa et al. (2022) reported that under saline conditions, the content of sugar and other osmolytes commonly increases to counterbalance the rise in osmotic potential in the vacuole. Shafieizargar et al. (2015) found that Cleopatra mandarin and Shaker rootstocks accumulate a higher content of soluble sugars and proline compared to other rootstocks, and this accumulation is relatively associated with their higher relative water content. The increase in leaf sugar content could be the result of starch degradation of rootstocks under salt stress, enabling the plant to maintain turgor level. The increased contents of osmoregulation substances, such as soluble sugar, soluble protein, and proline, effectively removed ROS, reducing the content of MDA in plants, and thus relieved the damage of salt stress to plants (Dien et al., 2019; Kučerová et al., 2019; Lin et al., 2019; Gurrieri et al., 2020; Sun et al., 2022). In this study, we found that both fresh and dry weights decreased on the different rootstocks, irrespective of NaCl concentration. However, the percentage of biomass reduction varied according to rootstock and salt stress intensity. The highest percentages of fresh weight reduction were recorded for rootstock *Poncirus Trifoliata* × *Citrus Reshni Hort. Ex Tan. (V5)* H6, and the lowest for rootstock *Poncirus Trifoliata* × *Citrus Reshni Hort. Ex Tan. (V1) H2*. For dry weight reduction, considered an index of plant sensitivity to salinity, the highest percentage of dry weight reduction was recorded in rootstock *Poncirus Trifoliata* × *Citrus Reshni Hort. Ex Tan. (V5)* H6, and the lowest in rootstock *Poncirus Trifoliata* × *Citrus Volkameriana* H1 and *Poncirus Trifoliata* × *Citrus Reshni Hort. Ex Tan. (V1)* H2. Similar findings were reported in the study of Othman et al. (2023), who found a reduction of fresh and dry weights by orders of 20% and 27%. With increasing salt stress, stem diameter, average leaf area, and dry weight of aboveground plant parts decrease (Sajyan et al., 2018; Sassine et al., 2022). Ünlükara et al. (2017) found that salinity significantly affects the fresh and dry leaf weight and fresh yield of spinach. The reduction of fresh and dry weights observed could be a result of photosynthetic activity and chlorophyll reduction.

CONCLUSION

We studied the effect of 0, 2, and 5 g L^{-1} NaCl in the irrigation solution (corresponding to 1.3, 4, and 9 dS m^{-1}, respectively) on six new hybrid citrus rootstocks for eight weeks. The findings indicated that the rootstock's response depends on the level of stress. The content of leaf chlorophyll, both fresh and dry weight, was noticeably reduced as the salt level increased. Additionally, as the salt level rose, the leaf chloride, proline, and soluble sugar content increased. According to the findings of our study, the hybrid *Poncirus Trifoliata× Citrus Reshni Hort. Ex Tan. (V1)* H2 is thought to be salt-tolerant since it accumulates 38.88 mg g^{-1} of dry weight leaf chloride, 1.72 mg g^{-1} of fresh weight chlorophyll content, and 1.59 mg g^{-1} of dry weight leaf sugar content. It also has the highest amount in both fresh and dry weight. In contrast, the hybrid *Poncirus Trifoliata× Citrus Reshni Hort. Ex Tan. (V5)* H6 exhibits salt sensitivity, accumulating high leaf chloride levels of 46.92 mg g^{-1} of dry weight and a low chlorophyll content of 1.12 mg g^{-1} of fresh weight associated with signs of leaf toxicity, leading to poor fresh and dry weight. Breeders aim to shorten selection time for new rootstocks using physiological, morphological, and biochemical indicators to understand tolerance mechanisms and quickly evaluate plant material. Conducting long-term studies to observe the effects of prolonged salt stress on the association of the tolerant hybrid with the scion could help to understand its resilience over multiple growing seasons. By adopting salt-tolerant rootstocks, farmers can maintain higher yields and better-quality produce in saline environments.

