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Original article

Efficient compartmentalization and translocation of toxic minerals lead tolerance in volkamer lemon tetraploids more than diploids under moderate and high salt stress

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Summary

Introduction – **Salt stress affects the growth and development of many crops. Citrus is a major global fruit crop and its production is strictly affected by salinity. The use of polyploid citrus rootstocks has been proposed as a strategy to improve salt tolerance. Although tetraploid rootstocks have been found to be more salt tolerant under low and moderate salinity. But, the mechanisms of these dynamics are unknown, including how they compartmentalize the toxic ions under moderate and high saline soils.** *Materials and methods* – **Exploring the differences in salt tolerance mechanism between tetraploid and diploid volkamer lemon rootstock, the plants were exposed to moderate (75 mM) and high (150 mM) salinity for 80 days. Various growth parameters (plant height and diameter, leaves number, dry biomass) and various minerals nutrients (N, P, K, Ca, Na and Cl) in leaves and roots of diploid and tetraploid rootstock were studied to understand tolerance mechanism.** *Results* – **The results exhibited that tetraploid rootstock behaved differently to cope with the salinity as compared to diploid. In both diploid and tetraploid rootstocks various growth traits were decreased under moderate and high salinity compared to the control. However the decrement was less in tetraploid as compared to diploid rootstocks in moderate and interestingly in high salinity.** *Conclusion* – **Tetraploid and diploid rootstocks compartmentalize the toxic ions differentially and/or different parts against moderate and high salt stress. These results suggest that the use of tetraploid citrus rootstocks will be useful and more beneficial for citrus cultivation in moderate and more importantly high saline soils.**

Keywords
citrus rootstock, nutrients, polyploidy, salinity, toxicity

Significance of this study

What is already known on this subject?

- Salinity is one of the major causes of yield decrease of many fruit crops including citrus. Citrus is the major fruit crop of the world but its production is decreasing by different factors, *i.e.*, salinity. Chloride is toxic for citrus. Tetraploids are more tolerant than diploids under moderate salt stress, however, under high salt level, diploids are more tolerant than tetraploids.

What are the new findings?

- Volkamer lemon tetraploids are more tolerant than diploids under moderate and specifically high salinity. The compartmentalization of toxic ions occur particularly in leaves under moderate salinity and in roots under high salt stress.

What is the expected impact on horticulture?

- The use of tetraploid rootstocks under moderate and high salt affected soils is a good strategy for better citrus production.

Introduction

A plant faces different abiotic stresses during its life span among which salinity is a major abiotic constraint that adversely affects plant growth and yield throughout the world (Byrt and Munns, 2008). Salinity decreases osmotic potential of soil solution, which perturbs plant-water relation. The excess of salts in soils ultimately increases the accumulation of toxic ions in plants, which causes imbalance in nutrients uptake and results in various physiological disorders (Forner-Giner *et al.*, 2011). The plants acquire essential nutrients from the rhizosphere. In saline soils, uptake of nutrients is disturbed in the presence of sodium chloride, mainly because of the interactive competition between various ions; for example, high sodium uptake can decrease potassium or calcium uptake which are among the main contributors to

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plant metabolism (Munns and Tester, 2008). Under salinity stress, different physiological disorders occur in citrus which restrict the growth and yield (Syvertsen and García-Sanchez, 2014). Salinity mainly causes decrement in water potential, photosynthesis rate, stomatal conductance and transpiration rate (Perez-Perez *et al.*, 2007).

Citrus exhibits wide diversity in the tolerance mechanism against salinity (Hussain *et al.*, 2015). Citrus is mostly affected by chloride ion accumulation and, therefore, the salt tolerance of citrus rootstocks is usually established by their capacity to cope with chloride ion (Hussain *et al.*, 2012b). Tolerant citrus rootstocks exclude chloride ion more efficiently than sensitive rootstocks (Hussain *et al.*, 2012b). Citrus species are mainly diploids $2n=18$, but tetraploid plants $4n=36$ can be considered as the clones of diploid ancestors, which were selected by nucellar seedlings from apomictic genotypes (Aleza *et al.*, 2011). Many investigators observed that tetraploids are different from their parent diploids in morphological, anatomical, physiological, biochemical and molecular traits (Romero-Aranda *et al.*, 1997; Hussain *et al.*, 2012b; Tan *et al.*, 2015; Fatima *et al.*, 2015; Oustric *et al.*, 2017).

Tetraploid plants are more tolerant against abiotic stresses as compared to their corresponding diploid plants (Saleh *et al.*, 2008; Allario *et al.*, 2013; Ruiz *et al.*, 2016a, b). They have more ability to exclude toxic ions from roots than diploid plants due to differences in morphological and histological traits of roots (García-Sanchez *et al.*, 2002; Ruiz *et al.*, 2016b). Yahmed *et al.* (2016) also observed that in roots compartment ion exclusion mechanism is present which influence on the status of salt tolerance. Further, it was observed that tetraploid rootstock does not affect fruit quality (Hussain *et al.*, 2012a). Hence, the use of tetraploid rootstocks has been suggested to cope with salinity more efficiently and also to maintain plant growth better than their parental diploid rootstocks. Previous studies concluded that, under low saline conditions, tetraploids thrived better than diploid plants (Saleh *et al.*, 2008), while under high saline conditions, tetraploids were more salt sensitive than their diploids (Mouhaya *et al.*, 2010). However, Grosser *et al.* (2012) stressed that tetraploid plants were more able to cope with sodium and chloride ions than diploids and maintained their growth and physiological attributes with less toxic symptoms even under high salinity. Therefore, this study was conducted to compare diploid and tetraploid volkamer lemon rootstock (*Citrus volkameriana* Tan. and Pasq.) for their growth, photosynthetic efficiency, and uptake and transportation of minerals under moderate and high salinity.

Materials and methods

The seeds of diploid and tetraploid volkamer lemon (Citrus volkameriana Tan. and Pasq.) were obtained in 2016 from INRA-CIRAD, France and sown in plastic seed containers. One-year-old healthy plants were selected for experimentation and transplanted in plastic container (ø30 cm) using sandy loam soil. The experiment was performed in protected environment where maximum day temperature was 28 °C and minimum night temperature was 14 °C, whereas relative humidity fluctuated between 50% and 80%.

