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RESEARCH ARTICLE

Effects of salt stress on tolerant accessions of quinoa at the morphological and metabolic levels

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ABSTRACT

Quinoa (*Chenopodium quinoa* Willd.) is a facultative halophyte recognized for its genetic variability and high tolerance to salinity. The objective of this study was to evaluate the effect of NaCl on three accessions of quinoa from the Camacani germplasm bank of the National University of the Altiplano, Perú, at three NaCl concentrations (0, 200 and 400 mM) under greenhouse conditions. Four morphological variables (plant height, root length, percentage of root DM, and aerial DM), as well as seven metabolic variables (chlorophyll, proline, phenols, flavonoids, saponins, amino acids and proteins content by spectrophotometric analysis) and electrolyte leakage (EL) in leaves were analyzed. Increasing NaCl exposure was positively correlated with increases in EL, proline, and saponin, while negatively correlated with leaf protein content. In accession PECQ 20037, for example, foliar protein content varied from 18.7% at 0 mM NaCl to 10.57% at 400 mM NaCl. The results indicated that accessions PECQ 20037 and Negra Oruro tolerated the 400 mM NaCl concentration better than Sajama.

Key words: *Chenopodium quinoa*, NaCl, morphological variables, metabolic variables.

INTRODUCTION

Quinoa (*Chenopodium quinoa* Willd.) is a native pseudocereal that was domesticated in the Andes over 7000 yr before the present (ybp) and has been cultivated continuously by pre-Hispanic civilizations and their indigenous descendants (Bazile et al., 2016). Andean South America is widely considered to be quinoa's center of conservation, diversity and genetic variability (Hinojosa et al., 2018); however, due to its adaptability to marginal conditions (Adolf et al., 2013) the crop has spread to North America, where it has spontaneously hybridized with its free-living sister pitseed goosefoot (*C. berlandieri* Moq.; Wilson and Manhart, 1993), and also to Europe, Africa, and Asia. Likewise, it has great nutritional properties, considered as a species that could safeguard food security, due to its composition rich in proteins, essential amino acids, fiber, vitamins, fatty acids and minerals (Maradini-Filho et al., 2017). In addition, this species belongs to a group of plants with the largest number of halophytic genera (Flowers and Colmer, 2015), that adapt to adverse abiotic factors, with the potential to prosper in conditions of salinity and water stress, being a species recognized for its tolerance to different concentrations of salinity (Adolf et al., 2013). This

characteristic has aroused the interest of researchers and a series of studies have been carried out, in recent years many researchers have shown that quinoa is a plant tolerant to salinity, being recognized for its great natural variability, these characteristics make it an attractive crop for agricultural activity in arid and semi-arid zones (Ruiz et al., 2016). In addition, Kaur et al. (2022) mention experiments carried out under salinity conditions ranging from 100 to 750 mM NaCl, where different accessions have been compared, demonstrating that quinoa has a complex trait, associated with mechanisms of tolerance to salinity under multiple processes and conditions (Ruiz et al., 2016).

Quinoa has a sophisticated salt exclusion system to counteract the ionic component of salinity, so it can be osmotically adjusted by abrupt increases in NaCl salt concentration (Terletskaya et al., 2023). Other studies have shown that in some accessions there is a reduction in plant height, root length, DM and leaf area under saline conditions (Qureshi and Daba, 2020). Likewise, in salinity studies, the increase in secondary metabolites is mentioned as a response to salt stress (Ruiz et al., 2016), and these would play an important role in the adaptation to the environment and overcoming stress conditions (Muscolo et al., 2016). Meanwhile, Mansour and Ali (2017) mention that the proline content is an indicator of adaptation in saline environments, likewise other authors mention that the secondary metabolites change according to the conditions in which the plant is developing (Sytar et al., 2018; Lin et al., 2019). According to the panorama presented, it would indicate that quinoa has great development potential under adverse conditions, involving biochemical, physiological and morphological pathways. Thus, the objective of this study was to evaluate the effect of NaCl concentrations in three quinoa accessions at the morphological and metabolic levels.

MATERIALS AND METHODS

Plant material

In this research, the quinoa (*Chenopodium quinoa* Willd.) accession PECQ 20037 from Puno-Peru and two accessions from Bolivia, Sajama and Negra de Oruro, were selected from the Camacani Germplasm Bank of the Universidad Nacional del Altiplano, Puno, Peru. These quinoa accessions were previously identified as the most salinity tolerant from among an experimental set of 50 accessions (Cueva, 2021).

Greenhouse experiment

The accessions were grown under greenhouse conditions at the Faculty of Agronomy of the Universidad Nacional de San Agustín de Arequipa, Perú, from December 2019 to march 2020 (day/night air temperature 35/10 ± 2 °C; relative humidity ranging 20%-65%). The accessions were planted in 8 L pots with sand-based substrate and fertilized with Osmocote Plus (ICL Group Ltd., Tel-Aviv, Israel), ferrous sulfate (500 g m^{-3}), additionally Ca and B (CaO 141 g L^{-1} and B_2O_3 9.5 g L^{-1}). Six to ten seeds per pot were sown and 4 plants per pot were thinned, watered every 3 d with 100-200 mL water. To ensure equal growth conditions, the pots were randomly distributed per block, the salt treatments began 15 d after direct sowing, incorporating the different salt concentrations gradually up to the desired concentration of 200 and 400 mM NaCl for 10 d, it was watered with a 100 mM NaCl solution and was evaluated by evaluating the EC and pH of the substrate. The experiment lasted 70 d, equivalent to the physiological phase of the flower primordium.