ACKNOWLEDGMENTS. The authors express their sincere gratitude to the staff of the Regional Centre for Agricultural Research of Kenitra, especially to the Centre's director, Dr. Hassan Benaouda, for the interest given to citrus improvement.

REFERENCE

Abbas, G., Saqib, M., Rafique, Q., Rahman, M.A., Akhtar, J., Haq, M.A. & Nasim, M. 2013. Effect of salinity on grain yield and grain quality of wheat (*Triticum aestivum* L.). *Pakistan Journal of Agricultural Sciences* **50**(2), 185–189.

Ait El Aouad, B., Fadli, A., Aderdour, T., Talha, A., Benkirane, R. & Benyahia, H. 2015. Investigating salt tolerance in citrus rootstocks under greenhouse conditions using growth and biochemical indicators. *Biolife* **4**, 820–826.

Alam, A., Ullah, H., Attia, A. & Datta, A. 2020. Effects of Salinity Stress on Growth, mineral Nutrient Accumulation and Biochemical Parameters of Seedlings of Three Citrus Rootstocks. *International Journal of Fruit Science* **20**(4), 786–804. doi: 10.1080/15538362.2019.1674762

Anjum, M.A. 2008. Effect of NaCl concentrations in irrigation water on growth and polyamine metabolism in two citrus rootstocks with different levels of salinity tolerance. *Acta Physiologiae Plantarum* **30**(1). doi: 10.1007/s11738-007-0089-3

Aragüés, R., Urdanoz, V., Çetin, M., Kirda, C., Daghari, H., Ltifi, W., Lahlou, M. & Douaik, A. 2011. Soil salinity related to physical soil characteristics and irrigation management in four Mediterranean irrigation districts. *Agricultural Water Management* **98**(6), 959–966. doi: 10.1016/j.agwat.2011.01.004

Arbona, V., Lopez-Climent, M.F., Mahouachi, J., Perez-Clemente, R.M., Abrams, S.R. & Gomez-Cadenas, A. 2006. Use of persistent analogs of abscisic acid as palliatives against salt-stress induced damage in citrus plants. *Journal of Plant Growth Regulation* **25**(1), 1–9. doi: 10.1007/s00344-005-0038-6

Arnon, D.I. 1949. Copper Enzymes in Isolated Chloroplasts. Polyphenoloxidase in Beta Vulgaris. *Plant Physiology* **24**(1), 1–15. doi: 10.1104/pp.24.1.1

Balal, R.M., Ashraf, M.Y., Khan, M.M., Jaskani, M.J. & Ashfaq, M. 2011. Influence of salt stress on growth and biochemical parameters of citrus rootstocks. *Pakistan Journal of Botany* **43**(4), 2135–2141.

Bañuls, J., Serna, M.D., Legaz, F., Talon, M. & Primo-Millo, E. 1997. Growth and gas exchange parameters of Citrus plants stressed with different salts. *Journal of Plant Physiology* **150**(1), 194–199. https://doi.org/10.1016/S0176-1617(97)80202-7

Bates, L.S., Waldren, R.P. & Teare, I.D. 1973. Rapid determination of free proline for water-stress studies. *Plant and Soil*, **39**(1), 205–207. https://doi.org/10.1007/BF00018060

Benyahia, H., Mustapha Ait Haddou Mouloud, Jrifi, A. & Lamsettef, Y. 2004. Effect of irrigation water salinity on the colonization of citrus rootstocks roots by Phytophthora parasitica. *Fruits* **59**(2). 101–108. https://doi.org/10.1051/fruits:2004010

Brito, M.E.B., da Silva, E.C.B., Fernandes, P.D., Filho, W.S.S., Filho, M.A.C., da Silva Sá, F.V.S., de Melo, A.S. & Barbosa, R. C.A. 2015. Salt balance in substrate and growth of 'Tahiti' acid lime grafted onto Sunki mandarin hybrids under salinity stress. *Australian Journal of Crop Science* **9**(10), 954–961.

