Physiological and Biochemical Responses of Quinoa (*Chenopodium Quinoa* Willd) Varieties to Salinity Stress

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ABSTRACT: *Quinoa (Chenopodium quinoa Willd) is recently introduced to Iran as a salt-tolerant crop of high nutritional value. To investigate the physiological and biochemical responses of three quinoa varieties ('NSRCQE', 'NSRCQB', and 'Titicaca') were compared at ≤ 2 as control, 10, 17 dS/m saline water in an experimental farm of Yazd Province, Iran in 2017-2018. This experiment was conducted as a split-plot based on a randomized complete block design with three replications, whereas the salinity treatment was in the main plots and the varieties in the subplots. Salinity and varieties significantly affected DPPH radical scavenging activity, phenol, anthocyanin, flavonoid, and Malondialdehyde (MDA) contents, accumulation of Na^+ and K^+, and Na^+/K^+ ratio, as well as seed protein and saponin contents. Salinity was caused by increasing DPPH radical scavenging activity, phenol, anthocyanin, flavonoid, and MDA contents, and accumulation of Na^+ in the leaves and seeds. 'NSRCQB' had the highest average of most measured traits under all salinity levels. The DPPH radical scavenging activity in leaves was significantly and positively correlated with phenol content, anthocyanin content, flavonoid content, MDA, Na^+ accumulation in the leaves, and DPPH activity, protein content, and Na^+ accumulation in the seeds. Results indicated that the salinity stress increased the amount of paracomaric, quercetin acid, and camphor acids in the leaves and seeds of quinoa; also, the highest amount of these compounds was found by 'NSRCQB', also, 'NSRCQE' had the lowest average of most of these compounds. Based on these findings, we conclude that the salt tolerance of quinoa grown on salt-affected soils of Yazd, Iran was linked with better crop stand establishment, low Na^+ accumulation in leaves as well as increased activities of enzymatic and non-enzymatic antioxidants, also, 'NSRCQB' variety showed the best potential under salinity conditions.*

KEYWORDS: *DPPH radical scavenging activity, Flavonoid content, Na^+/K^+ ratio, Phenol content, Salt-tolerant crop, Seed protein.*

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INTRODUCTION

Salinity is one of the major threats to crop production, especially in arid and semi-arid regions of the world. Increasing salinity is a major cause of land degradation over the world, 7% of the total land area is salt-affected while sodic soils are even more prominent [13]. In Iran, more than 800 Mha of land throughout the world are salt-affected, with estimated to vary between 16 and 23 Mha [1]. The term salinity implies a high concentration of salts in the soil. It is NaCl that constitutes most the soil salinity. That is why all plants have evolved several mechanisms to regulate NaCl accumulation or exclusion [1]. Face this situation, utilizing salt-tolerant halophytes directly in agriculture as a potential source of new crops or genotypes is a solution to expanding the limited agricultural area in the arid and semi-arid regions of the world [6].

Quinoa (*Chenopodium quinoa* Willd.) a seed crop native to the Andean mountain, is one of the most promising halophyte plants that has high potential as a human food source and high tolerance to salinity stress with high nutritional quality that traditionally called the mother of grains [19]. The interest in the crop is increasing globally, both due to its superior nutritional profile and its stress tolerance. Quinoa grain is gluten-free, rich in essential amino acids, vitamins (A, B_2, and E), minerals (K, Ca, Fe, and Mn), oil containing large amounts of linoleate, and natural antioxidants, polyunsaturated fatty acids such as omega 3 and 6, and carbohydrates [29]. The crop has begun to be known as the grain of the 21st century' due to its high protein content with a balanced presence of essential amino acids [10]. Quinoa has recently been received as an alternative crop in arid and semi-arid agriculture worldwide [3]. In Iran, there is considerable attention to the quinoa plant and recently been tested successfully in many different parts of the country [26].

The salinity threshold of quinoa varies during its growth. Quinoa is very sensitive to salinity at the seedling stage, but the sensitivity is reduced by growth and is at the minimum level at the flowering stage. The salinity threshold value of quinoa in sandy loam soil is measured at 8, 20, and 15 dS/m at each seedling establishment, flowering, and seed-filling growth stages, respectively [16]. Quinoa tolerates salinity stresses depending on the genotype [29].