The plants of each ploidy level were exposed to 80 days of salt stress treatments: control (0 mM NaCl), moderate salinity (75 mM NaCl) and high salinity (150 mM NaCl). All treatments were applied through irrigating with Hoagland nutrient solution ($Ca(NO_3)_2.4H_2O$: $MgSO_4.7H_2O$: KNO_3, $NH_4H_2PO_4$: $(NH_4)_2HPO_4$: H_3BO_3: $CuSO_4$: $MnSO_4$: $NaMoO_4$: FeNaEDTA), one L solution was applied in each application and three applications per week. One plant in each container and two plants of each genotype were used for destructive and two plants for non-destructive sampling in each experimental unit. The experiment was performed in completely randomized design and replicated thrice.

Plant height and diameter, leaves number and dry biomass

Plant height, diameter and leaves number were measured at the start and end of the experiment, while dry biomass was measured only at the end of the experiment. Plant height was measured using measuring tape and diameter was measured using LCD Digital Vernier Caliper. The increment in plant height, diameter and leaves number was calculated using the equation given below. Plant dry biomass was estimated using oven drying (65 °C) the whole plant and weighing with electronic weighing balance.

$$Increment\ (\%) = \frac{Final\ Value - Initial\ Value}{Initial\ Value} \times 100$$

Initial value = Start of experiment (day 0); Final value = End of experiment (after 80 days).

Minerals analysis

The leaves (at middle of the stem) and roots (primary and secondary) samples were harvested at the end of the experiment and oven dried at 70 °C for 48 hours. For total nitrogen (N), phosphorus (P), potassium (K), calcium (Ca) and sodium (Na) estimation, 100 mg dry samples were added in 2 mL H_2SO_4 and digested at 300 °C. After cooling the samples at room temperature 0.2 mL H_2O_2 (stabilized for phosphorus) were added continuously until the colourless digestate appeared. Total N in leaves and roots were measured using the colorimetric method (Martin *et al.*, 1983). For P estimation, malachite green method was followed (Ohno and Zibilske, 1991). K, Ca and Na were determined using flame photometer as described by Ryan *et al.* (2001). For chloride (Cl) analysis, a 100 mg oven-dried sample of leaves or roots was placed in muffle furnace at 400 °C and Cl content was measured using Cl electrode (Thermo Scientific Orion 9617BNWP ionplus Sure-Flow) as described by Hussain *et al.* (2012b).

Statistical analysis

The analysis of variance (CRD, two way factorial and three way factorial), Pearson's correlation by using XLSTAT software (v. 2018.7). Two way factorial analyses were done on growth attributes to observe the relationship between salinity and polyploidy. However, three way factorial analyses were performed on minerals data to observe the relationship between salinity, polyploidy and plant parts. At 5% probability level, least significant difference test was used for the comparison of means (Gomez and Gomez, 1984). Graphical representation was performed using SigmaPlot version 12.5 software (Systat Software, San Jose, CA).

Results

Plant height and diameter, leaves number and dry biomass

Diploid and tetraploid plants under control treatment exhibited more increment in plant height and diameter than

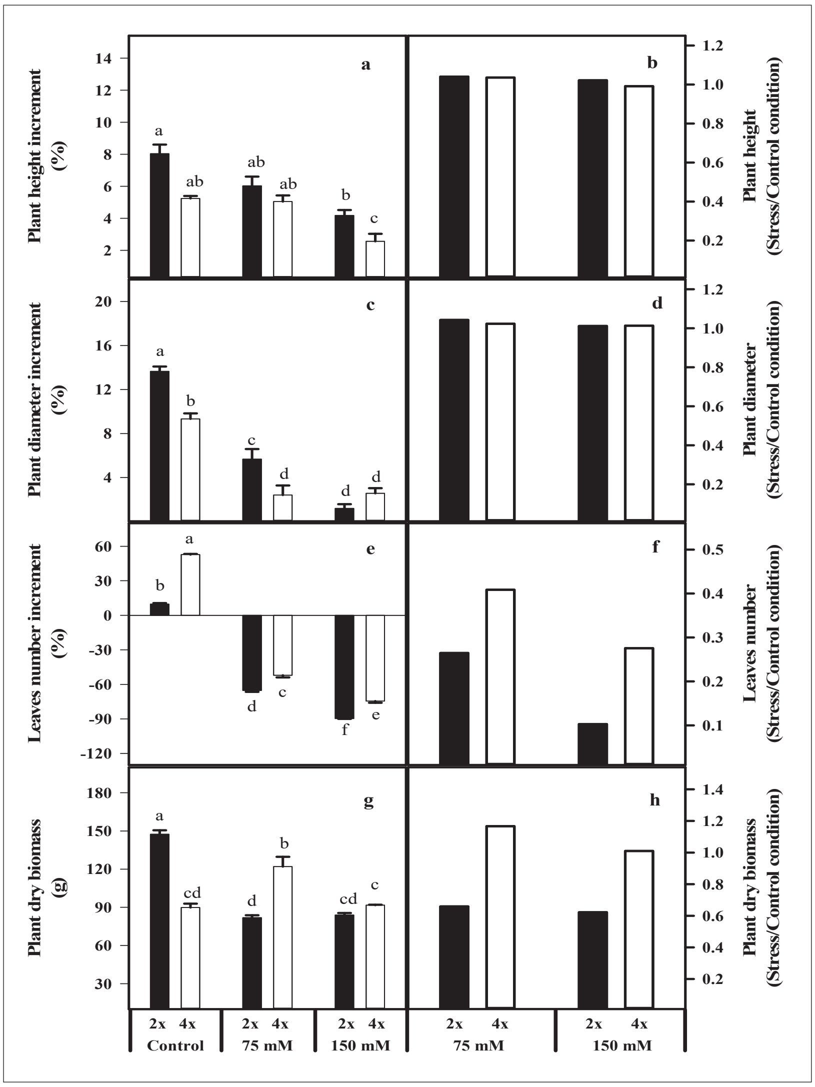

FIGURE 1. (a) Plant height increment; (c) Plant diameter increment; (e) Leaves number increment; (g) Plant dry biomass of diploid and tetraploid volkamer lemon plants grown under moderate and high salinity for 80 days. (b) Plant height increment; (d) Plant diameter increment; (f) Leaves number increment; (h) Plant dry biomass was based on the ratio of the increment over control condition after 80 days of salt stress. Bars indicate ±S.E. at $p < 0.05$ ($n = 3$).