Morphological variables

The accessions were evaluated 17 d after starting the saline stress, one pot per treatment and accessions were evaluated for the variables fresh weight (FW) and dry weight (DW) of the aerial part and root were evaluated, then % DM (dry material) was calculated, in addition to the length of the stem and root. In addition, the leaf area (LA) was determined using the ImageJ software (Wayne Rasband, Research Services Branch, National Institute of Mental Health, Bethesda, Maryland, USA).

Metabolic variables and electrolyte leakage

For the determination of chlorophyll (CHL) content, 30 mg fresh leaves were extracted and ground with 3 mL acetone (85% v/v) and 2.5 mL acetone (80% v/v) was added. Absorbance was measured at 662 and 644 nm in the Lambda 365 UV-Vis spectrophotometer (Perkin Elmer, Waltham, Massachusetts, USA). The chlorophylls *a* and *b* were calculated according to the formulas of Redžić et al. (2005) and expressed in mg g^{-1} FW. The determination of chlorophyll was evaluated out 15 d and 42 d after the start of saline stress, in four plants of each treatment and accession, young leaves were taken from the middle third.

To determine electrolyte leakage (EL), 1 cm^2 leaf discs were washed with Type I water, left to stand for 6 h in 10 mL at room temperature, and the final electrical conductivity was measured after the water bath at 90 °C with a duration of 1 h. Electrolyte leakage was estimated using an electrical conductivity meter (EZDO 8200, Gondo Electronic, Taiwan), and expressed as a percentage of total conductivity (Restrepo et al., 2014). The determination of LE was carried out 27 d after the start of saline stress, in 4 plants of each treatment and accession, young leaves were taken from the middle third and old leaves from lower third.

Proline (PROL) was determined according to the protocol of Bates et al. (1973) modified, 100 mg fresh leaf was crushed in 5 mL aqueous sulfosalicylic acid (3% w/v) and centrifuged at 6000 rpm for 30 min at 10 °C, 1 mL supernatant was taken and 1 mL ninhydrin and 1 mL glacial acetic acid were added and placed in a water bath for 1 h, the reaction was completed by exposure to ice and the addition of 3 mL toluene. The supernatant was taken with a micropipette and the absorbance at 520 nm was measured with the UV-Vis spectrophotometer. Likewise, proline in leaves was evaluated 21 d after starting the stress, samples were taken from the middle third.

Flavonoids (FLAV) were determined according to the Caldwell (1971) protocol modified, 5 mg powdered dry leaves were weighed, then 5 mL hot pure methanol were added and homogenized with 5 mL acidified methanolic solution (HCl:water:methanol 1:29:79), and subsequently the sample was centrifuged at 3000 rpm for 15 min, then the absorbance of the supernatant was measured at 300 and 360 nm in the UV-Vis spectrophotometer.

Determination of amino acids (AMIN) was carried out according to the methodology of Yemm et al. (1955) modified, 0.002 g dry leaves were weighed and ground in 1.5 mL 70% ethanol, then 500 µL 1 M sodium citrate were added. To 500 µL the macerate, 1 mL ninhydrin at 1% and vortexed for 60 s at 3200 rpm. Subsequently, it was heated at 95 °C for 20 min, then it was centrifuged for 2.5 min at 9000 rpm, finally the supernatant was extracted and the reading was made at 570 nm in the UV-Vis spectrophotometer.

For protein (PROT) determination, the protocol of Bradford (1976) was followed, 0.5 g lyophilized ground leaves were weighed and 5 mL 100 mM potassium phosphate buffer pH 7.5 were added. Then, it was homogenized for 2 min in a vortex on an ice bed for 1 h, then it was centrifuged for 30 min at 6000 rpm at 4 ºC. The supernatant (1.5 mL) was extracted, finally 200 µL protein extract and 800 µL Bradford's reagent were added, left to stand for 15 min. Finally, the absorbance at 590 and 450 nm was determined in the UV-Vis spectrophotometer.

For the determination of saponins (SAP), 0.1 g leaves were weighed and macerated in 1 mL ethanol (50%) for 30 min, 0.05 mL macerated sample was taken and 1 mL color reagent was added (sulfuric acid: acetic anhydride 5:1) and allowed to stand for 50 min, the absorbance was determined in the UV-Vis spectrophotometer at 528 nm (Galindo et al., 1989).

Phenolic compounds (PHEN) determination was carried out according to the method of Goulart et al. (2022) modified, 0.5 g dry sample and 2 mL methanol mixed uniformly for 1 min were used; it rested for 24 h in refrigeration (4 °C); then it was centrifuged for 15 min at 3000 rpm; 0.5 mL supernatant was taken, 8 mL Type I water and 0.5 mL Folin-Ciocalteu 0.25 N reagent were added; mixed and allowed to react for 3 min, 1 mL 1 N Na_2CO_3 was added. The absorbance was measured at 725 nm and the content of total phenols was estimated from the equation developed for chlorogenic acid, to express the results in mg gallic acid equivalent GAE 100 g^{-1} DW.

Likewise, the flavonoid, amino acid, protein, and saponins and phenol content were evaluated 45 d after starting the stress, the samples were taken from the middle third.

Statistical analysis

The experiment was conducted under a randomized complete block design with a 3×3 factorial arrangement with four replicates. The data for each variable were subjected to ANOVA, evaluating the main effects and simple effects for each variable and using a Duncan mean comparison test (0.05). The term significant was used in the text only when the variable in question was confirmed to be significant ($p < 0.05$). To process the data, the free access software SAS on demand for academics was used (SAS Institute, Cary, North Carolina, USA). For the multivariate analysis, correlation and principal components analysis (PCA) were performed with the free access software RStudio (Posit PBC, Boston, Massachusetts, USA).