Cole, P.J. 1985. Chloride toxicity in citrus. *Irrigation Science* **6**(1), 63–71. doi: 10.1007/BF00272476

Cotlove, E. 1964. Determination of Chloride in Biological Materials. In *Methods of Biochemical Analysis* (pp. 277–391). John Wiley & Sons, Ltd. doi: 10.1002/9780470110300.ch6

Davies, F.S. & Albrigo, L.G. 1994. Citrus. Wallingford: CAB International. *Crop Production Science in Horticulture* **2**, 254.

Denaxa, N.-K., Nomikou, A., Malamos, N., Liveri, E., Roussos, P.A. & Papasotiropoulos, V. 2022. Salinity Effect on Plant Growth Parameters and Fruit Bioactive Compounds of Two Strawberry Cultivars, Coupled with Environmental Conditions Monitoring. *Agronomy* **12**(10). https://doi.org/10.3390/agronomy12102279

Dien, D.C., Mochizuki, T. & Yamakawa, T. 2019. Effect of various drought stresses and subsequent recovery on proline, total soluble sugar and starch metabolisms in Rice (Oryza sativa L.) varieties. *Plant Production Science* **22**(4). 530–545. doi: 10.1080/1343943X.2019.1647787

Dong, S. & Beckles, D.M. 2019. Dynamic changes in the starch-sugar interconversion within plant source and sink tissues promote a better abiotic stress response. *Journal of Plant Physiology* **234–235**, 80–93. https://doi.org/10.1016/j.jplph.2019.01.007

Douglas, T.S. & Walker, R.R. 1983. 4-Desmethylsterol composition of citrus rootstocks of different salt exclusion capacity. *Physiologia Plantarum* **58**(1). doi: 10.1111/j.1399-3054.1983.tb04145.x

DuBois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A. & Smith, F. 1956. Colorimetric Method for Determination of Sugars and Related Substances. *Analytical Chemistry* **28**(3), 350–356. https://doi.org/10.1021/ac60111a017

Fadli, A., Chetto, O., Talha, A., Benkirane, R., Morillon, R. & Benyahia, H. 2014. Characterization in greenhouse conditions of two salt tolerant citrumelo (Citrus paradisi Macf. X Poncirus trifoliata (L.) Raf.) cultivars. *Journal of Life Sciences* **8**, 955–966. doi: 10.17265/1934-7391/2014.12.005

FAOSTAT. 2021. https://www.fao.org/faostat/en/#data/QCL. Accessed 04.10.2023.

Forner-Giner, M.A., Legaz, F., Primo-Millo, E. & Forner, J. 2011. Nutritional responses of citrus rootstocks to salinity: Performance of the new hybrids forner-alcaide 5 and forner-alcaide 13. *Journal of Plant Nutrition* **34**(10). 1437–1452. doi: 10.1080/01904167.2011.585202

Forner-Giner, M.A., Primo-millo, E. & Forner, J.B. 2009. Performance of Forner-Alcaide 5 and Forner-Alcaide 13, hybrids of Cleopatra mandarin x Poncirus trifoliata, as Salinity-Tolerant Citrus Rootstocks. *Journal of The American Pomological Society* **63**, 72–80.

