

Guava seedlings with rootstocks or interstocks and their reaction to salinity

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ABSTRACT: The introduction of rootstocks for the guava has become necessary due to the advance of guava decline. Among them, *Psidium* spp. or their hybrids have been evaluated. In addition to disease resistance, tolerance to salinity must be evaluated in the new rootstocks. The aim of this study was to verify the effect of rootstocks and intergrafts on the mineral composition of *Psidium* spp. submitted to salt stress. The experiment was setup in a randomized block design, with the following treatments (scion/rootstock): *P. guineense*/*P. cattleianum*; *P. guajava*/*P. guineense*/*P. cattleianum*; *P. guajava* ‘Paluma’/*P. guajava*; *P. guajava* ‘Paluma’/*P. guineense*; *P. guajava* ‘Cortibel 1’/*P. guajava*; *P. guajava* ‘Cortibel 1’/*P. guineense*; *P. cattleianum* (purple) and *P. cattleianum* (yellow), with three replications and three plants per plot. The plants underwent irrigation with saline solution, and the levels of macronutrients, micronutrients and sodium in the scion leaves were evaluated. As rootstock, *P. cattleianum* results in greater Na uptake, with higher levels in the leaves of the scions, leading to less tolerance to salt stress.

Key words: accumulation of sodium in leaves, mineral nutrient contents in *Psidium* spp., interstocked seedlings.

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INTRODUCTION

In 2017, guava production in Brazil was 460,000 tons on a cultivated area of 20,000 hectares (ha), and the states of São Paulo and Pernambuco were the largest domestic producers. The crop is grown in several states in the country, which demonstrates its wide adaptability to a large range of climatic conditions and soil types. The state of Rio de Janeiro has an area of 642 ha with guava, concentrated in the districts of Cachoeiras de Macacu, São Francisco de Itabapoana, Guapimirim and São João da Barra, with 350, 70, 35 and 30 ha respectively (IBGE 2019). Guava decline has been detected in the state, a disease with a high destructive capacity, considered to be polyphagous in nature (Marques et al. 2012).

Guava decline is a complex disease, caused by a synergistic interaction between the phytonematode *Meloidogyne enterolobii*, and the fungus *Fusarium solani* (Mart.) Sacc. (Gomes et al. 2010; Gomes et al. 2011). Most nematodes in association with *Fusarium* predispose the formation of morphophysiological changes in the plant, hampering the absorption and/or translocation of water and nutrients, causing yellowing and reddish leaf margins, followed by wilting, necrosis and premature abscission, resulting in the death of the plants (Freitas et al. 2014).



No source of resistance to the nematode was found in *Psidium guajava*; however, resistance was found in plants of the genus *Psidium* spp. (Carneiro et al. 2007). Costa et al. (2012) identified a genotype of *P. guineense* with resistance to the nematode and developed hybrids between *P. guajava* and *P. guineense* for possible use as rootstocks. Sources of resistance to *M. enterolobii* were also identified in genotypes of *P. cattleianum* by Carneiro et al. (2007), Miranda et al. (2012) and Biazatti et al. (2016). However, in guava grafted onto *P. cattleianum*, graft incompatibility was seen one year after planting in the field (Robaina et al. 2015).

The introduction of new rootstocks should be evaluated in relation to several factors, among the most important are their resistance to biotic features, rooting capacity and compatibility with the scion. However, the rootstocks may also influence plant response to abiotic stress, such as salinity. Water and salt stress can be induced by rainfall scarcity and inadequate irrigation management, and in the Brazilian semi-arid region this has caused serious problems to crops, also affecting guava production (Souza et al. 2007; Cavalcante et al. 2010). In a study carried out with salinity levels in plants of the 'Paluma' guava, Silva et al. (2008) found symptoms of leaf toxicity as well as reduced growth and productivity at the lowest evaluated level (electrical conductivity of 2 dS·m⁻¹).

Sá et al. (2016) reported that increased salinity restricted the emergence, growth and accumulation of biomass and the number of leaves in guava rootstocks, during the initial stages of development, with the most drastic effects at levels greater than 1.8 dS·m⁻¹. Silva et al. (2017) reported that an increase in electrical conductivity (EC) from 0.3 dS·m⁻¹ onwards negatively affected the morphophysiology of the guava rootstock 'Paluma', especially during the earliest phase, also affecting the rate of growth of the stem diameter, shoot dry matter and root to shoot ratio (Souza et al. 2017).