RESULTS AND DISCUSSION

Effect of salt stress on morphological variables

During the effects of incremental NaCl concentrations, for the ANOVA, significant differences can be found from the different sources of variation, with the exception of the source of variation DM percentage in the root, associated with morphological traits such as leaf area, root length, plant height, DM percentage, dry root and aerial part (Table 1).

Table 1. Analysis of variance for morphological variables. ^{ns}Nonsignificant; *Significant at $P < 0.05$.

		F Value					
Source	DF	Leaf area	Root length	Plant height	DM root	DM aerial part	F value table ($\alpha = 0.05$)
		cm^2	cm	cm	%	%	
Block	3	0.34^{ns}	0.70^{ns}	1.12^{ns}	0.18^{ns}	4.39^{ns}	3.01
Accession (A)	2	9.27^{*}	0.58^{ns}	0.08^{ns}	0.34^{ns}	11.50^{*}	3.40
Concentration (C)	2	140.28^{*}	22.78^{*}	83.86^{*}	0.13^{ns}	3.33^{ns}	3.40
A×C	4	3.91^{*}	0.55^{ns}	0.26^{ns}	0.23^{ns}	1.05^{ns}	2.78
Error	35						
CV, %		28.55	24.36	19.04	10.75	6.86	

Table 2 shows that 'Negra Oruro' presented the largest leaf area, being significantly different from the other accessions, while significant differences can be found from the different sources of variation (Table 3). This accession also presented the highest values of leaf area (Table 4), even in absence of NaCl and also for the concentration of 200 mM NaCl, showing significant differences with the other accessions. However, for 400 mM NaCl nonsignificant differences were found between any of the accessions. This is because at 400 mM the accessions present greater sensitivity in the development of their foliar primordia, being able to affect the expansion of the leaves by reducing the turgor pressure and extensibility of the cell wall (Fghire et al., 2015), due to the decrease in water potential related to a better adaptation. In addition, quinoa presents great genetic variability and has a high plasticity for tolerance to stress situations. Therefore, many of the existing accessions tolerate concentrations of 100 to 200 mM NaCl optimally (Table 2). With respect to the DM percentage of the plant, 'Sajama' showed the lowest value being significantly different from the other accessions (Table 2), which would indicate that first mentioned accession would have a greater resistance to the loss of water, this would explain why the accessions have different expression to adverse conditions (Hinojosa et al., 2018), so we could infer that the accessions would be influenced by salinity and that it would be expressing tolerance mechanisms in its morphology (Maestro-Gaitán et al., 2022).

With respect to root length and plant length, a decrease in values is observed as salinity levels increase (Table 3). This would be explained by the toxic impact of the NaCl used and the inequality of nutrient absorption (Hussain et al., 2020) and this was evidenced mainly by a reduction in plant height and root length at 400 mM (Terletskaya et al., 2023).

Table 2. Leaf area and percentage of aerial part DM in average concentration NaCl for quinoa accessions. Means ± standard deviation within the same column followed by different letters is significantly different ($P < 0.05$).

Accessions	Leaf area	DM aerial part
	cm^2	%
PECQ 20037	341.62 ± 317.96^b	10.64 ± 1.21^a
Sajama	260.81 ± 214.09^b	9.31 ± 0.29^b
Negra Oruro	434.05 ± 370.36^a	10.15 ± 0.72^a

Table 3. Leaf area, root length and plant height in different concentrations of NaCl. Means ± standard deviation within the same column followed by different letters is significantly different ($P < 0.05$).

NaCl	Leaf area	Root length	Plant height
	cm^2	cm	cm
0 mM	711.76 ± 197.86^a	31.84 ± 7.58^a	49.73 ± 8.09^a
200 mM	276.94 ± 114.44^b	27.54 ± 5.55^a	28.98 ± 4.51^b
400 mM	47.79 ± 9.37^c	15.64 ± 3.33^b	17.81 ± 3.40^c

Table 4. Leaf area (cm^2) in different concentrations of NaCl. Means ± standard deviation within the same column followed by different letters is significantly different ($P < 0.05$).

NaCl	PECQ 20037	Sajama	Negra Oruro	Accessions	0 mM	200 mM
0 mM	750.18 ± 124.36^a	520.08 ± 118.86^a	865.02 ± 177.99^a	PECQ 20037	750.18 ± 124.36^a	220.28 ± 54.15^b
200 mM	220.28 ± 54.15^b	214.52 ± 30.37^b	396.02 ± 125.63^b	Sajama	520.08 ± 118.86^a	214.52 ± 30.37^b
400 mM	54.42 ± 11.56^c	47.82 ± 5.57^c	41.12 ± 6.29^c	Negra Oruro	865.02 ± 177.99^a	396.02 ± 125.63^b

Effect of salt stress on metabolic variables and electrolyte leakage

Table 5 shows the significant differences of different sources of variation associated with metabolic variables such as chlorophyll *a*, chlorophyll *b*, and EL percentage in young and old leaves.

With respect to chlorophyll *b*, the highest value was found in 'PECQ 20037' and 'Negra Oruro' being significantly equal to each other, however, only 'Negra Oruro' showed to be significantly equal to 'Sajama', which obtained the lowest value. This is due to that chlorophyll decreases due to the destruction of chloroplasts by salt concentrations, affecting the synthesis of chlorophylls (Shu et al., 2013). In addition, Fghire et al. (2015) mentions that at high concentrations of salinity quinoa presents changes in chlorophyll content, which would be influencing the metabolism of each accession.