González et al. (2011) [20] reported that under abiotic stress conditions, the growth and grain yield of quinoa is dependent mainly on the soil conditions, but genotypic variations related to growth and grain yield have also been reported. Some quinoa genotypes can grow well at 100 mM NaCl [8]. However, higher salinity considerably reduced plant growth, with a maximum reduction of 82% observed at 500 mM NaCl [8]. High adaptability to soil saline stress may be achieved at the early development stage by improved metabolic regulation based on ion absorption to adjust water potential in tissues and limit transpiration under saline conditions [11]. Besides, *Rosa et al*. (2009) [20] found that quinoa can maintain Ca^{2+}/Na^+ and K^+/Na^+ selectivity under saline stress. This evidence suggests that quinoa can be a good candidate for a cropping system under soil salinization and salinity tolerance studies [25].

Salt stress induces ionic toxicity and osmotic stress which adversely affect plant physiology, growth, and development. Salinity perturbs cytosolic enzymatic activity and may cause oxidative damage and nutritional imbalance, all of which eventually lead to a significant reduction in crop yield [9]. *Waqas et al*. (2017) [28] reported that the concentration of proline and soluble sugars increased in root and leaf tissues under salinity. Also, salinity stress-induced oxidative damage by increasing lipid peroxidation (MDA) levels in roots and more specifically, in leaf tissues. *Hariadi et al*. (2012) [11] reported a 5-fold increase in salinity level (from 100 to 500 mM) resulted in only a 50% increase in the sap Na^+ content, suggesting either a very strict control of xylem Na^+ loading or an efficient Na^+ removal from leaves.

One major aspect of plant adaptation to saline environments is the utilization of a massive accumulation of inorganic ions (mainly Na^+ and Cl^-) to osmotically adjust [8]. Osmotic adjustment in terms of salt accumulation is energetically efficient but requires a combination of several tolerance and/or avoidance strategies that act in concert to avert ion toxicity and imbalance [14]. Cai and Gao (2020) [5] reported that substantial variations were found in plant size (biomass) and overall salinity tolerance (plant biomass in salt treatment as % of control) among the different quinoa cultivars. Also, by increasing salinity levels, leaf superoxide dismutase activity and lipid peroxidation generally increased, but catalase and peroxidase activities showed non-linear patterns. Organic solutes (soluble sugar, proline, and protein) accumulated in leaves, whereas inorganic ions (Na^+ and K^+) increased but K^+/Na^+ decreased in both leaves and roots.

Table 1: Physico-chemical characteristics of soil

EC	Na	Mg	Ca	Cl	P	K	Zn	Mg	Fe	Cu	N	Organic carbon
(dS/m)	(mEq/l)				µg/g						%	
1.675	106.74	27.22	19.89	125.31	11.1	136	1.34	1.07	4.49	0.05	0.24	0.97

Some varieties can grow in salt concentrations similar to those found in seawater and even higher, well above the threshold for any known crop species. The physiological mechanisms behind this remarkable abiotic stress tolerance remain unclear. Despite an apparent interest from agronomists, the underlying physiological mechanisms conferring this tolerance are still not understood [11]. Furthermore, a few field studies have been reported related to the physiological and biochemical responses of quinoa to NaCl stress that are available, especially in Iran. In the light of above, this experiment aimed to investigate the physiological and biochemical responses of quinoa varieties ('NSRCQE', 'NSRCQB', and 'Titicaca') to salinity stress (control, 10, and 17 dS/m saline water) conducted.

EXPERIMENTAL SECTION

Plant materials and experimental design

The present study was conducted in an experimental farm in Hosseinabad, Yazd province, Iran (1134 m above sea level, the latitude of 32° 03′ 15″ N and longitude of 54° 14′ 11″ E) during the growing seasons in 2017–18 to evaluate the physiological and biochemical responses of quinoa varieties ('NSRCQE': V1, 'NSRCQB': V2, and 'Titicaca': V3) to saline water stress (2±0.5 as control, 10±2, and 17±2 dS/m). This experiment was conducted as a split plot based on a randomized complete block design (RCBD) with three replications, whereas the salinity treatment was in the main plots and the varieties in the ub-plots. Soil analysis results are demonstrated in Table 1. Based on soil analysis results, the supply of the elements (NPK) was conducted during planting.