the plants exposed to salt stress (Figure 1). Diploid plants showed significantly more plant height increment under control and high salt stress condition (Table 3), while diploids showed minimum increase in plant height under high level of salinity. However, the interaction between polyploidy and salt stress was statistically non-significant. Moreover, the increase in plant diameter was also observed higher in diploid plants than tetraploids under control and moderate salinity (Figure 1), while under high salt stress diploid and tetraploid plant diameter increment was statistically significant. Diploid and tetraploid plants showed a decrease in the leaves number when exposed to salt stress which was statistically significant (Table 3), while showing an increase when kept under control condition. The maximum leaf drop was observed in diploid plants under high salinity condition (Figure 1). Diploid plants showed high dry biomass in non-stressed plants, while at moderate and high salinity, tetraploid plants exhibited more biomass than diploid plants and the interaction between polyploidy and salinity levels was statistically significant. The values for the ratio of plant height, diameter, leaves number and dry biomass were measured after 80 days of stress compared with control (Figure 1). The ratio of plant height and diameter increment did not show significant change among treatments, while the ratio of leaves number and plant dry biomass was higher in tetraploid plants as compared to diploids under stressed condition.

Minerals analysis

Total N was more in leaves and roots of tetraploid plants than diploid ones under control condition. At moderate salt stress, N was more in diploid plants. However, tetraploids showed a decrease in N when exposed to salt stress as compared to control. The low total N was estimated in the leaves and roots of tetraploid plants grown under high salinity (Figures 2 and 4).

Diploid plants showed the maximum P content under control condition in leaves, while, under moderate and high salt stressed condition, P decreased in leaves and roots. Tetraploid plants exhibited more P in leaves when exposed to moderate salinity than under high salinity concentration, whereby P decreased in leaves and roots (Figures 2 and 4).

K content was higher in leaves of diploid and tetraploid volkamer lemon rootstocks under control than under stress conditions. K concentration decreased under moderate and high salinity. Moreover, tetraploids showed the maximum K at moderate and high salinity levels as compared to diploid. However, Ca was higher in the leaves of diploid plants under control and higher salinity than in the leaves of tetraploids, while under moderate saline condition, tetraploids leaves showed more content than diploids leaves but the differences in Ca concentration were statistically non-significant (Figure 2). Roots of diploid and tetraploid volkamer lemon showed a decrease in potassium and Ca concentrations when exposed to moderate and high salinity. Tetraploid plants ex-

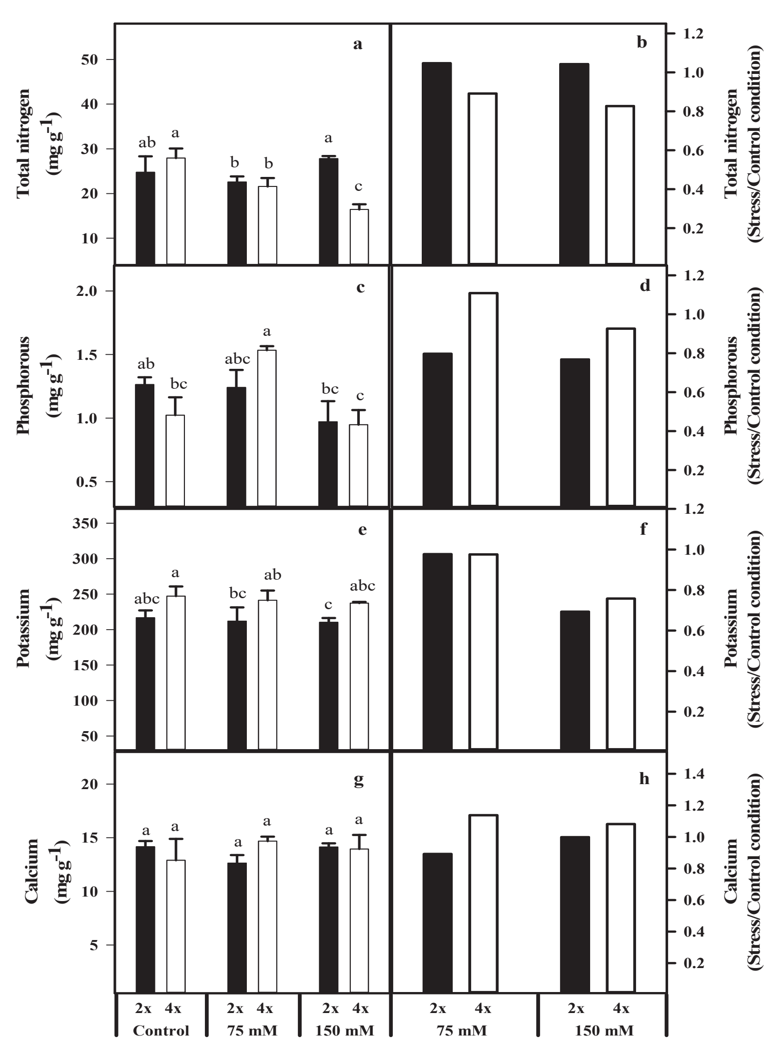

FIGURE 2. (a) Total nitrogen; (c) Phosphorus; (e) Potassium; (g) Calcium of diploid and tetraploid volkamer lemon leaves grown under moderate and high salinity for 80 days. (b) Total nitrogen; (d) Phosphorus; (f) Potassium; (h) Calcium was based on the ratio of the stressed over control leaves after 80 days of salt stress. Bars mean ± S.E. at $p < 0.05$ ($n = 3$).