Goell, A. 1969. Salinity Effects on Citrus Trees. *Proceedings of the 1st International Citrus Symposium* **3**, 1819–1824.
Goharrizi, K.J., Baghizadeh, A., Afroushteh, M., Amirmahani, F. & Kermani, S. G. 2020. Effects of salinity stress on proline content and expression of Δ1-pyrroline-5-carboxylate synthase and vacuolar-type H+ subunit E genes in wheat. *Plant Genetic Resources* **18**(5), 334–342. https://doi.org/10.1017/S1479262120000350
Grattan, S.R., Díaz, F.J., Pedrero, F. & Vivaldi, G.A. 2015. Assessing the suitability of saline wastewaters for irrigation of Citrus spp.: Emphasis on boron and specific-ion interactions. *Agricultural Water Management* **157**, 48–58. https://doi.org/10.1016/j.agwat.2015.01.002
GuetaDahan, Y., Yaniv, Z., Zilinskas, B.A. & BenHayyim, G. 1997. Salt and oxidative stress: Similar and specific responses and their relation to salt tolerance in Citrus. *Planta* **203**(4), 460–469. https://doi.org/10.1007/s004250050215
Gurrieri, L., Merico, M., Trost, P., Forlani, G. & Sparla, F. 2020. Impact of Drought on Soluble Sugars and Free Proline Content in Selected Arabidopsis Mutants. *Biology* **9**(11). doi: 10.3390/biology9110367
Hoagland, D.R. & Arnon, D.I. 1950. The water-culture method for growing plants without soil. *Circular. California Agricultural Experiment Station*, Circular 347. (2nd edit).
Huang, Z., Zhao, L., Chen, D., Liang, M., Liu, Z., Shao, H. & Long, X. 2013. Salt Stress Encourages Proline Accumulation by Regulating Proline Biosynthesis and Degradation in Jerusalem Artichoke Plantlets. *PLOS ONE* **8**(4), e62085. doi: 10.1371/journal.pone.0062085
Hussain, S., Luro, F., Costantino, G., Ollitrault, P. & Morillon, R. 2012. Physiological analysis of salt stress behaviour of citrus species and genera: Low chloride accumulation as an indicator of salt tolerance. *South African Journal of Botany* **81**, 103–112. doi: 10.1016/j.sajb.2012.06.004
Issa, D.B., Alturki, S.M., Sajyan, T.K. & Sassine, Y.N. 2020. Sorbitol and lithovit-guano25 mitigates the adverse effects of salinity on eggplant grown in pot experiment. *Agronomy Research* **18**(1), 113–126. https://doi.org/10.15159/AR.20.075
Ivushkin, K., Bartholomeus, H., Bregt, A.K., Pulatov, A., Kempen, B. & de Sousa, L. 2019. Global mapping of soil salinity change. *Remote Sensing of Environment* **231**, 111260. doi: 10.1016/j.rse.2019.111260
Kamran, M., Parveen, A., Ahmar, S., Malik, Z., Hussain, S., Chattha, M.S., Saleem, M.H., Adil, M., Heidari, P. & Chen, J.-T. 2020. An Overview of Hazardous Impacts of Soil Salinity in Crops, Tolerance Mechanisms, and Amelioration through Selenium Supplementation. *International Journal of Molecular Science* **21**(1), 148. 1–27 doi: 10.3390/ijms21010148
Khalil, H.A., Eissa, A.M., El-Shazly, S.M. & Aboul Nasr, A.M. 2011. Improved growth of salinity-stressed citrus after inoculation with mycorrhizal fungi. *Scientia Horticulturae* **130**(3), 624–632. https://doi.org/10.1016/j.scienta.2011.08.019
Khoshbakht, D., Ramin, A.A. & Baninasab, B. 2015. Effects of sodium chloride stress on gas exchange, chlorophyll content and nutrient concentrations of nine citrus rootstocks. *Photosynthetica* **53**(2), 241–249. https://doi.org/10.1007/s11099-015-0098-1
Kučerová, K., Henselová, M., Slováková, Ľ. & Hensel, K. 2019. Effects of plasma activated water on wheat: Germination, growth parameters, photosynthetic pigments, soluble protein content, and antioxidant enzymes activity. *Plasma Processes and Polymers* **16**(3), 1800131. https://doi.org/10.1002/ppap.201800131
Lin, Q., Xie, Y., Guan, W., Duan, Y., Wang, Z. & Sun, C. 2019. Combined transcriptomic and proteomic analysis of cold stress induced sugar accumulation and heat shock proteins expression during postharvest potato tuber storage. *Food Chemistry* **297**, 124991. doi: 10.1016/j.foodchem.2019.124991
López-Climent, M.F., Arbona, V., Pérez-Clemente, R.M. & Gómez-Cadenas, A. 2008. Relationship between salt tolerance and photosynthetic machinery performance in citrus. *Environmental and Experimental Botany* **62**(2), 176–184. doi: 10.1016/j.envexpbot.2007.08.002