Plants can accumulate salts in the leaves, stems and roots. Genotypes of *P. cattleianum* that have been used in crosses aimed at the production of rootstocks for the guava (Gomes et al. 2016) should therefore be evaluated for sodium uptake.

The hypothesis of this study was to test whether *P. cattleianum* as rootstock enhances Na uptake to the leaves of scions inducing less tolerance to salt stress. The aim was to evaluate whether rootstocks of *Psidium* spp. interfere with the tolerance of the guava to salt stress.

MATERIAL AND METHODS

The experiment was conducted under greenhouse conditions, in a randomized block design, including eight treatments and three replications, with one experimental unit consisting of three plants. The treatments were scion/rootstock: (I) *P. guineense*/*P. cattleianum*, (II) *P. guajava*/*P. guineense*/*P. cattleianum*, (III) *P. guajava* 'Paluma'/*P. guajava*, (IV) *P. guajava* 'Paluma'/*P. guineense*, (V) *P. guajava* 'Cortibel 1'/*P. guajava*, (VI) *P. guajava* 'Cortibel 1'/*P. guineense*, (VII) *P. cattleianum* (purple) and (VIII) *P. cattleianum* (yellow).

The plants were 28 months old, and grown in Basaplant substrate in conical flasks with a capacity of 3.8 L. Residue analysis showed the following: pH 5.95, N 6.20, P₂O₅ 3.48, K₂O 0.59, Ca 18.23, Mg 3.51 - in g·kg⁻¹; Fe 10,811, Cu 53.50, Zn 255, Mn 447, S 0.46, B 17.02 - in mg·kg⁻¹; and an EC of 1.28 dS·m⁻¹. The plants from each treatment were pruned and then irrigated with saline solution, which was prepared by dissolving sodium chloride (NaCl) in the irrigation water at a concentration of 50 mmol·L⁻¹ at an EC of 5.8 dS·m⁻¹. On average, 200 mL of saline solution was applied in one irrigation every three days (Ferreira et al. 2001). To monitor the EC, three flasks from each working plot were selected before irrigation, and a plastic bag tied to the lower end of the flasks so that the irrigation water could be collected after passing over the root system. When the EC was above the maximum concentration stipulated for the experiment, the system was washed with irrigation water.

After a period of 37 days, the new emerged branches were collected. These branches were separated into leaves, and cleaned with cotton wool and deionized water. They were later dried in a forced air circulation oven at 70 °C for 48 h and then ground in a Wiley mill, sieved using a 20-mesh screen and stored in hermetically sealed and

identified bottles. The same procedure was done prior and follow induction of salinity stress, then leaves from the two collecting times were compared for nutrients composition.

To determine the levels of N and Na, 0.1 g of the dry matter was subjected to sulphuric acid digestion. Nitrogen was analyzed by the Nessler method, while Na was analyzed in a flame photometer. To determine the levels of P, K, Ca, Mg, S, B, Cu, Fe, Mn and Zn, 0.1 g of dry matter was submitted to open digestion with HNO₃ and H₂O₂, using the Shimadzu ICPE-9000 plasma spectrometer. To extract the chloride, 1 g of dry matter was used. The Cl content was extracted by calcium nitrate solution in the form of the chloride ion titrated with a standard silver nitrate solution in the presence of potassium chromate as indicator. The constant application of copper fungicides prevents a precise determination of Cu before submission to salt stress, and this was therefore excluded from the analysis.

The data were evaluated in split-plot design with subplots corresponding to data obtained prior to and following salt stress. The variables were submitted for normality and homogeneity tests; if there were no restrictions, an analysis of variance was carried out and the mean values for each characteristic were compared by Tukey's test ($p > 0.05$), using the SANEST statistical analysis software.

RESULTS AND DISCUSSION

The first symptoms of toxicity were noted after 18 days in the seedlings irrigated with NaCl solution. Initially, the apex of the distal part of the leaves presented a brownish-yellow coloration (Fig. 1a), developing into dried leaf margins, mainly in the older leaves (Fig. 1b,c), followed by leaf rolling and necrosis (Fig. 1d-f), leading to premature fall.