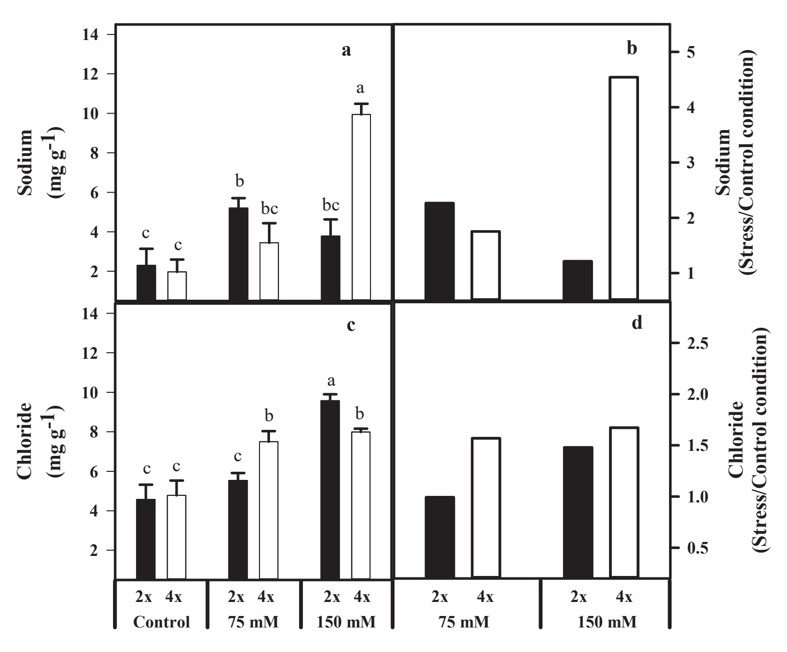

FIGURE 3. (a) Sodium; (c) Chloride of diploid and tetraploid volkamer lemon leaves grown under moderate and high salinity for 80 days. (b) Sodium; (d) Chloride was based on the ratio of the stressed over control leaves after 80 days of salt stress. Bars mean ± S.E. at $p < 0.05$ ($n = 3$).

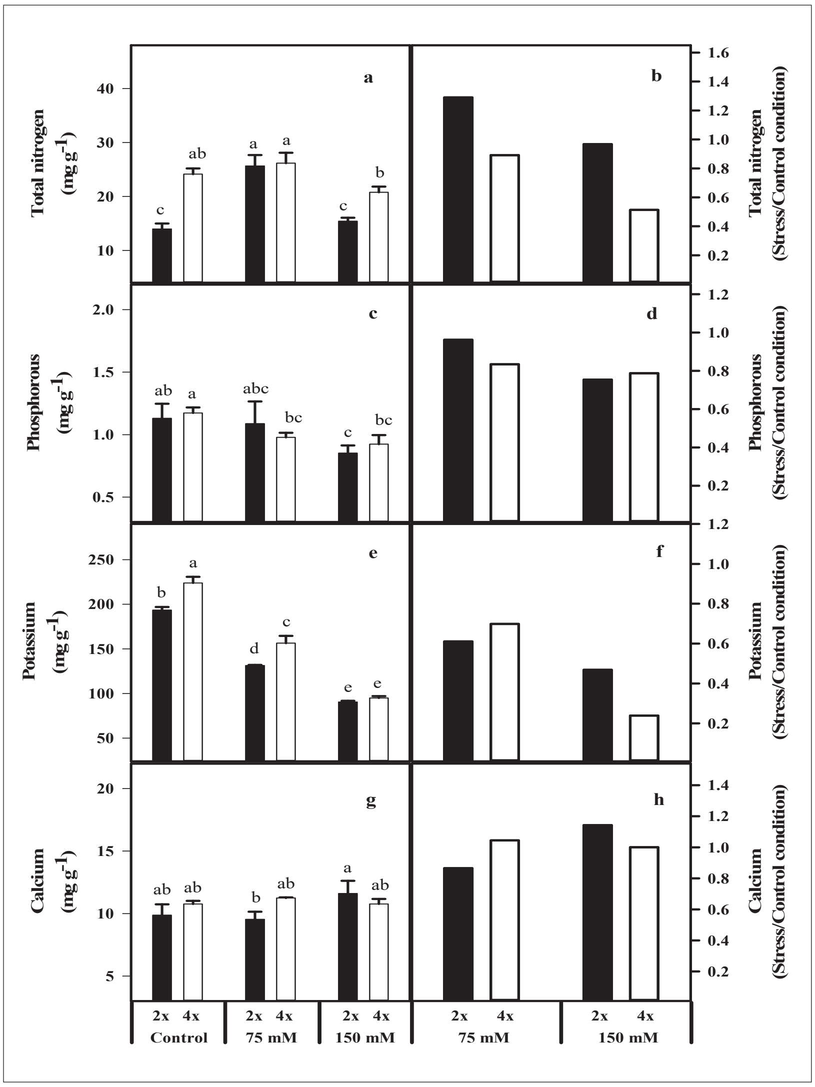

Figure 4. (a) Total nitrogen; (c) Phosphorus; (e) Potassium; (g) Calcium of diploid and tetraploid volkamer lemon roots grown under moderate and high salinity for 80 days. (b) Total nitrogen; (d) Phosphorus; (f) Potassium; (h) Calcium was based on the ratio of the stressed over control roots after 80 days of salt stress. Bars mean ± S.E. at $p < 0.05$ ($n = 3$).