Maaroufi-Dguimi, H., Gamal Mohammed, S., Abdalgadir, H. & Omari al Zahrani, F. 2024. Effects of chemical seed priming on germination performance and seedling growth of Lycopersicon esculentum (Mill.) under salt stress. *Agronomy Research* **22**(S2), 672–684. https://doi.org/10.15159/AR.24.035

Maas, E.V. 1993. Salinity and citriculture. *Tree Physiology* **12**(2). doi: 10.1093/treephys/12.2.195

Marathe, R.A., Murkute, A.A., Kolwadkar, J. & Deshpande, C.P. 2022. Salinity tolerance potential of Citrus (Citrus spp.) rootstock genotypes. *Indian Journal of Agricultural Sciences* **92**(11), 1311–1315. https://doi.org/10.56093/ijas.v92i11.120698

Martínez-Cuenca, M.-R., Primo-Capella, A. & Forner-Giner, M.A. 2021. Screening of 'King' mandarin (Citrus nobilis Lour) × Poncirus trifoliata ((L.) Raf.) hybrids as salt stress-tolerant citrus rootstocks. *Horticulture Environment and Biotechnology* **62**(3), 337–351. https://doi.org/10.1007/s13580-020-00291-1

Moya, J.L., Gomez-Cadenas, A., Primo-Millo, E. & Talon, M. 2003. Chloride absorption in salt-sensitive Carrizo citrange and salt-tolerant Cleopatra mandarin citrus rootstocks is linked to water use. *Journal of Experimental Botany* **54**(383), 825–833. doi: 10.1093/jxb/erg064

Moya, J.L., Primo-Millo, E. & Talon, M. 1999. Morphological factors determining salt tolerance in citrus seedlings: The shoot to root ratio modulates passive root uptake of chloride ions and their accumulation in leaves. *Plant, Cell & Environment* **22**(11). https://doi.org/10.1046/j.1365-3040.1999.00495.x

Moya, J.L., Tadeo, F. R., Gomez-Cadenas, A., Primo-Millo, E. & Talon, M. 2002. Transmissible salt tolerance traits identified through reciprocal grafts between sensitive Carrizo and tolerant Cleopatra citrus genotypes. *Journal of Plant Physiology* **159**(9), 991–998. https://doi.org/10.1078/0176-1617-00728

Murkute, A.A., Sharma, S. & Singh, S.K. 2005. Citrus in terms of soil and water salinity: A review. *Journal of Scientific and Industrial Research* **64**(6), 393–402.

Murkute, A.A., Sharma, S. & Singh, S.K. 2006. Studies on salt stress tolerance of citrus rootstock genotypes with arbuscular mycorrhizal fungi. *Horticultural Science* **33**(2), 70–76. https://doi.org/10.17221/3742-HORTSCI

Murkute, A.A., Sharma, S., Singh, S.K. & Patel, V.B. 2009. Response of mycorrhizal citrus rootstock plantlets to salt stress. *Indian Journal of Horticulture* **66**(4), 456–460.

Navarro, J.M., Pérez-Tornero, O. & Morte, A. 2014. Alleviation of salt stress in citrus seedlings inoculated with arbuscular mycorrhizal fungi depends on the rootstock salt tolerance. *Journal of Plant Physiology* **171**(1), 76–85. https://doi.org/10.1016/j.jplph.2013.06.006

Othman, Y.A., Hani, M.B., Ayad, J.Y. & St Hilaire, R. 2023. Salinity level influenced morpho-physiology and nutrient uptake of young citrus rootstocks. *Heliyon* **9**(2). https://doi.org/10.1016/j.heliyon.2023.e13336