The EL (%) in young leaves is higher in 'PECQ 20037', showing a significant difference with the other accessions, however, for the EL (%) in old leaves, 'PECQ 20037' and 'Negra Oruro' showed the highest value, being significantly equal between them, but different from 'Sajama', which showed the lowest value, this is due to the fact that old leaves show greater damage to cell membranes and that each accession presents a different behavior to stress (Table 6). Likewise, it is inferred that the mechanisms to regulate the loss of

ions would be activated (Bajji et al., 2002; Restrepo et al., 2014). Likewise, it is observed that as salinity levels increase, EL (%) in young leaves increases, being significantly equal at 200 and 400 mM and different from the control treatment (Table 7). Tables 8 and 9 show that 'PECQ 20037' presented the highest values of EL (%) in young leaves when subjected to 200 and 400 mM NaCl, while 'Sajama' and 'Negra Oruro' showed the lowest values of EL (%) in young leaves, this possibly is because cell membranes cannot maintain their integrity and stability as NaCl content increases (Bajji et al., 2002), so it could be inferred that the changes in membrane permeability are essential for gene expression that confer tolerance; while EL (%) in old leaves of 'Sajama' was the one that showed the lowest value and 'PECQ 20037' showed the highest value of EL (%) in old leaves This is probably due to the fact that this accession presents disorganized membranes (Saddiq et al., 2021), while it can be inferred that the accessions that lose less electrolytes would be more tolerant to saline conditions, and that the results vary according to the accession since each one has a different degree of expression. In addition, Bajji et al. (2002) mentions that membrane stability is an important component for tolerance in plants, while in studies on quinoa salt vesicles are structures that would be contributing to salt tolerance (Ruiz et al., 2016), being able to infer that glands in the evaluated accessions provide mechanisms of tolerance, therefore avoiding the destruction of the membranes due to the NaCl contents in the medium in which they develop.

Table 5. Analysis of variance for chlorophyll and electrolyte leakage. EL: Electrolyte leakage; ^{ns}nonsignificant; *Significant at $P < 0.05$.

		F Value				F value table ($\alpha = 0.05$)
Source	DF	Chlorophyll *a*	Chlorophyll *b*	Young leaves EL	Old leaves EL	
		mg g^{-1} FW	mg g^{-1} FW	%	%	
Block	3	0.92^{ns}	0.45^{ns}	0.88^{ns}	0.47^{ns}	3.01
Accession (C)	2	2.94^{ns}	3.64*	20.30*	4.66*	3.40
Concentration (C)	2	2.88^{ns}	1.80^{ns}	52.26*	1.84^{ns}	3.40
A×C	4	3.70*	2.11^{ns}	18.76*	7.00*	2.78
Error	35					
CV, %		19.23	21.84	4.66	10.46	

Table 6. Chlorophyll *b*, percentage of electrolyte leakage (EL) in young and old leaves of quinoa accession. Means ± standard deviation within the same column followed by different letters is significantly different ($P < 0.05$).

Accessions	Chlorophyll *b*	Young leaves EL	Old leaves EL
	mg g^{-1} FW	%	%
PECQ 20037	0.12 ± 0.03^{a}	51.31 ± 8.23^{a}	67.60 ± 6.09^{a}
Sajama	0.10 ± 0.02^{b}	42.47 ± 11.45^{c}	58.97 ± 14.84^{b}
Negra Oruro	0.11 ± 0.02^{ab}	46.28 ± 4.51^{b}	70.34 ± 12.40^{a}

Table 7. Percentage of electrolyte leakage (EL) in young leaves in different concentrations of NaCl. Means ± standard deviation within the same column followed by different letters is significantly different ($P < 0.05$).

NaCl	EL young leaves
	%
0 mM	38.48 ± 8.90b
200 mM	50.19 ± 6.63a
400 mM	51.38 ± 5.29a

Table 8. Chlorophyll *a*, percentage of electrolyte leakage (EL) in young and old leaves in different concentrations of NaCl. Means ± standard deviation within the same column followed by different letters is significantly different ($P < 0.05$).

NaCl	Chlorophyll *a*	Young leaves EL			Old leaves EL	
	PECQ 20037	PECQ 20037	Sajama	Negra Oruro	Sajama	Negra Oruro
	mg g^{-1} FW	%			%	
0 mM	0.11 ± 0.02^{a}	41.78 ± 6.88^{b}	27.78 ± 1.11^{c}	45.88 ± 1.41^{b}	47.76 ± 7.89^{b}	72.95 ± 9.76^{a}
200 mM	0.09 ± 0.01^{a}	55.56 ± 3.29^{a}	53.08 ± 2.64^{a}	41.92 ± 1.63^{b}	54.48 ± 2.68^{b}	79.31 ± 11.95^{a}
400 mM	0.06 ± 0.01^{b}	56.58 ± 2.86^{a}	46.54 ± 3.56^{b}	51.03 ± 3.76^{a}	74.68 ± 14.66^{a}	58.77 ± 5.52^{b}

Table 9. Chlorophyll *a*, percentage of electrolyte leakage (EL) in young and old leaves of quinoa accession. Means ± standard deviation within the same column followed by different letters is significantly different ($P < 0.05$). EL: electrolyte leakage.