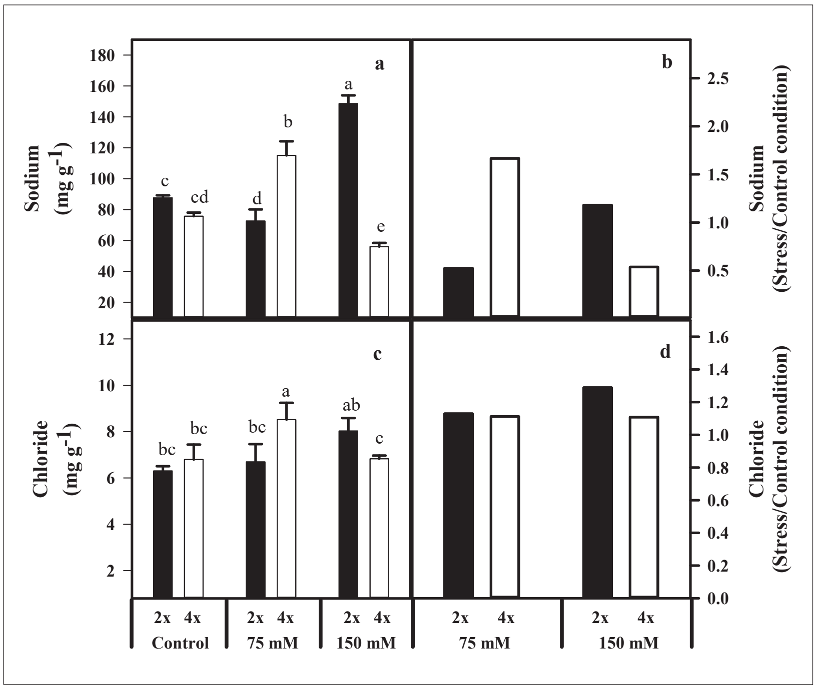

Figure 5. (a) Sodium; (c) Chloride of diploid and tetraploid volkamer lemon roots grown under moderate and high salinity for 80 days. (b) Sodium; (d) Chloride was based on the ratio of the stressed over control roots after 80 days of salt stress. Bars mean ± S.E. at $p < 0.05$ ($n = 3$).

TABLE 1. Pearson's correlation matrix among leaves and roots parameters of diploid and tetraploid volkamer lemon seedlings under moderate and high salinity.

Variables	PH	PD	NOL	DB	N-L	P-L	K-L	Ca-L	Na-L	Cl-L	N-R	P-R	K-R	Ca-R	Na-R
PH	**1.000**														
PD	0.206	**1.000**													
NOL	0.330	0.707	**1.000**												
DB	**0.842**	-0.221	-0.229	**1.000**											
N-L	-0.596	0.642	0.436	**-0.882**	**1.000**										
P-L	**0.888**	0.027	-0.105	**0.967**	-0.732	**1.000**									
K-L	0.768	0.710	**0.829**	0.310	0.038	0.460	**1.000**								
Ca-L	0.529	-0.359	-0.616	**0.889**	**-0.816**	**0.848**	-0.078	**1.000**							
Na-L	0.223	-0.794	-0.171	0.368	-0.721	0.135	-0.171	0.205	**1.000**						
Cl-L	0.131	-0.673	**-0.892**	0.644	-0.761	0.526	-0.512	**0.893**	0.325	**1.000**					
N-R	-0.136	**0.888**	0.787	-0.608	**0.872**	-0.410	0.522	-0.747	-0.684	**-0.910**	**1.000**				
P-R	0.364	0.622	**0.993**	-0.185	0.354	-0.088	**0.822**	-0.597	-0.054	**-0.864**	0.715	**1.000**			
K-R	0.410	**0.962**	0.606	0.044	0.426	0.294	0.768	-0.094	-0.756	-0.472	0.729	0.525	**1.000**		
Ca-R	-0.381	-0.247	**-0.857**	0.073	-0.070	0.081	-0.667	0.517	-0.358	0.697	-0.403	**-0.912**	-0.158	**1.000**	
Na-R	0.298	0.407	-0.276	0.426	-0.061	0.591	0.156	0.610	-0.647	0.395	0.003	-0.356	0.588	0.632	**1.000**
Cl-R	**-0.870**	0.058	-0.422	-0.676	0.631	-0.625	-0.662	-0.275	-0.601	0.008	0.206	-0.501	-0.067	0.681	0.208

Significant coefficients were those higher than |0.8| and they are marked as bold.

PH = Plant height; PD = Plant diameter; NOL = Leaves number; DB = Dry biomass; N-L, P-L, K-L, Ca-L, Na-L, Cl-L = Nitrogen, phosphorus, potassium, calcium, sodium and chloride in leaves; N-R, P-R, K-R, Ca-R, Na-R, Cl-R = Nitrogen, phosphorus, potassium, calcium, sodium and chloride in roots. Bold values indicate the significant correlation.

TABLE 2. Variables affected by salinity levels (control, moderate and high), genotypes (2*x* and 4*x*) and plant part (leaves and roots).

Variables	Control (0 mM)				Moderate salinity (75 mM)				High salinity (150 mM)			
	Diploid (2*x*)		Tetraploid (4*x*)		Diploid (2*x*)		Tetraploid (4*x*)		Diploid (2*x*)		Tetraploid (4*x*)	
Plant height (%) ^{ns}	8.03		5.23		6.02		5.05		4.18		2.56	
Plant diameter (%) ***	13.65		9.33		5.67		2.40		1.19		2.56	
Leaves number (%) ***	9.65		52.78		-65.24		-52.15		-89.71		-74.48	
Dry biomass (g) ***	147.44		89.83		81.82		122.03		83.90		91.73	
	Leaves	*Roots*	*Leaves*	*Roots*	*Leaves*	*Roots*	*Leaves*	*Roots*	*Leaves*	*Roots*	*Leaves*	*Roots*
Nitrogen (mg g^{-1}) ***	24.76	13.97	27.93	24.13	22.59	25.66	21.57	26.16	27.81	15.41	16.43	20.77
Phosphorus (mg g^{-1}) **	1.26	1.13	1.02	1.17	1.24	1.08	1.53	0.97	0.97	0.85	0.94	0.92
Potassium (mg g^{-1}) ^{ns}	216.64	193.31	247.11	223.91	211.80	131.32	241.20	156.36	210.20	90.50	237.24	95.02
Sodium (mg g^{-1}) ***	2.30	87.63	1.97	75.72	5.20	72.56	3.45	115.08	3.79	148.56	9.94	56.07
Chloride (mg g^{-1}) ^{ns}	4.57	6.30	4.78	6.79	5.53	6.69	7.49	8.51	9.57	8.03	7.99	6.82
Calcium (mg g^{-1}) ^{ns}	14.15	9.87	12.89	10.76	12.62	9.53	14.68	11.25	14.13	11.61	13.94	10.77

^{ns} = non-significant; ** = significant ($p < 0.05$); *** = highly significant ($p < 0.01$).

TABLE 3. Analysis of variance of different studied parameters of diploid and tetraploid volkamer lemon under different level of salt stress and plant parts.