Romero-Aranda, R., Moya, J.L., Tadeo, F.R., Legaz, F., Primo-Millo, E. & Talon, M. 1998. Physiological and anatomical disturbances induced by chloride salts in sensitive and tolerant citrus: Beneficial and detrimental effects of cations. *Plant Cell and Environment* **21**(12), 1243–1253. https://doi.org/10.1046/j.1365-3040.1998.00349.x

Sajyan, T.K., Shaban, N., Rizkallah, J. & Sassine, Y.N. 2018. Effects of Monopotassium-phosphate, Nano-calcium fertilizer, Acetyl salicylic acid and Glycinebetaine application on growth and production of tomato (Solanum lycopersicum) crop under salt stress. *Agronomy Research* **16**(3), 872 883. https://doi.org/10.15159/AR.18.079

Sassine, Y.N., Sajyan, T.K., El Zarzour, A., Abdelmawgoud, A.M.R., Germanos, M. & Alturki, S.M. 2022. Integrative effects of biostimulants and salinity on vegetables: Contribution of bioumik and Lithovit®-urea50 to improve salt-tolerance of tomato. *Agronomy Research* **20**(4), 793–804. https://doi.org/10.15159/AR.22.074

Shafieizargar, A., Awang, Y., Ajamgard, F., Juraimi, A.S., Othman, R. & Ahmadi, A.K. 2015. Assessing five citrus rootstocks for NaCl salinity tolerance using mineral concentrations, proline and relative water contents as indicators. *Asian Journal of Plant Sciences* **14**(1). 20–26. https://doi.org/10.3923/ajps.2015.20.26

Shrivastava, P. & Kumar, R. 2015. Soil salinity: A serious environmental issue and plant growth promoting bacteria as one of the tools for its alleviation. *Saudi Journal of Biological Sciences* **22**(2), 123–131. https://doi.org/10.1016/j.sjbs.2014.12.001

Sohby, M.K., Khalil, H.A., Eissa, A.M. & Fekry, W.M.E. 2023. influence of nano-silicon and nano-chitosan on growth, ion content, and antioxidant defense enzyme of two citrus rootstocks under salinity conditions. *mesopotamia journal of agriculture* **51**(2), 147–166. https://doi.org/10.33899/magrj.2023.179915

Srivastava, A.K. & Singh, S. 2009. Citrus Decline: Soil Fertility and Plant Nutrition. *Journal of Plant Nutrition* **32**(2), 197–245. https://doi.org/10.1080/01904160802592706

Storey, R. & Walker, R.R. 1998. Citrus and salinity. *Scientia Horticulturae* **78**(1), 39–81. https://doi.org/10.1016/S0304-4238(98)00190-3

Sun, R., Zheng, H., Yin, S., Zhang, X., You, X., Wu, H., Suo, F., Han, K., Cheng, Y., Zhang, C. & Li, Y. 2022. Comparative study of pyrochar and hydrochar on peanut seedling growth in a coastal salt-affected soil of Yellow River Delta, China. *Science of The Total Environment* **833**, 155183. https://doi.org/10.1016/j.scitotenv.2022.155183

Syvertsen, J.P. & Garcia-Sanchez, F. 2014. Multiple abiotic stresses occurring with salinity stress in citrus. *Environmental and Experimental Botany* **103**, 128–137. doi: 10.1016/j.envexpbot.2013.09.015

Ünlükara, A., Yurtyeri, T. & Cemek, B. 2017. Effects of Irrigation water salinity on evapotranspiration and spinach (Spinacia oleracea L. Matador) plant parameters in Greenhouse Indoor and Outdoor Conditions. *Agronomy Research* **15**(5), 2183–2194, https://doi.org/10.15159/AR.17.041

Vives-Peris, V., Gómez-Cadenas, A. & Pérez-Clemente, R.M. 2018. Salt stress alleviation in citrus plants by plant growth-promoting rhizobacteria Pseudomonas putida and Novosphingobium sp. *Plant Cell Reports* **37**(11), 1557–1569. doi: 10.1007/s00299-018-2328-z