Field preparation operations such as plowing, disc, and the like were conducted before planting. The size of each plot was 2 × 3 m (with five rows of cultivars). The distance between blocks and the distance plot in each block were 2 and 1 m, respectively. Seeds were sown at depths of 1-2 cm on 6 August. Then, the first irrigation was given after sowing and the second was performed after 3 days with low saline water (≤2 dS/m). The saline irrigation method was applied in the experiment and the proposed method of *Shahverdi et al*. (2018) [23] which was performed on stevia plants, was used to irrigate with salt ware. The rate of daily water consumed was calculated to fill the soil profile of the farm to a depth of 0.5 m in terms of field capacity.

Malondialdehyde assay

The malondialdehyde (MDA) is a lipid peroxidation product and in measured by estimating the MDA content by thiobarbituric acid (TBA) reaction. A 1 mL aliquot of the supernatant and 4 mL trichloroacetic acid (20%) containing 0.5% TBA were mixed. The mixture was heated for 30 min at 95 °C, quickly cooled, and then centrifuged for 10 min at 10000 rpm. The MDA contents were assessed Eq. (1) [7].

$$MDA = 6.45 \times (A532\text{-}A600) - 0.56 \times A450 \quad (1)$$

Na^+ and K^+ contents in leaves and seeds

The measurement of Na^+ and K^+ contents in leaves and seeds was performed using flame photometry. Using the standard curve in this method, Eq. (2) calculated Na^+ and K^+ contents in different organs [1].

$$A = y \times 100/1000 \times 1000/w \quad (2)$$

Whereas y is: the number of achieve the curve (mg/kg); A: is Na^+ or K^+ content; w: is primary dry weight (g).

Saponin analysis

Saponin analysis was performed on defatted flour. The defatted sample was hydrolyzed under reflux for 3 h with a methanolic solution of hydrochloric acid. After hydrolysis, sapogenins were extracted with ethyl acetate (3 × 20 mL). The ethyl acetate extracts were combined and then derivatized with dry pyridine and bis (trimethylsilyl) trifluoroacetamide and derivatized for 20 min at 50 °C. Total sapogenins were estimated by the Gas-Chromatographic (GC) method described by [20] with slight modification. The derivatized samples were the injected into a Perkin Elmer GC (Perkin-Elmer, Norwalk, CT, USA) fitted with a capillary column (ZB-5HT; Phenomenex, Torrance, CA, USA). The temperatures of

the injector and detector (FID) were set to 370 °C whereas the oven temperature was programmed with increments of 8 °C min) 1 from 180 to 230 °C and with increments of6 °C min) 1 from 230 to 350 °C.

Total soluble protein content

Total soluble protein content was measured according to the method of [4] Fresh samples (100 mg) were homogenized with ice-cold sodium phosphate buffer (50 mM, pH 7.2) containing 1 mM EDTA. Na_2 and 2% (w/v) PVPP and then centrifuged at 13.000 × g for 40 min at 4 °C. The supernatant was collected and stored in small aliquots at -80 °C. The supernatant was mixed with Bradford reagent (B6916) and incubated thereafter in the dark for 5 min for protein quantification. The absorbance was measured at 595 nm using a UV/VIS spectrophotometer. Soluble protein concentration in enzyme extract was estimated according to [4] using bovine serum albumin (BSA) as standard.

DPPH radical scavenging activity

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity was determined according to the method described by [12]). The reaction mixture (total volume 3 mL), consisting of 0.5 mL of 0.5 M acetic acid buffer solution at pH 5.5, 1 mL of 0.2 mM DPPH in ethanol, and 1.5 mL of 50% (v/v) aqueous ethanol solution, was shaken vigorously with various samples. After incubation at room temperature for 30 min, the remaining DPPH was determined by absorbance at 517 nm, and the radical scavenging activity of each sample was expressed using the ratio of the absorption decrease of DPPH (%) to that of the control DPPH solution (100%) in the absence of the sample. The radical scavenging activity was calculated as (%) =100(A-B)/A, where A and B are the 517 nm absorption of the control and the corrected absorption of the sample reaction mixture.

Total phenol content

The total phenol content was spectrophotometrically measured using the Folin-Ciocalteu method described previously [22], with slight modifications. Cotyledons (50 mg fresh weight) were homogenized with 500 µl of 90% methanol. The sample was then centrifuged at 10,000 × g for 5 min. The supernatant (20 µl) was diluted with 680 µl of distilled water, and 50 µl of phenol reagent was mixed. After adding 300 µl of 5% sodium carbonate, the mixture was incubated at 25 °C for 30 min in the dark. The absorbance of the supernatant was measured at 765 nm, and a standard curve was prepared using gallic acid.