Source	Degree of freedom	F-value	Probability
Plant height increment			
Treatment (*0, 75, 150 mM*) (T)	2	12.81	0.0011 ***
Genotype (*2x, 4x*) (G)	1	12.16	0.0045 ***
T × G	2	2.07	0.1691 ^{ns}
Error	12		
Total	17		
Plant diameter increment			
Treatment (*0, 75, 150 mM*) (T)	2	189.74	0.0000 ***
Genotype (*2x, 4x*) (G)	1	24.00	0.0004 ***
T × G	2	17.07	0.0003 ***
Error	12		
Total	17		
Leaves number			
Treatment (*0, 75, 150 mM*) (T)	2	7618.24	0.0000 ***
Genotype (*2x, 4x*) (G)	1	90.5.57	0.0000 ***
T × G	2	149.48	0.0000 ***
Error	12		
Total	17		
Dry biomass			
Treatment (*0, 75, 150 mM*) (T)	2	50.61	0.0000 ***
Genotype (*2x, 4x*) (G)	1	1.62	0.2267 ^{ns}
T × G	2	132.01	0.0000 ***
Error	12		
Total	17		
Nitrogen			
Treatment (*0, 75, 150 mM*) (T)	2	7.94	0.0023 ***
Genotype (*2x, 4x*) (G)	1	1.95	0.1757 ^{ns}
Plant part (*Leaf, root*) (P)	1	9.47	0.0052 ***
T × G	2	12.58	0.0002 ***
T × P	2	16.53	0.0000 ***
G × P	1	26.84	0.0000 ***
T × G × P	2	7.52	0.0029 ***
Error	24		
Total	35		
Phosphorus			
Treatment (*0, 75, 150 mM*) (T)	2	11.71	0.0003 ***
Genotype (*2x, 4x*) (G)	1	0.01	0.9089 ^{ns}
Plant part (*Leaf, root*) (P)	1	7.48	0.0115 **
T × G	2	1.21	0.3165 ^{ns}
T × P	2	4.68	0.0192 **
G × P	1	0.01	0.9363 ^{ns}
T × G × P	2	4.04	0.0308 **
Error	24		
Total	35		
Potassium			
Treatment (*0, 75, 150 mM*) (T)	2	68.40	0.0000 ***
Genotype (*2x, 4x*) (G)	1	31.89	0.0000 ***
Plant part (*Leaf, root*) (P)	1	330.92	0.0000 ***
T × G	2	1.06	0.3622 ^{ns}
T × P	2	51.48	0.0000 ***
G × P	1	1.06	0.3145 ^{ns}
T × G × P	2	0.64	0.5381 ^{ns}
Error	24		
Total	35		

Table 3. Continued.

Source	Degree of freedom	F-value	Probability
Sodium			
Treatment (*0, 75, 150 mM*) (T)	2	15.69	0.0000 ***
Genotype (*2x, 4x*) (G)	1	27.00	0.0000 ***
Plant part (*Leaf, root*) (P)	1	2260.80	0.0000 ***
T × G	2	98.79	0.0000 ***
T × P	2	6.27	0.0065 ***
G × P	1	35.13	0.0000 ***
T × G × P	2	125.75	0.0000 ***
Error	24		
Total	35		
Chloride			
Treatment (*0, 75, 150 mM*) (T)	2	31.45	0.0000 ***
Genotype (*2x, 4x*) (G)	1	1.20	0.2846 ns
Plant part (*Leaf, root*) (P)	1	4.33	0.0482 **
T × G	2	13.58	0.0001 ***
T × P	2	14.24	0.0001 ***
G × P	1	0.12	0.7347 ns
T × G × P	2	0.10	0.9069 ns
Error	24		
Total	35		
Calcium			
Treatment (*0, 75, 150 mM*) (T)	2	0.95	0.4019 ns
Genotype (*2x, 4x*) (G)	1	0.80	0.3806 ns
Plant part (*Leaf, root*) (P)	1	49.02	0.0000 ***
T × G	2	2.85	0.0774 ns
T × P	2	0.09	0.9180 ns
G × P	1	0.19	0.6636 ns
T × G × P	2	1.00	0.3818 ns
Error	24		
Total	35		

ns = non-significant; ** = significant ($p < 0.05$); *** = highly significant ($p < 0.01$).

hibited more potassium and Ca content under control and moderate salt stress than diploids, while at high salinity the decrease was more in the roots of tetraploids (Figure 4).

Tetraploids leaves under high salinity and tetraploids roots under moderate salinity exhibited more Na concentration, while the leaves and roots of diploids under moderate and high salinity, respectively, showed more Na content. The roots of tetraploids showed the minimum concentration of Na under high salinity (Figures 3 and 5). Cl accumulation increased in diploid and tetraploid plants when exposed to salt stress. Cl accumulation was higher in leaves and roots of tetraploid plants under moderate salinity than in those of diploids. Moreover, the interaction between polyploidy and salinity levels was found statistically significant in N, Na and Cl, while non-significance was observed in K, P and Ca. However, under high salt stress, diploid plants showed the maximum Cl contents in leaves and roots. The stressed/control ratio of different minerals in leaves and roots was measured after 80 days of salt stress.

Pearson's correlation analysis

The plant height was significantly and positively correlated with dry biomass and phosphorus in leaves, while negatively correlated with Cl in roots. Cl in leaves were negatively correlated with leaves number, N and P in roots, while positively with Ca in leaves (Table 1 and Supplemental Information – Table S1). The leaves number and K in leaves were positively correlated with P in roots.