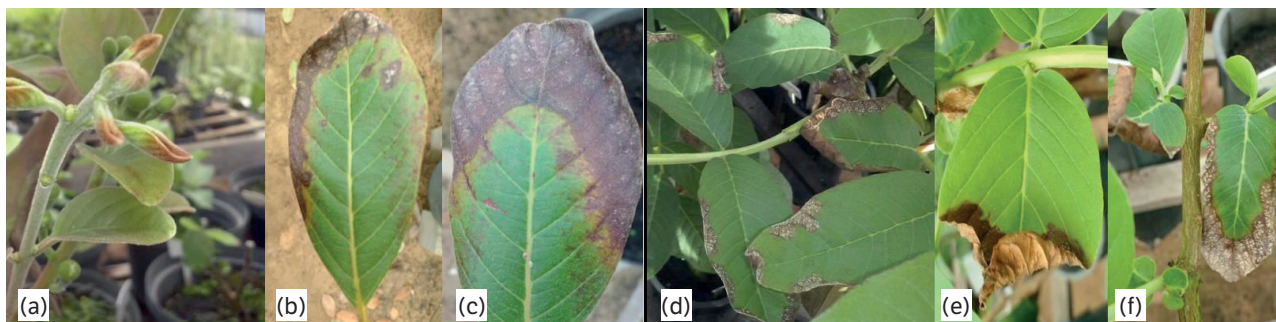


Figure 1. Initial symptoms of sodium toxicity after 18 days of induced salt stress in leaves of *P. guineense* (a), advanced symptoms of sodium toxicity in leaves of *P. guineense* grafted onto *P. cattleianum* (treatment I) after 37 days of induced salt stress (b and c) and severe symptoms in leaves of *P. guajava* grafted onto *P. cattleianum* with *P. guineense* interstock that comprised treatment II (d, e, and f).

The treatments *P. guineense*/*P. cattleianum* (I), and the interstocked seedlings that comprised treatment II, both using *P. cattleianum* as rootstock, were the most sensitive to salinity and presented the greatest damage among the treatments under evaluation.

Typical symptoms of salt-stress toxicity in more sensitive species are characterized by chlorosis, followed by necrosis of the leaf tips and margins, with damage increasing from the younger to the older leaves. These symptoms were seen in each treatment irrigated with saline water (EC between 2 and 8 dS·m⁻¹) in an experiment conducted by Silva et al. (2008), and were most severe for irrigation water at 8 dS·m⁻¹. These symptoms were correlated with Na and Cl toxicity. The authors state that the damage seen at such low concentrations as 2 dS·m⁻¹, indicates that the 'Paluma' guava is sensitive to salinity. Such symptoms may simply be a consequence of changes in the water relations of the cells.

Symptoms of an excess of Na and Cl are more frequent and more serious than those of a deficiency. There may be cases of toxicity, especially in places where evaporation exceeds leaching, and leaching of the anion does not

occur. The effects of salt stress on plant growth are dependent on exposure time and the ionic nature of the salts. In this case, quantifying tolerance to salinity among correlated species is very difficult, and after a short period of induced salinity, species with different levels of tolerance may present the same reduction in growth. Plants that are sensitive to salinity may display specific symptoms when subjected to elevated salinity levels for several days. The inability of plants to exclude NaCl causes damage and abscission in older leaves. In addition, the concentration of Na and Cl in the leaves increases throughout the period of salt exposure, and the leaves may be discarded by the plant due to an internal tolerance mechanism (Willadino and Camara 2010).

The use of *P. cattleianum* as rootstock in interstocked seedlings of guava plants, enabled the uptake of Na to the leaves, reducing the tolerance of the plants to salt stress. Even under conditions of low substrate salinity, the level of 2.8 g·kg⁻¹ Na seen in the leaves of the 'Paluma' guava interstocked with tissue from *P. guineense*, and with *P. cattleianum* as rootstock demonstrates that this rootstock led to the accumulation of Na in the guava leaves (Table 1).

Table 1. Mean values in g·kg⁻¹ of Na and Cl in the leaf dry matter of *Psidium* spp. prior to (P) and following (F) induction to salt stress.