The anthocyanin content

The anthocyanin content was spectrophotometrically measured as described previously [21] Fresh cotyledons were promptly dried in an oven at 90 °C for 1 day. Dried cotyledons were weighed (about 15 mg) and soaked in 1 mL of methanol containing 1% HCl and were incubated at 95 °C for 15 min. The sample was then cooled to room temperature. After removing the cotyledons, the absorbance of the supernatant was measured at 533 nm, and a standard calibration curve was prepared using cyanidin-3-glucoside.

Determination of total flavonoid

The total flavonoid content of extracts from leaves of quinoa with different levels of salinity stress was determined by the method of [2]. A 0.5 mL aliquot of 20 g/l $AlCl_3$ ethanolic solution was added to 0.5 mL of extract solution. After 1 h at room temperature, the absorbance at 240 nm was measured. The yellow color indicated the presence of flavonoid. Extract samples were evaluated at a final concentration of 0.1 mg/mL, Total flavonoids content expressed as Eq (mg Eq /g Dry Weight (DW)).

Analyses of phenolic and flavonoid acids by HPLC

To measure gallic acid content, chlorogenic acid, caffeic acid, paracomaric acid, quercetin acid, and camphor acid in the sample, 20 µl of phenolic and flavonoid extracts were extracted after concentrating with a rotary evaporator and passing a separate 0.2-micron filter into the HPLC column. By comparing the delay time and subsurface level of the sample with standard samples at a wavelength of 280 nm, the amount of phenolic and flavonoid acids was identified and determined [27].

Statistical analysis

Results were expressed as mean value ± standard deviation of two extractions. A two-way analysis of variance (ANOVA) was used to compare the means. Differences were considered significant at $P \leq 0.05$. All statistical analyses were performed with the Statistical Analysis System software (SAS Institute, Cary, NC, USA, Version (9.2). A comparison of means of the components were performed by two-way analysis

Table 2: Analysis of variance the effect of salinity stress on leaves physiological traits of three seedlings of quinoa (Chenopodium quinoa Willd) varieties

SOV	df	Mean square (MS)							
		DPPH	Phenol	Anthocyanin	Flavonoid	MDA	Na^+	K^+	Na^+/K^+ ratio
Block	2	30.04 ns	57.80 ns	366.5 ns	2.88 ns	0.30 ns	20.98 ns	5.41 ns	0.0003ns
Salinity (S)	2	6544.9**	4497.1**	384705.0**	1293.6**	77.36**	1018.30**	71.14**	0.065**
Block × S	4	19.20	62.40	571.9	13.97	0.17	23.49	0.31	0.0003
Varieties (V)	2	227.8*	263.1**	2797.2*	17.12 ns	2.44**	54.57*	58.59**	0.009**
S × V	4	63.64 ns	19.96 ns	995.3 ns	7.04 ns	0.66**	203.4**	14.24*	0.005**
Error	12	40.38	32.05	501.2	8.03	0.11	8.67	2.94	0.0006
CV (%)	-	14.40	8.24	5.29	13.86	8.42	4.11	7.89	8.13

ns: non-significant; * and **: significant at 5 and 1% probably levels, respectively

of variance with LSD test. A comparison of assays was made by correlation and linear regression analysis. Differences were considered significant if $P \leq 0.05$.

RESULTS AND DISCUSSION

DPPH radical scavenging activity

The antioxidant activity of the lipophilic compounds in quinoa seeds and leaves was assessed using the DPPH method. Results indicated that the antioxidant activity was significantly different under salinity stress and quinoa varieties in seeds and leaves (Tables 4 and 5). The highest DPPH activity in leaves and seeds (71.1% and 15.9%, respectively) was observed under severe salinity stress (17 dS/m), and the lowest activity by free salinity stress (control) in both organs (Table 5). 'NSRCQB' showed the highest DPPH activity in leaves and seeds (49.5% and 11.4%, respectively), and 'NSRCQE' had the lowest activity in both organs (39.6% and 9.90%, respectively) (Table 5).

Phenol content

The effect of salinity and varieties were significant on phenol content (Table 3). Salinity stress-induced significantly increased the content of phenol in the leaves. The highest phenol content (91.1 mg/g gallic acid) was achieved under severe stress (17 dS/m) and 'NSRCQB' variety (47.0 mg/g gallic acid) and the lowest content (45.5 and 63.2 mg/g gallic acid) by free salinity stress and 'NSRCQE' (Table 5).