Discussion

Diploid and tetraploid volkamer lemon plants showed remarkable differences under moderate and high salt stress (Figure 6). Tetraploid plants appeared to be more tolerant against salinity than their corresponding diploids (Khalid *et al.*, 2020). Diploid and tetraploid plants under salt stress condition showed less increase in plant height and diameter as compared to those under control. However, the interaction between treatment and genotypes was statistically non-significant in plant height and significant in plant diameter. Salinity decreases the growth of the plant by ceasing the leaf formation and development of internodes and increase in leaf abscission (Khalid *et al.*, 2019). The decrease in plant growth is linked with accumulation of toxic ions (Hatfield and Prueger, 2015). Brumos *et al.* (2009) suggested that under stress condition, sensitive genotype showed more growth by overexpression of the genes which are responsible in carbon metabolism under stress. Also, Hussain *et al.* (2012b) suggested that the maximum decrease in plant height and plant diameter was observed by tolerant genotype under salinity as compared to control. The leaves number increment was observed more in tetraploid plants under control condition, while under salt stress condition, both ploidy levels showed a decrease in leaves number. Diploid volkamer lemon plants exhibited the maximum decrease at high salinity level which showed its sensitivity to salt stress. Similar findings were observed by Saleh *et al.* (2008) and Hussain *et al.* (2012b) that under salinity leaf drop was more in sensitive genotypes. Salt stress significantly affected the plant dry biomass and the decrease was more in tetraploid plants than in diploids. The interactions showed that leaves number and dry biomass were statistically affected by the ploidy level under moderate and high salinity (Table 3). Ruiz *et al.* (2016a) also observed that under salinity tetraploid plants of *Citrus macrophylla* showed more decrease in biomass as compared to their diploids which might be due to the decrease in net CO_2 assimilation rate.

Under saline conditions, Cl and nitrate form of nitrogen (NO_3-N) were shown to have an antagonistic relationship, so the increased Cl^- accumulation in root may result in the decrease of total nitrogen in leaves and roots of citrus rootstocks (Lea-Cox and Syvertsen, 1993). In this study, total nitrogen concentration decreased in leaves and roots. A similar and high decrement in nitrogen concentration was noticed in roots at high salinity, for diploid and tetraploid plants. The interaction between diploid, tetraploid and moderate, high salinity was statistically significant related to nitrogen con-

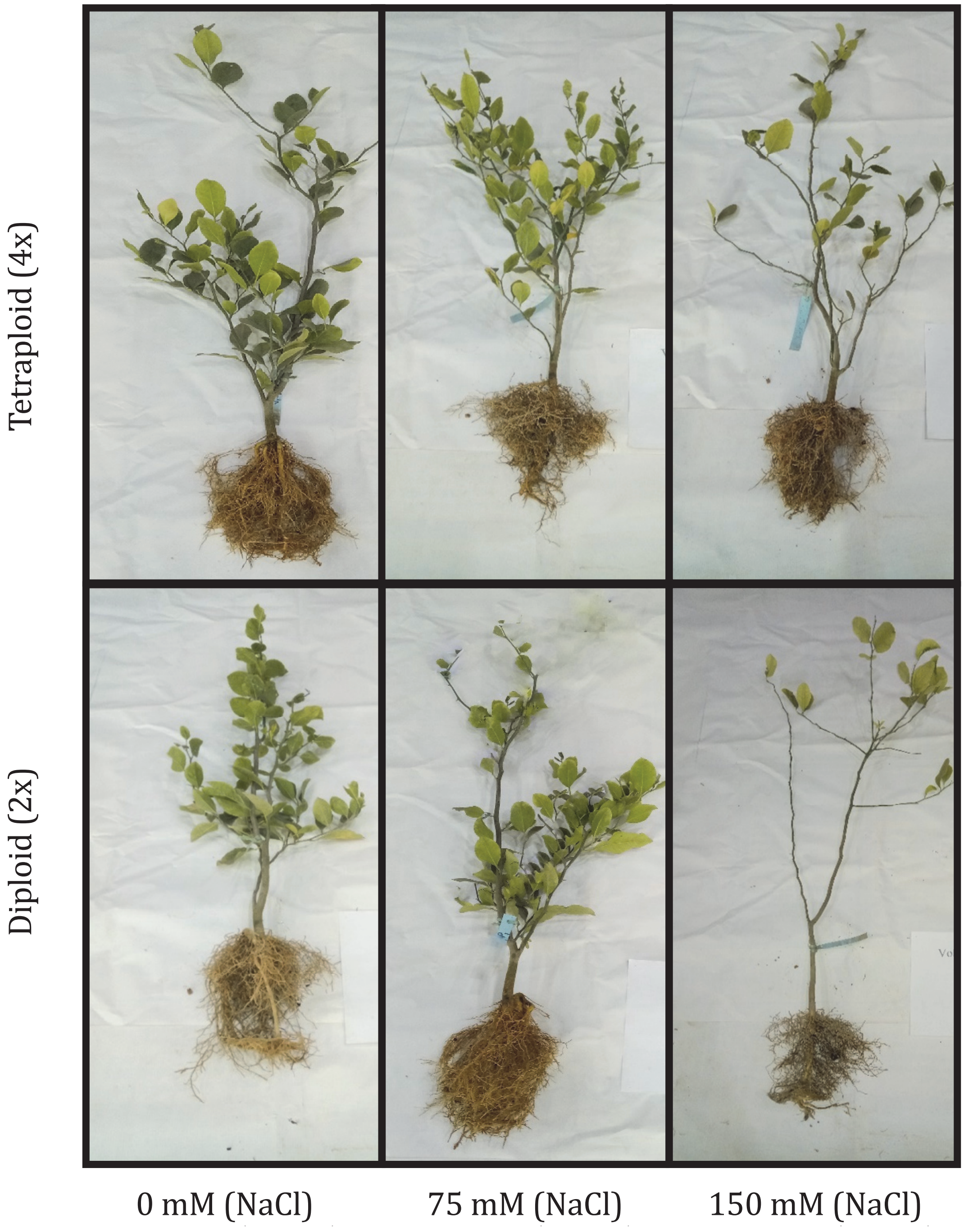

Figure 6. Volkamer lemon diploid (2×) and tetraploid (4×) seedlings grown under 0 mM, 75 mM and 150 mM NaCl for 80 days.

centration. The decrease in nitrogen concentration was also due to increase in biomass and abundance of nitrogen dilution by growth (García-Sánchez *et al.*, 2002). Total nitrogen was also significantly and negatively correlated with plant biomass (Table 1). Phosphorus concentration did not significantly decrease in leaves and roots of diploid and tetraploid volkamer lemon under moderate salinity. However, under high salinity both ploidy levels showed a significant decrease. The decrease in uptake and assimilation of phosphorus at high salinity may be due to reduction in PO_4^{3-} activity and solubility of Ca-P (Qadir and Schubert, 2002; Hussain *et al.*, 2018). Furthermore, the positive correlation of phosphorus with plant height, leaves number, biomass and potassium concentration was observed (Table 1).