Accessions	Chlorophyll *a*	Young leaves EL			Old leaves EL	
	0 mM	0 mM	200 mM	400 mM	0 mM	200 mM
	mg g^{-1} FW	%			%	
PECQ 20037	0.11 ± 0.02^{a}	41.78 ± 6.88^{a}	55.56 ± 3.29^{a}	56.58 ± 2.86^{a}	64.40 ± 1.50^{a}	71.22 ± 10.00^{a}
Sajama	0.06 ± 0.00^{b}	27.78 ± 1.11^{b}	53.08 ± 2.64^{a}	46.54 ± 3.56^{b}	47.76 ± 7.89^{b}	54.48 ± 2.68^{b}
Negra Oruro	0.08 ± 0.02^{b}	45.88 ± 1.41^{a}	41.92 ± 1.63^{b}	51.03 ± 3.76^{b}	72.95 ± 9.76^{a}	79.31 ± 11.95^{a}

However, there are significant differences of the different sources of variation, with the exception of content of phenols, associated with metabolic variables such as content of proline, flavonoids, amino acids, saponins and protein percentage (Table 10). Likewise, the highest proline content was found in 'Negra Oruro', being significantly different from the other accessions (Table 11). In addition, Table 12 also shows that the highest proline value was found with a salt concentration of 400 mM, being significantly different from the other concentrations; however, at 0 and 200 mM there

was nonsignificant difference, this is because metabolites such as proline have a role against salt stress, and this accumulates as salinity increases (Kaur et al., 2022).

Table 10. Analysis of variance for metabolic variables. EAG: Equivalents of gallic acid; ^{ns}nonsignificant; *Significant at $P < 0.05$.

Source	DF	F value					Phenols	F value table ($\alpha = 0.05$)
		Proline	Flavonoids	Amino acids	Proteins	Saponins		
		µg mg^{-1} FW	mg mg^{-1} DW	mg g^{-1} DW	%	mg g^{-1} DW	mg EAG 100 g^{-1} DW	
Block	3	0.48^{ns}	1.91^{ns}	2.33^{ns}	0.92^{ns}	0.44^{ns}	1.39^{ns}	3.01
Accessions	3	13.35*	0.92^{ns}	38.73*	23.67*	126.33*	2.50^{ns}	3.40
C	2	74.54*	5.32*	51.10*	9.91*	79.70*	1.83^{ns}	3.40
A×C	2	1.32^{ns}	5.32*	23.71*	3.17*	11.70*	0.95^{ns}	2.78
Error	4							
CV, %	35	28.89	16.51	19.91	17.59	8.84	20.98	

Table 11. Proline, amino acids, proteins, and saponins of quinoa accession. Means ± standard deviation within the same column followed by different letters is significantly different ($P < 0.05$).

Accessions	Proline	Amino acids	Proteins	Saponins
	µg mg^{-1} FW	mg g^{-1} DW	%	mg g^{-1} DW
PECQ 20037	0.14 ± 0.11^{b}	25.69 ± 15.32^{c}	14.93 ± 3.88^{a}	2.60 ± 1.04^{c}
Sajama	0.16 ± 0.12^{b}	39.29 ± 16.28^{b}	12.90 ± 2.28^{b}	4.70 ± 1.00^{a}
Negra Oruro	0.24 ± 0.13^{a}	54.06 ± 28.17^{a}	8.97 ± 2.19^{c}	3.67 ± 0.33^{b}

Table 12. Proline, flavonoids, amino acids, proteins, and saponins in different concentrations of NaCl. Means ± standard deviation within the same column followed by different letters is significantly different ($P < 0.05$).

NaCl	Proline	Flavonoids	Amino acids	Proteins	Saponins
	µg mg^{-1} FW	mg mg^{-1} DW	mg g^{-1} DW	%	mg g^{-1} DW
0 mM	0.12 ± 0.06^{b}	0.15 ± 0.03^{a}	40.89 ± 19.06^{b}	14.04 ± 4.29^{a}	3.00 ± 1.11^{c}
200 mM	0.09 ± 0.06^{b}	0.13 ± 0.03^{ab}	55.34 ± 26.01^{a}	12.58 ± 3.69^{a}	3.37 ± 0.96^{b}
400 mM	0.33 ± 0.08^{a}	0.12 ± 0.03^{b}	22.81 ± 10.63^{c}	10.16 ± 2.17^{b}	4.60 ± 0.97^{a}

The content of flavonoids at 0 mM NaCl presents the highest value (Table 12), being significantly equal to 200 mM NaCl but significantly different from 400 mM. Similarly, in Table 13 it is observed that in 'PECQ 20037' the highest value of flavonoids was given at 0 mM showing a decrease in amount as the concentration of NaCl increases. The PECQ 20037 accession showed the highest content of flavonoids (Table 14); however, at 200 mM there were nonsignificant differences between accessions and at 400 mM flavonoid concentration diminished in 'PECQ 20037' to lower value of flavonoids compared to 'Sajama'. This is explained by the genetic factor and the environment in which it develops, producing metabolic changes induced by salinity (Nguyen et al., 2020).

Table 13. Flavonoids, amino acids, proteins and saponins in different concentrations of NaCl. Means ± standard deviation within the same column followed by different letters is significantly different ($P < 0.05$).

NaCl	Flavonoids	Amino acids			Proteins	Saponin		
	PECQ 20037	PECQ 20037	Sajama	Negra Oruro	PECQ 20037	PECQ 20037	Sajama	Negra Oruro
	mg mg^{-1} DW	mg g^{-1} DW			%	mg g^{-1} DW		
0 mM	0.18 ± 0.04^{a}	20.63 ± 6.46^{b}	57.89 ± 12.09^{a}	44.14 ± 14.06^{b}	18.70 ± 1.51^{a}	1.58 ± 0.12^{c}	3.76 ± 0.60^{c}	3.67 ± 0.26^{ab}
200 mM	0.13 ± 0.03^{b}	44.39 ± 7.51^{a}	32.32 ± 2.51^{b}	89.30 ± 3.76^{a}	15.50 ± 2.85^{b}	2.28 ± 0.16^{b}	4.48 ± 0.35^{b}	3.36 ± 0.14^{b}
400 mM	0.11 ± 0.02^{b}	12.04 ± 3.72^{b}	27.64 ± 10.62^{b}	28.73 ± 7.43^{c}	10.57 ± 0.11^{c}	3.94 ± 0.22^{a}	5.86 ± 0.42^{a}	4.00 ± 0.22^{a}

Table 14. Flavonoids, amino acids, proteins and saponins of quinoa accession. Means ± standard deviation within the same column followed by different letters is significantly different ($P < 0.05$).