Anthocyanin content

Anthocyanin content was changed significantly by the salinity stress and variety treatments (Table 2). Salinity significantly increased anthocyanin content by 65.4% compared to the control. As shown in Table 4, the highest anthocyanin content was observed under severe stress and 'NSRCQB' (633.3 and 439.7 µg/g DW) and the lowest content by free stress conditions and 'NSRCQE' (218.5 and 404.5 µg/g DW).

Flavonoid content

The results showed that the effect of salinity was significant on flavonoid content ($P \leq 0.01$) (Table 2). Salinity stress significantly enhanced the content of flavonoids. Flavonoids ranged from 7.07 mg/g quercetin in non-stressed conditions to 30.2 mg/g quercetin during severe stress conditions (Table 4).

MDA content

Different experimental treatments, including salinity and quinoa varieties as well as their interactions significantly affected MDA (Table 2). As shown in Table 6, the results indicated that salinity was caused by increasing MDA content in all varieties. The content of MDA ranged from 1.05 mmol/g DW by 'NSRCQE' under non-stressed conditions to 7.98 mmol/g DW by 'NSRCQB' under severe stress (17 dS/m).

Contents of Na^+ and K^+ ions, and Na^+/K^+ ratio

The contents of Na^+ and K^+ ions, and the Na^+/K^+ ratio in the leaves and seeds of quinoa are affected by salinity and variety (Tables 2 and 3). Na^+ content increased by salinity while K^+ and Na^+/K^+ ratios decreased. The highest Na^+ content in leaves was found by 'NSRCQE' and 'Titicaca' varieties under severe stress (86.5 and 86.5 mg/kg, respectively) and in seeds by 'NSRCQE' varieties under severe stress (50.97 mg/kg).

Table 3: Analysis of variance the effect of salinity stress on seed physiological traits of three quinoa (Chenopodium quinoa Willd) varieties

SOV	df	Mean square (MS)					
		DPPH	Protein	Saponin	Na^+	K^+	Na^+/K^+ ratio
Block	2	1.15 ns	0.04 ns	0.007 ns	12.16 ns	0.008 ns	0.002 ns
Salinity (S)	2	246.7**	41.06**	0.42**	1196.7**	29.9**	0.17**
Block × S	4	1.07	1.78	0.001	1.56	0.57	0.001
Varieties (V)	2	6.67*	20.79**	0.01**	33.6ns	3.54**	0.002 ns
S × V	4	2.25 ns	2.19 ns	0.008*	67.4*	0.92ns	0.001 ns
Error	12	1.99	0.92	0.002	19.43	0.44	0.003
CV (%)	-	12.96	5.87	14.69	13.60	10.43	24.08

ns: non-significant; * and **: significant at 5 and 1% probably levels, respectively.

Table 4: Interaction effect of salinity and varieties on quinoa (Chenopodium quinoa Willd) seed physiological traits

	DPPH (%)	Phenol (mg/g gallic acid)	Anthocyanin (µg/g DW)	Flavonoid (mg Eq/g DW)	DPPH (%)	Seed protein (%)	K^+ (mg/kg)	Na^+/K^+ ratio
Salinity (dS/m)	Leaves				Seeds			
Control	17.2±1.8 c	45.5±7.9 c	218.5±47.2 c	7.07±1.1 c	5.47±1.4 c	14.1±3.4 c	7.97±0.9 a	0.38±0.09 a
10	43.9±7.2 b	70.3±13.4 b	417.1±62.9 b	24.0±3.4 b	11.2±2.4 b	16.4±5.6 b	6.73±1.1 b	0.21±0.05 b
17	71.1±11.4 a	91.1±10.1 a	633.3±58.7 a	30.2±5.2 a	15.9±3.7 a	18.4±4.1 a	4.38±0.8 c	0.10±0.01 c
LSD (P ≤0.05)	6.52	5.81	22.9	2.91	1.44	0.98	0.68	0.05
Varieties								
V1	39.6±4.8 b	63.2±8.4 b	404.5±20.4 b	19.2±2.9 a	9.90±1.5 b	14.5±4.7 b	5.71±1.2 b	0.22±0.05 a
V2	49.5±6.9 a	47.0±5.2 a	439.7 ±71.2 a	20.1±4.7 a	11.4±2.0 a	17.3±3.9 a	6.69±1.6 a	0.25±0.04 a
V3	43.1±7.1 ab	68.7±10.4 ab	424.7±50.9 ab	21.9±3.6 a	11.2±3.1 ab	17.1±4.5 a	6.40±1.8 a	0.22±0.04 a
LSD (P ≤0.05)	6.52	5.81	22.99	2.91	1.44	0.98	0.68	0.05