Vives-Peris, V., López-Climent, M.F., Moliner-Sabater, M., Gómez-Cadenas, A. & Pérez-Clemente, R.M. 2023. Morphological, physiological, and molecular scion traits are determinant for salt-stress tolerance of grafted citrus plants. *Frontiers in Plant Science* **14**. https://doi.org/10.3389/fpls.2023.1145625

Xue, X., Liu, A. & Hua, X. 2009. Proline accumulation and transcriptional regulation of proline biothesynthesis and degradation in Brassica napus. *BMB Reports* **42**(1), 28–34. https://doi.org/10.5483/BMBRep.2009.42.1.028

Yang, Y. & Guo, Y. 2018. Elucidating the molecular mechanisms mediating plant salt-stress responses. *New Phytologist* **217**(2), 523–539. https://doi.org/10.1111/nph.14920

Zhang, M., Li, X., Wang, X., Feng, J. & Zhu, S. 2023. Potassium fulvic acid alleviates salt stress of citrus by regulating rhizosphere microbial community, osmotic substances and enzyme activities. *Frontiers in Plant Science* **14**. https://doi.org/10.3389/fpls.2023.1161469

Ziogas, V., Tanou, G., Morianou, G. & Kourgialas, N. 2021. Drought and Salinity in Citriculture: Optimal Practices to Alleviate Salinity and Water Stress. *Agronomy* **11**(7), 1283. https://doi.org/10.3390/agronomy11071283

Agronomy Research **23**(S2), 628–636, 2025
https://doi.org/10.15159/AR.25.001

Toxicity of insecticides for adults of *Diceraeus melacanthus* Dallas, 1851 (Hemiptera: Pentatomidae) in three exposure modes

E.C.S. Vieira[1,*], C.J. Ávila[2], M.C.S. Vieira[1] and P.G. Silva[1]

[1]Universidade da Grande Dourados, Entomologia e Conservação da Biodiversidade, Departamento de Entomologia. Rodovia Dourados – Itahum, km 12, Cidade Universitária, Mato Grosso do Sul, Brasil
[2]Embrapa Agropecuária Oeste, Rodovia BR 163, km 253, Dourados, Mato Grosso do Sul, Brasil
[*]Correspondence: elizete.cavalcanteufgd@gmail.com

Received: July 10th, 2024; Accepted: November 11st, 2024; Published: January 2nd, 2025

Abstract. Phytophagous stink bugs are considered the important pest in second-crop corn cultivation in Brazil, especially when they occur during the early stage of plant development. This study aimed to evaluate the efficiency of insecticides in controlling adults of the *Diceraeus melacanthus* (Dallas, 1851) stink bug when applied separately in three different modes of exposure. The treatments were evaluated in three modes of exposure of insecticides to *D. melacanthus* adults as described below: direct contact (direct application of the insecticide to the insects); tarsal contact (exposure through their walking on the treated surface) and ingestion (contact through their feeding on previously treated fresh bean pods). Mortality was assessed at 1-, 5-, 24- and 48-hours post-exposure. We observed that the percentage of accumulated mortality of *D. melacanthus* adults was significant through direct contact with chemical treatments. However, if the target insect does not receive direct spraying on its body, indirect contact through its tarsus walking on the treated surface can guarantee a significant final mortality of the stink bugs. In addition, although the mode of exposure through ingestion has shown low mortality, it may also contribute to the final mortality of stink bugs in the field depending on the chemical treatment applied to the crop.

Key words: chemical control, green belly stink bug, mortality, neonicotinoids.

INTRODUCTION

Phytophagous stink bugs are the primary entomological issue in second-crop corn cultivation in Brazil from January to July, particularly when they appear during the early stages of plant development (Ávila & Panizzi, 1995). The green-bellied stink bug *Diceraeus melacanthus* Dallas, (Heteroptera: Pentatomidae) is considered a pest in several crops such as soybeans (Panizzi, 1997), corn (Guedes et al., 2017) and wheat (Manfredi-Coimbra et al., 2005). However, in corn, this species inflicts the most damage