Treatment	Na		Cl	
	P	F	P	F
I	1.9 aA	2.6 bA	3.2 aA	2.5 dA
II	2.8 aB	7.8 aA	4.1 aB	5.9 bcA
III	0.1 bA	0.1 cA	3.8 aA	4.3 cdA
IV	0.1 bA	0.2 cA	5.7 aA	5.9 bcA
V	0.1 bA	0.3 cA	4.3 aA	3.88 cdA
VI	0.2 bA	0.2 cA	5.5 aA	6.00 bcA
VII	2.1 aB	7.7 aA	5.4 aB	7.3 abA
VIII	3.3 aB	8.3 aA	4.9 aB	9.7 aA
CVA (%)	15.17		15.02	
CVB (%)	27.07		23.27	

Mean values followed by the same lowercase letter in a column, and uppercase letter on a row, do not differ by Tukey's test (5%). I = *P. guineense*/*P. cattleianum*; II = *P. guajava*/*P. guineense*/*P. cattleianum*; III = *P. guajava* 'Paluma'/*P. guajava*; IV = *P. guajava* 'Paluma'/*P. guineense*; V = *P. guajava* 'Cortibel 1'/*P. guajava*; VI = *P. guajava* 'Cortibel 1'/*P. guineense*; VII = *P. cattleianum* (purple); VIII = *P. cattleianum* (yellow). CVA = coefficient of variation in the plot and CVB = coefficient of variation in the subplot.

Increased of Na content in plants of *Psidium* spp. grown without rootstock was also observed. However, the sodium content of the leaves from treatment I (*P. guineense*/*P. cattleianum*) showed no significant increase following the induction of salt stress. In this case, it can be deduced that the rootstock was not the only genotype responsible for the increase in the sodium content of the leaves, but that the grafting combination also played an important role (Table 1).

The leaves of *P. cattleianum* have high concentrations of sodium, and their use as rootstock or interstock has a negative effect on the tolerance of guava scions to salt stress. The *P. guineense*/*P. cattleianum* combination can be used to form interstocked plants, however, there is no study that reports the use of interstocked guava seedlings in the field, and further research should therefore be carried out. This combination accumulates sodium and chlorine in the leaves, in smaller amounts and, when used as rootstock in the guava, their content increases by approximately three times. This fact shows that the use of *P. cattleianum* as rootstock for other species of *Psidium* can lead to a lower tolerance of these plants to salt stress. Such plants should be evaluated in areas contaminated with the nematode, but should be avoided in saline areas.

Levels of sodium greater than 10 g·kg⁻¹ in leaves of guava plants grown in nutrient solution with 0, 25, 50, 75, 100, 125 and 150 mmol·L⁻¹ NaCl and harvested 30 and 50 days after stress, were seen in concentrations greater

than 50 mmol·L⁻¹ NaCl, and correlated with negative effects on the produced dry matter, as well as an imbalance between the other absorbed cations (Ferreira et al. 2001). In the present study, the interstocked seedlings (II) and the yellow *P. cattleianum* (VIII) reached Na levels of 7.8 g·kg⁻¹ and 8.3 g·kg⁻¹ respectively in the leaf dry matter harvested 37 days following the induction of salt stress.

Chlorine accumulated in the leaves following the induction of salt stress, and was larger in treatments II, VII and VIII (Table 1). It is possible that *P. cattleianum* has a different root system from the other genotypes of *Psidium* spp., and therefore absorbs Cl more quickly and allocates it to the plant canopy. The Na content was higher than the Cl content, up to three times more in treatments I and VII (Table 1). Studies relating the presence of an interstock to plant tolerance to salinity differ from the results obtained in the present study.

Zapata et al. (2004) found that in citrus plants, the biomass distribution of Cl and Na between the organs is variable, both when interstocked and under saline conditions. Interstocked plants had a higher biomass allocation in the leaves and roots than plants with no interstock. They also found that when the xylem vessels of an interstock have a mean lumen diameter less than that of the rootstock, graft or both, the interstock may contribute to restrict water and increase resistance to salt. The Cl and Na concentration in the leaves increased after 60 and 75 days of saline treatment respectively in interstocked plants, compared to 30 days in plants with only one graft region. However, it should be considered that the anatomy of citrus plants and *Psidium* spp. is different and, as such, the translocation of Na and Cl between rootstocks, interstocks and shoots must take place differently.