Means ± SD; Means followed by the same letter in each column are not significantly different according to the LSD test at a 5 % level, V1: 'NSRCQE'; V2: 'NSRCQB'; V3: 'Titicaca'

Table 5: Interaction effect of salinity and varieties on leaves and seeds physiological traits of quinoa (Chenopodium quinoa Willd)

Salinity (dS/m)	Varieties	MDA (mmol/ g DW)	Na^+ (mg/kg)	K^+ (mg/kg)	Na^+/K^+ ratio	Saponin (%)	Na^+ (mg/kg)
		Leaves				Seeds	
Control	V1	1.05±0.02 f	51.83±0.8 e	19.83±0.72 cd	0.38±0.02 a	0.17±0.03 c	17.97±1.52 e
	V2	1.35±0.05 f	65.07±2.5 d	27.47±1.1 a	0.42±0.01 a	0.22±0.03 c	23.03±1.75 e
	V3	1.27±0.08 f	62.77±1.2 d	26.27±0.6 a	0.42±0.01 a	0.23±0.05 c	22.87±1.59 e
10	V1	3.18±0.2 e	73.03±1.5 bc	21.43±0.5 bc	0.29±0.01 bc	0.51±0.05 b	31.4±1.56 cd
	V2	3.86±0.08 e	75.67±1.2 b	22.57±1.4 bc	0.3±0.02 bc	0.67±0.03 a	29.84±0.66 d
	V3	3.75±0.2 de	74.0±3.6 bc	21.43±0.6 bc	0.29±0.01 bc	0.52±0.02 b	33.54±2.89 cd
17	V1	5.91±0.4 c	86.5±1.6 a	16.23±0.5 e	0.19±0.01 d	0.15±0.02 c	50.97±2.32 a
	V2	7.98±0.03 a	69±3.1 cd	22.77±1.5 b	0.33±0.03 b	0.2±0.01 c	37.63±3.38 c
	V3	7.26±0.3 b	86.5±1.1 a	17.7±0.5 de	0.2±0.01 d	0.17±0.01 c	44.33±2.84 b
LSD (P ≤0.05)		0.65	6.26	2.78	0.04	0.08	6.56

Means ± SD; Means followed by the same letter in each column are not significantly different according to the LSD test at a 5% level, V1: 'NSRCQE'; V2: 'NSRCQB'; V3: 'Titicaca'

The lowest Na^+ content was observed by 'NSRCQE' under non-stressed conditions in leaves (51.83 mg/kg) and, in seeds, by all varieties under non-stressed conditions (Tables 4 and 5).

Table 6: Correlation coefficients among physiological characteristics of quinoa (Chenopodium quinoa Willd) under salinity stress and varieties

		1	2	3	4	5	6	7	8	9	10	11	12	13
Leaves	2	0.94**												
	3	0.96**	0.93**											
	4	0.93**	0.95**	0.92**										
	5	0.97**	0.94**	0.98**	0.91**									
	6	0.68**	0.73**	0.73**	0.71**	0.67**								
	7	-0.48*	-0.42*	-0.56**	-0.52*	-0.51*	-0.47*							
	8	-0.70**	-0.70**	-0.77**	-0.76**	-0.70**	-0.86**	0.84**						
Seeds	9	0.96**	0.95**	0.96**	0.94**	0.95**	0.72**	-0.45*	-0.71**					
	10	0.81**	0.82**	0.78**	0.78**	0.82**	0.48*	-0.12ns	-0.38*	0.84**				
	11	-0.05ns	0.04ns	-0.09ns	0.19ns	-0.14ns	0.09ns	0.14ns	-0.06ns	0.01ns	0.04ns			
	12	0.79**	0.78**	0.85**	0.76**	0.79**	0.87**	-0.64**	-0.87**	0.78**	0.50*	-0.14ns		
	13	-0.77**	-0.70**	-0.83**	-0.71**	-0.79**	-0.61**	0.77**	0.79**	-0.74**	-0.49*	0.28ns	-0.83**	
	14	-0.85**	-0.83**	-0.89**	-0.84**	-0.84**	-0.78**	0.61**	0.81**	-0.84**	-0.62**	0.01ns	-0.92**	0.88**

ns: non-significant; * and **: significant at 5 and 1% probably levels, respectively. Leaves = 1: DPPH, 2: phenol, 3: anthocyanin, 4: flavonoid, 5: MDA, 6: Na^+, 7: K^+, 8: Na^+/K^+ ratio, Seeds = 9: DPPH, 10: protein, 11: saponin, 12: Na^+, 13: K^+, 14: Na^+/K^+ ratio