Accessions	Flavonoids		Amino acids			Protein		Saponin		
	0 mM	400 mM	0 mM	200 mM	400 mM	0 mM	200 mM	0 mM	200 mM	400 mM
	mg mg^{-1} DW		mg g^{-1} DW			%		mg g^{-1} DW		
PECQ 20037	0.18 ± 0.04^{a}	0.11 ± 0.02^{b}	20.63 ± 6.46^{c}	44.39 ± 7.51^{b}	12.04 ± 3.72^{b}	18.70 ± 1.51^{a}	15.50 ± 2.85^{a}	1.58 ± 0.12^{b}	2.28 ± 0.16^{c}	3.94 ± 0.22^{b}
Sajama	0.13 ± 0.01^{b}	0.15 ± 0.02^{a}	57.89 ± 12.09^{a}	32.32 ± 2.51^{c}	27.64 ± 10.62^{a}	13.47 ± 3.16^{b}	13.67 ± 1.87^{a}	3.76 ± 0.60^{a}	4.48 ± 0.35^{a}	5.86 ± 0.42^{a}
Negra Oruro	0.14 ± 0.02^{b}	0.11 ± 0.01^{b}	44.14 ± 14.06^{b}	89.30 ± 3.76^{a}	28.73 ± 7.43^{a}	9.96 ± 1.89^{c}	8.57 ± 1.94^{b}	3.67 ± 0.26^{a}	3.36 ± 0.14^{b}	4.00 ± 0.22^{b}

'Negra Oruro' has the highest value of amino acids (Table 11), showing a significant difference with the other accessions; however, for the control treatment, the highest amino acid value was obtained by 'Sajama', being significantly different from 'Negra Oruro' and 'PECQ 20037' which showed the lowest value (Table 14). Nevertheless, for 200 and 400 mM, 'Negra Oruro' showed the highest content of amino acids, evidencing an increase as the level of salinity increases, in the same way in Tables 12 and 13 it is observed that the highest value of amino acids occurred at 200 mM and that 'Negra Oruro' obtained its highest value, this is due to the water potential of the plant (Al-Naggar et al., 2018) causing metabolic changes in the accessions, as a response to adaptation in an adverse condition by means of synthesis and accumulation of organic solutes.

'PECQ 20037' present the highest protein percentage (Table 11), showing a significant difference with the other accessions; however, at 0 and 200 mM, 'PECQ 20037' showed the highest protein percentage (Table 14), being under this last concentration significantly equal to 'Sajama', while at 400 mM nonsignificant differences were observed in terms of protein percentage for the different accessions. Similarly, in Tables 12 and 13 it is observed that the protein percentage decreases as the salinity level increases, this is due to the osmotic stress caused by concentrations 200 and 400 mM that causes protein degradation. In addition, tolerance mechanisms would generate osmoprotective compounds that would reduce protein degradation, being able to infer that at 400 mM there would be osmoprotectors such as proline that would be preventing the total degradation of proteins (Hamilton and Heckathorn, 2001). Likewise, there are genes such as dehydrins that would be induced by the NaCl content and environmental factors (Burrieza et al., 2017), so protein content would be variable in the different accessions.

The highest saponin value was found with 'Sajama', showing a significant difference from the other accessions (Table 11), in addition, in the Table 14 also shows that for all NaCl concentrations, 'Sajama' showed the highest saponin values, likewise, Tables 12 and 13 show that the level of saponins increases as the salinity level increases, showing significant differences between those levels, this is due to the fact that

the NaCl content affects the presence and availability of water (Hussain et al., 2020), stimulating the metabolic expression of the saponin content, being able to infer that depending on the accession and environment, metabolic effects are generated by increasing the saponin content, corroborating what was mentioned by Gómez-Caravaca et al. (2012).

Pearson correlation

According to Pearson's correlation analysis (Table 15), the root length (RL) variable is correlated with leaf area (LA) (r = 0.71); and plant height (PH) (r = 0.84) is positively correlated with LA (r = 0.84) and RL (r = 0.83). Similar results were found in a study carried out on maize, where there was a positive correlation between LA and PH (Ogunniyan and Olakojo, 2014). Likewise, it is observed that fresh weight (FW_P) is positively correlated with LA (r = 0.89), RL (r = 0.73) and PH (r = 0.91).

Table 15. Pearson correlation of three quinoa accessions between morphological, physiological and biochemical variables under salt stress conditions. LA: Leaf area (cm^2); RL: root length (cm); PH: plant height (cm); FW_P: fresh weight (g); DW_P: dry weight (g); CHL_1: total chlorophyll 15 d after the stress (mg g^{-1} FW); CHL_2: total chlorophyll 42 d after stress (mg g^{-1} FW); EL_YL: electrolyte leakage in young leaves (%); EL_OL: electrolyte leakage in old leaves (%); PHE: phenols (mg EAG 100 g^{-1} DW); PROL: proline (µg mg^{-1} FW); FLAV: flavonoids (mg mg^{-1} DW); SAP: saponins (mg g^{-1} DW); AMIN: amino acids (mg g^{-1} DW); PROT: proteins (%).