Sá et al. (2016) conducted an experiment with the objective of evaluating the tolerance of three guava rootstocks ('Crioula', 'Paluma' and 'Ogawa') under salt stress during the early stages of development where the treatments consisted of five levels of irrigation water salinity (0.6, 1.2, 1.8, 2.4 and 3.0 dS·m⁻¹). The experiment showed that an increase in water salinity affected growth, biomass accumulation and number of leaves in the cultivars, having the most extreme effects at levels greater than 1.8 dS·m⁻¹. Guava plants diagnosed with decline had higher levels of sodium found in the leaves (Gomes et al. 2008). The authors concluded that no ranges for Na content considered phytotoxic to the guava have been reported, and as such, could not relate observed levels to the symptomatology of the disease in the aerial part of the plants.

In order to evaluate the tolerance of grapevine rootstocks under saline conditions, Viana et al. (2001) conducted a greenhouse experiment with five rootstock cultivars grown in nutrient solution at five levels of NaCl, and demonstrated that the cultivars presented different patterns of nutrient concentration in the organs under analysis (root, stem and leaves). The most sensitive cultivars, 420-A and IAC 313, allocated a large amount of Na to the leaves, and displayed restrictions on the uptake of K, Mg and Ca. The IAC 572, IAC 766 and Ripária do Traviú cultivars, which proved to be more tolerant, were preferentially able to exclude Na from metabolically active regions, allocating it to the roots and stems.

For the macronutrient content, it was found that between the periods of induction, the levels of N and K did not differ significantly in the leaf dry matter; however, there was a difference between treatments. Induction to salt stress positively influenced P levels for each of the treatments under evaluation, except for treatments I and II (Table 2).

In treatments II, VII and VIII, the levels of Ca, Mg and S decreased following induction to salt stress, except for S in treatment VIII (Table 2). In seedlings of the yellow passion fruit, concentrations of N, P and Ca were not affected by salinity. On the other hand, the K concentration decreased in all parts of the plant, whereas that of S only decreased in the roots (Cruz et al. 2006). Tomaz et al. (2008) evaluated genetic differences in uptake efficiency, and the transport and use of K, Ca and Mg in grafted coffee seedlings. The authors found that there were variations in uptake efficiency for these nutrients in coffee plants depending on the graft/rootstock combination. As for uptake efficiency, and the translocation and utilization of K, Ca and Mg, as well as dry-matter production, in most cases the performance of the grafted plants was worse than that of the ungrafted plants.

Calcium is a macronutrient that is extracted and accumulated in greatest quantity in the tissue of guava plants. The levels of Ca in treatments II and VII were reduced, as were the levels of Mg in treatments I, II, VII and VIII following induction to salt stress (Table 2). When evaluating the levels of S in the leaf, uptake is seen to be significantly lower

in treatment II and higher in treatment VIII following induction to salt stress (Table 2). Cruz et al. (2006) found that salinity stimulated the transport of S in passion fruit from the roots to the shoots and from the stem to the leaves, the leaves appearing to be the more effective drain for this nutrient. As for the micronutrient content, it was found that levels of B decreased significantly in treatments II and VII following induction to salt stress, and that the Cu content, evaluated following induction to salt stress, did not differ between treatments (Table 3).

The Fe content only differed for induction period, whereas the levels of Mn only differed between treatments. The Zn content in treatments I, II and III decreased significantly following induction to salt stress, with the lowest values being found in treatments VII and VIII (Table 3).

This study suggests that the reduction in nutrient levels in plants cultivated in saline environments may be related to the higher concentrations of Na and Cl in the soil and, consequently, in their tissue, since the presence of these elements in the substrate has been seen to reduce plant growth. This is because, among other n.

Table 2. Mean values in g·kg⁻¹ for macronutrients in the leaf dry matter of *Psidium* spp. prior to (P) and following (F) induction to salt stress.