The K concentration in leaves of diploid and tetraploid volkamer lemon was not significantly affected by moderate salt stress, but K content was decreased at high salinity. However, the interaction between polyploidy and salt levels was statistically non-significant. Tetraploid plants showed more K concentration which justifies its tolerance against salinity. As K is positively correlated with E, which shows that K maintained the opening of stomata. Increase in K concentration shows the proper functioning of the physiological mechanism under salinity (Table 1).

The Na and K were negatively correlated with each other (Table 1). The high Na concentration decreased the concentration of K (Anjum, 2008). Moreover, the roots of diploid and tetraploid rootstocks showed a significant decrease in Na content at both moderate and high salinity levels. But for K in roots, tetraploid plants showed more decrease in K content at high salinity than diploids and the interaction between genotype and salt levels was statistically non-significant. García-Sánchez *et al.* (2002) reported that tolerant genotype maintains K concentration in leaves by reducing K in roots as observed in this study. The salinity significantly affects Ca concentration. The leaves of diploid and tetraploid rootstocks showed a decrease in Ca concentration; the decrease was noted more in diploid plants as compared to tetraploids. However, the roots of diploid rootstock under salinity showed decrease in Ca content while, tetraploid showed no significant change. The tolerant citrus genotype maintains root Ca content under salinity, which shows its ability to maintain root growth and cope with the effect of salinity (García-Sánchez *et al.*, 2002).

Under moderate and high salinity both ploidy levels of volkamer lemon showed a significant increase in Cl and Na contents. At moderate salinity, roots of the diploid plants transported Na ion to the leaves, and when the level of salinity increased, the compartmentalization of Na ions also increased in roots, which resulted in less transportation of Na ions to the leaves. However, in tetraploid plants, roots maintained the concentration of Na ions instead of transferring them to the leaves. Under high salinity, tetraploid roots transferred the Na ions to the leaves where they compartmentalize easily. Na ion concentration was limited in the roots of tolerant genotypes (Ruiz *et al.*, 2016a). Regarding the effect of Cl concentration in leaves and roots of diploid and tetraploid volkamer lemon plants under salinity, both ploidy levels showed different mechanism. Under moderate salinity, leaves and roots of the tetraploid plants accumulated more Cl content than those of diploids, but showed no such accumulation under high salinity. However, in diploid plants, concentration of Cl in both leaves and roots increased with increasing salinity level. The interaction of Na and Cl between diploid and tetraploid volkamer lemon and moderate and high salinity was statistically significant. The rapid uptake and accumulation of Cl ions in the leaves with increasing salt concentration may underline the inhibitory effects of salinity on growth in citrus rootstocks (Moya *et al.*, 2003; Anjum, 2008). At moderate salinity, tetraploids showed an increase in Cl concentration, while by increasing the salt concentration the compartmentalization of Cl ions in tetraploids also increased, which maintains the better uptake of nutrients even under high salinity (Khoshbakht *et al.*, 2015).

Our results indicated that volkamer lemon tetraploid plants are more tolerant to high salt stress than their corresponding diploids. Our results are in contradiction with the previous findings that tetraploid plants are more sensitive under high saline environment than diploids (Mouhaya *et al.*, 2010). Tetraploid plants have the ability to maintain their plant height and diameter as these plants showed a less decrement in leaves number by compartmentalization of Na and Cl ions in leaves and roots as compared to their corresponding diploids. Although tetraploid plants are more tolerant than diploids, they performed worse when compared to tetraploid control plants.

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SUPPLEMENTAL INFORMATION

SUPPLEMENTAL INFORMATION – TABLE S1. R squared values among leaves and roots parameters of diploid and tetraploid volkamer lemon seedlings under moderate and high salinity.

Variables	PH	PD	NOL	DB	N-L	P-L	K-L	Ca-L	Na-L	Cl-L	N-R	P-R	K-R	Ca-R	Na-R	Cl-R
PH	1.000															
PD	0.043	1.000														
NOL	0.109	0.500	1.000													
DB	0.709	0.049	0.053	1.000												
N-L	0.356	0.413	0.191	0.778	1.000											
P-L	0.788	0.001	0.011	**0.934**	0.536	1.000										
K-L	0.589	0.503	0.688	0.096	0.001	0.212	1.000									
Ca-L	0.280	0.129	0.379	0.791	0.666	0.720	0.006	1.000								
Na-L	0.050	0.630	0.029	0.135	0.520	0.018	0.029	0.042	1.000							
Cl-L	0.017	0.453	0.796	0.415	0.579	0.277	0.263	0.797	0.106	1.000						
N-R	0.018	0.789	0.619	0.369	0.760	0.168	0.272	0.558	0.468	**0.828**	1.000					
P-R	0.132	0.387	**0.986**	0.034	0.125	0.008	0.675	0.357	0.003	0.746	0.511	1.000				
K-R	0.168	**0.925**	0.367	0.002	0.182	0.086	0.591	0.009	0.571	0.223	0.532	0.275	1.000			
Ca-R	0.145	0.061	0.735	0.005	0.005	0.007	0.445	0.267	0.128	0.485	0.162	**0.831**	0.025	1.000		
Na-R	0.089	0.165	0.076	0.181	0.004	0.349	0.024	0.372	0.419	0.156	0.000	0.126	0.345	0.399	1.000	
Cl-R	0.756	0.003	0.178	0.457	0.398	0.391	0.439	0.076	0.361	0.000	0.043	0.251	0.004	0.463	0.043	1.000

Significant coefficients were those higher than |0.8| and they are marked as bold.

PH = Plant height; PD = Plant diameter; NOL = Leaves number; DB = Dry biomass; N-L, P-L, K-L, Ca-L, Na-L, Cl-L = Nitrogen, phosphorus, potassium, calcium, sodium and chloride in leaves; N-R, P-R, K-R, Ca-R, Na-R, Cl-R = Nitrogen, phosphorus, potassium, calcium, sodium and chloride in roots. Bold values indicate the significant correlation.