	LA	RL	PH	FW P	DW P	CHL 1	CHL 2	EL YL	EL OL	PHE	PROL	FLAV	SAP	AMIN	PROT
LA	1	0.71**	0.84**	0.89**	0.89**	-0.40	0.22	-0.48	0.05	0.21	-0.49	0.44**	-0.50	0.21	0.28
RL		1	0.83**	0.73**	0.70**	-0.27	0.19	-0.28	-0.03	0.33	-0.59	0.30	-0.48	0.25	0.43**
PH			1	0.91**	0.88**	-0.34	0.19	-0.53	-0.17	0.19	-0.59	0.36*	-0.48	0.16	0.44**
FW P				1	0.99**	-0.40	0.32	-0.39	-0.04	0.23	-0.59	0.46**	-0.63	0.04	0.53**
DW P					1	-0.40	0.34*	-0.36	0.02	0.22	-0.58	0.47**	-0.65	0.04	0.51**
CHL 1						1	-0.34	0.18	0.04	0.06	0.29	-0.49	0.32	-0.09	-0.34
CHL 2							1	0.05	0.19	0.18	-0.24	0.24	-0.55	0.07	0.27
EL YL								1	0.23	0.05	0.17	-0.20	0.07	-0.43	-0.11
EL OL									1	0.04	0.06	-0.09	-0.02	0.23	-0.20
PHE										1	-0.15	-0.07	-0.33	0.12	0.21
PROL											1	-0.36	0.47**	-0.24	-0.54
FLAV												1	-0.3	-0.08	0.43**
SAP													1	-0.09	-0.47
AMIN														1	-0.26
PROT															1

The dry weight of the plant (DW_P) is positively correlated with LA (r = 0.89), RL (r = 0.70), PH (r = 0.88) and FW_P (r = 0.99). Chlorophyll at 42 d after stress presents a correlation with DW_P (r = -0.34). Aycan et al. (2021) carried out a study on salinity in wheat about physiological response during development, they found reduced DM production related to a photosynthetic activity.

The electrolytes leakage in young leaves (EL_YL) is negatively correlated with the variables LA (r = -0.48), RL (r = -0.28), PH (r = -0.53), FW_P (r = -0.39) and DW_P (r = -0.36); and proline (PROL) content is negatively correlated with LA (r = -0.49), RL (r = -0.59), PH (r = -0.59), FW_P (r = -0.59) and DW_P (r = -0.58); Singhal et al. (2021) in a study of salinity in wheat found a negative correlation between PH and PROL.

The flavonoids (FLAV; mg mg^{-1} DW) showed a correlation with LA (r = 0.44), PH (r = 0.36), FW_P (r = 0.46) and DW_P (r = 0.47). In a study carried out by Le et al. (2021) found that there is a correlation with FLAV and PH.

Amino acids (AMIN) are negatively correlated with EL_YL (r = -0.43) and the content of protein (PROT) is positively correlated with RL (r = 0.43), PH (0.44), FW_P (0.53), DW_P (0.51) and FLAV (r = 0.43).

Saponins (SAP) correlates positively with PROL (r = 0.47), and negatively with morphological variables, phenols (PHE) (r = -0.33) and FLAV (r = -0.30), which is similar to what was found by Lopez et al. (2016) where mineral nutrition influenced the content of some metabolites, being able to infer that salinity would be affecting the assimilation of nutrients.

Multivariate analysis

The principal component analysis (PCA) (Figure 1) showed that the first two dimensions explain 56.0% of the variance, PC1 represents around 43.9% of the total variance and PC2 represents 12.1% of the total variance, being the most significant.