Treatment	N (ns)		P		K (ns)		Ca		Mg		S	
	P	F	P	F	P	F	P	F	P	F	P	F
I	13.3	10.3	1.4 cA	1.7 dA	12.7	14.6	11.1 bcA	14.0 bcA	2.3 cA	1.7 cB	2.0 bcA	1.6 dA
II	14.6	12.7	1.8 bcA	2.2 cdA	16.2	15.6	18.1 aA	13.7 bcB	2.6 bcA	1.6 cB	2.8 aA	2.2 bcdB
III	13.8	13.5	2.2 bB	3.2 abA	18.4	18.7	8.7 cB	12.1 cA	2.7 bcA	2.6 bA	2.7 abA	2.6 abA
IV	16.3	14.7	3.2 aB	4.0 aA	21.2	19.7	13.8 abB	17.8 abA	2.7 bA	2.5 bA	2.7 aA	2.6 abcA
V	14.9	15.2	2.5 abB	3.9 aA	19.5	21.4	9.1 cB	12.0 cA	2.5 bcA	2.5 bA	3.3 aA	3.3 aA
VI	16.3	14.8	2.5 abB	3.7 aA	18.1	19.4	14.8 abB	21.0 aA	2.8 bA	2.7 bA	2.9 aA	3.2 aA
VII	14.4	10.8	1.5 cB	2.2 cdA	25.1	23.5	16.6 aA	12.6 cB	2.5 bcA	1.7 cB	1.8 cA	1.7 dA
VIII	9.3	9.7	1.4 cB	2.5 bcA	20.9	19.8	17.4 aA	15.1 bcA	5.0 aA	3.9 aB	1.3 cB	1.9 cdA
CVA (%)	7.91		9.23		7.41		10.38		5.24		9.21	
CVB (%)	9.99		7.50		6.22		8.63		4.82		9.18	

Mean values followed by the same lowercase letter in a column, and uppercase letter on a row, do not differ by Tukey's test (5%). (ns) = not significative; I = *P. guineense*/*P. cattleianum*; II = *P. guajava*/*P. guineense*/*P. cattleianum*; III = *P. guajava* 'Paluma'/*P. guajava*; IV = *P. guajava* 'Paluma'/*P. guineense*; V = *P. guajava* 'Cortibel 1'/*P. guajava*; VI = *P. guajava* 'Cortibel 1'/*P. guineense*; VII = *P. cattleianum* (purple); VIII = *P. cattleianum* (yellow). CVA = coefficient of variation in the plot and CVB = coefficient of variation in the subplot.

Table 3. Mean content in mg·kg⁻¹ of micronutrients in the leaf dry matter of *Psidium* spp. prior to (P) and following (F) induction to salt stress.

Treatment	B		Cu	Fe (ns)		Mn (ns)		Zn	
	P	F	F	P	F	P	F	P	D
I	33.8 abA	33.7 abA	9.1 a	129.7	79.4	32.4	26.3	19.4 bA	11.4 bB
II	40.1 aA	31.5 abB	9.4 a	109.7	59.5	50.8	60.9	19.3 bA	11.9 bB
III	29.4 bA	33.1 abA	10.6 a	71.3	60.3	14.6	14.6	28.4 aA	21.0 aB
IV	31.3 abA	36.2 aA	12.1 a	71.8	55.7	19.3	30.7	25.2 abA	21.4 aA
V	25.76 bA	26.7 bA	13.0 a	86.6	54.3	13.9	13.7	26.7 aA	24.3 aA
VI	31.5 abA	34.2 abA	13.9 a	117.8	63.1	21.5	29.0	28.2 aA	25.3 aA
VII	38.3 aA	31.2 abB	6.2 a	62.6	45.7	23.7	18.2	10.6 cA	8.8 bA
VIII	31.5 abB	39.2 aA	12.6 a	52.9	90.5	22.3	28.2	9.5 cA	10.9 bA
CVA (%)	7.23		26.89	29.84		39.63		9.97	
CVB (%)	10.42		37.54	39.84		21.03		13.12	

Mean values followed by the same lowercase letter in a column, and uppercase letter on a row, do not differ by Tukey's test (5%). (ns) = not significative; I = *P. guineense*/*P. cattleianum*; II = *P. guajava*/*P. guineense*/*P. cattleianum*; III = *P. guajava* 'Paluma'/*P. guajava*; IV = *P. guajava* 'Paluma'/*P. guineense*; V = *P. guajava* 'Cortibel 1'/*P. guajava*; VI = *P. guajava* 'Cortibel 1'/*P. guineense*; VII = *P. cattleianum* (purple); VIII = *P. cattleianum* (yellow). CVA = coefficient of variation in the plot and CVB = coefficient of variation in the subplot.

CONCLUSION

P. cattleianum as rootstocks for *Psidium* spp. increases the concentration of Na in the leaves of the scions, inducing less tolerance to salt stress. The presence of an interstock does not prevent an increase in the sodium concentration in the scion leaves.

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AUTHORS' CONTRIBUTION

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