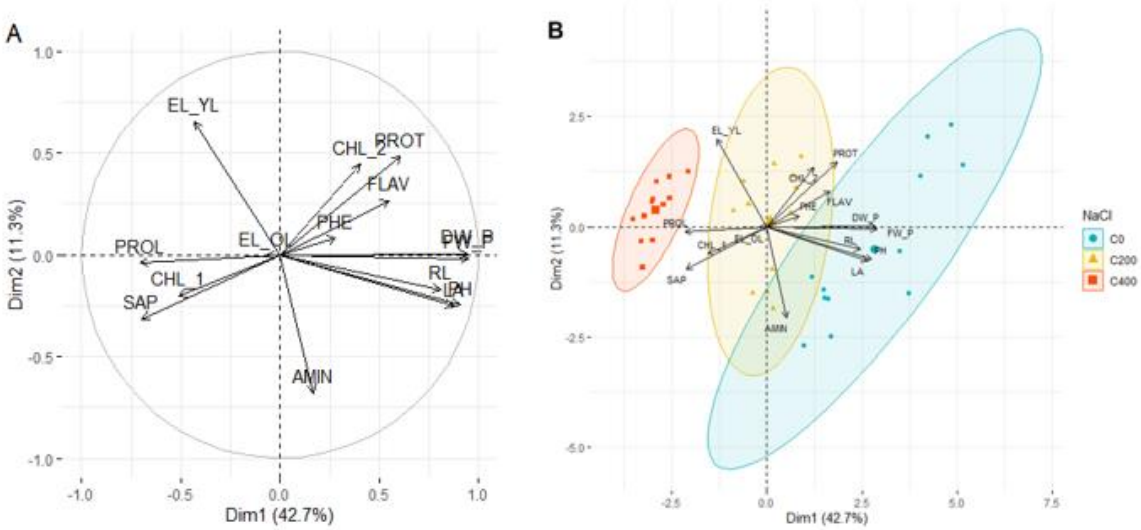

Figure 1. Principal component analysis (PCA) of the measured variables of three quinoa accessions under salt stress conditions. (A) PCA for the variables. (B) PCA for the concentrations. LA: Leaf area (cm^2), RL: root length (cm), PH: plant height (cm), FW_P: fresh weight (g), DW_P: dry weight (g), CHL_1: total chlorophyll 15 d after the stress (mg g^{-1} FW), CHL_2: total chlorophyll 42 d after stress (mg g^{-1} FW), EL_YL: electrolyte leakage in young leaves (%), EL_OL: electrolyte leakage in old leaves (%), PHEN: phenols (mg EAG 100 g^{-1} DW), PROL: proline (µg mg^{-1} FW), FLAV: flavonoids (mg mg^{-1} DW), SAP: saponins (mg g^{-1} DW), AMIN: amino acids (mg g^{-1} DW), PROT: proteins (%).

Therefore, we can indicate that the variables FW_P, DW_P, PH, LA, RL, PROL and SAP provide more data to the main component 1; and AMIN, EL_YL, PROT and total chlorophyll 42 d after stress (CHL_2) provide more data for component 2.

The variables DW_P, FW_P, PH, RL, AMIN are inversely proportional to EL_YL, indicating that the higher the fresh and dry weight of leaves, the lower EL_YL. It would also indicate that the higher the AMIN, the lower EL_YL, this would correspond to the fact that amino acids constitute proteins and these in turn fulfill specific functions in the membrane, being able to infer that the increase in amino acids contributes to the stability of the membrane, in addition to being subjected to a osmotic stress by saline concentrations this causes changes in the membrane (Al-Naggar et al., 2018). Likewise, it is mentioned in a study carried out by Zhang et al. (2022), that the response to NaCl in the membrane causes a rapid denaturation of proteins in the membranes in response to osmotic stress, giving a rapid response and allowing the cell to remain

alive, while quinoa, being a halophyte plant, has tolerance mechanisms within which salt bladders are mentioned, which would help in the process of storage and excretion of excess salts (Zhang et al., 2022). Therefore, we speculate that an osmotic regulation would be taking place in the different accessions subjected to NaCl concentrations. Likewise, it could be inferred that the greater the EL, the lower the AMIN, which suggests that the greater the saline stress, the membranes would be destroyed, causing EL.

While it is also observed (Figure 1.A) that there is a correlation with the variables PHEN, FLAV, PROT and CHL_2, these being inversely proportional with total chlorophyll 15 d after the stress (CHL_1), SAP and PROL, that is, the lower the content of phenols, flavonoids, protein and total chlorophyll will be higher the SAP and PROL content, while it is observed that the correlation is positive with the chlorophyll content (15 d) and turns out to be negative with a longer elapsed time (45 d), due to the effect of the incremental concentrations of salts over time (Table 15).

The existing relationship between the secondary metabolites suggests that in saline stress they tend to accumulate to counteract the osmotic potential. Thus, in Figure 1 it is observed that the contents of phenols, flavonoids and proteins present higher expression in 200 mM NaCl, being able to infer that in saline stress the plant modulates the levels of secondary metabolites to protect itself, likewise Sytar et al. (2018), mentions that secondary metabolites can participate in the balance of the oxidative state of the plant, acting as antioxidants in the development of responses to salt stress. On the other hand, Lin et al. (2019) mentions that a great variety of secondary metabolites has been identified and each one plays a different or shared role in physiological and biochemical processes. Meanwhile, we can speculate that the content of metabolites found is expressed according to genetic variability and the conditions in which it develops.

In the figure on the right side, it can be seen that the variables SAP, PROL, CHL_1, EL_YL and EL in old leaves (EL_OL) have greater expression at 200 and 400 mM NaCl, and the variables DW_P, FW_P, RL, PH and LA have greater expression at 0 mM NaCl. Likewise, it is observed that the content of proline and saponins tend to be better expressed at 400 mM NaCl, so we can infer that these metabolites are influenced by the presence of salts, similar to what was described by Xu and Fu (2020).

CONCLUSIONS

Considering the morphological and metabolic traits evaluated under salt stress, it is concluded that the quinoa morphological variables were affected by salt stress. Likewise, in metabolic variables it was observed that there is a correlation between the loss of electrolytes and the content of amino acids related to the stability of the membrane under salt stress conditions, while the contents of flavonoids, amino acids, proteins, and chlorophyll are inversely proportional to the contents of proline and saponins, likewise the contents of these secondary metabolites increase according to the NaCl content, being an indicator of tolerance in the quinoa accessions, it could also be pointed out that there was greater tolerance at 200 mM NaCl. The PECQ 20037 and Negra Oruro accessions showed tolerance to salinity in the growth and vegetative development stage, so these two accessions, under greenhouse conditions, would have promising behavior to be considered in programs of genetic improvement of tolerance to salinity.

Author contributions

Conceptualization: C.F.L., M.E.M.A. Methodology: C.F.L., LGP, L.L.M., C.A.J. Data curation: C.F.L., O.Z. Software: O.Z., R.G.R., C.F.L. Formal analysis: O.Z., R.G.R., C.F.L. Investigation: C.F.L. Funding acquisition: M.E.M.A. Resources: C.F.L., M.E.M.A., L.L.M. Validation: C.F.L., M.E.M.A. Writing-original draft preparation: C.F.L. and R.G.R. Review and editing: C.F.L., O.Z., M.E.M.A., R.G.R., J.C.A., C.A.J., E.N.J., C.Z. All authors have read and agreed to the published version of the manuscript.

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