Table 8: The amount of phenolic and flavonoid acids compound (µg/ g DW) in quinoa varieties (seed and leaves extract)

	Gallic acid		Chlorogenic acid		Caffeic acid		Paracomaric acid		Quercetin acid		Camphor acid	
Standard retention time (min)	3.2		13.4		16.9		34.1		14.9		13.2	
Salinity levels (dS/m)	Leaves	Seeds	Leaves	Seeds	Leaves	Seeds	Leaves	Seeds	Leaves	Seeds	Leaves	Seeds
Control	154.5±8.4	83.0±4.7	56.4±2.5	-	-	-	32.3±3.1	12.0±0.24	7.5±0.6	4.5±1.2	40.3±4.5	23.3±3.2
10	170.4±5.3	88.3.±2.1	70.6±5.6	-	-	-	34.5±5.9	19.5±1.2	8.9±0.8	4.9±0.9	40.5±4.9	24.6±6.3
17	158.1±6.7	85.7±4.6	72.3±3.9	-	-	-	41.5±7.3	24.3±2.3	10.3±4.9	5.4±1.5	48.3±6.3	29.7±4.9
Varieties												
V1	142.6±3.2	70.6±4.5	54.2±2.2	-	-	-	30.1±2.8	13.3±4.2	7.1±1.2	4.4±0.8	37.2±3.4	22.2±1.8
V2	155.1±1.4	79.1±4.1	59.4±5.3	-	-	-	35.6±4.4	16.5±4.8	8.2±0.9	4.9±0.6	42.5±4.8	26.5±3.1
V3	148.6 ±2.6	74.5±6.8	52.7±7.1	-	-	-	33.8±6.1	14.2±2.7	7.4±1.1	4.8±1.1	40.2±3.3	25.6±2.4

Means ± SD; V1: 'NSRCQE'; V2: 'NSRCQB'; V3: 'Titicaca'

Salinity stress was caused by decreasing K^+ content in leaves and seeds. The highest K^+ content was observed by 'NSRCQB' and 'Titicaca' varieties under non-stressed conditions in the leaves. In the leaves, the ranged content of K^+ from 27.47 mg/kg by 'NSRCQB' variety under non-stressed conditions to 16.23 mg kg^{-1} by 'NSRCQB' under severe stress. In the seeds, the highest K^+ content related to non-stressed conditions and 'NSRCQB' and 'Titicaca' varieties (Tables 4 and 5).

All varieties achieved the highest Na^+/K^+ ratio in leaves under non-stressed conditions. Salinity significantly decreased the Na^+/K^+ ratio in all varieties. Na^+/K^+ ratio ranged from 0.42 to 0.19 mg/kg in the leaves (Tables 4 and 5).

Correlation coefficients

There were significant negative and positive correlations between plant physiological and biochemical properties. For example, the DPPH radical scavenging activity in leaves was significantly and positively correlated with phenol content, anthocyanin content, flavonoid content, MDA, Na^+ in leaves, and DPPH activity, protein, and Na^+ in seeds. However, the seed protein percentage was negatively and significantly correlated with the K^+ content and Na^+/K^+ ratio in leaves and seeds while significantly and positively correlated with DPPH activity, phenol content, anthocyanin content, flavonoid content, MDA, and Na^+ content in leaves, and DPPH activity, and Na^+ content in seeds (Table 6).

Phenolic and flavonoid acids compound

Results indicated that the salinity stress increased the amount of paracomaric acid, quercetin acid, and camphor acid in leaves and seeds of quinoa. The highest amount of paracomaric acid (41.5 and 24.3 µg/g DW), quercetin acid (10.3 and 5.4 µg/g DW), and camphor acid (48.3 and 29.7 µg/g DW) related to severe stress (17 dS/m) in the leaves and seeds, respectively. Free salinity stress treatment (control) showed the lowest amount of gallic, chlorogenic, paracomaric, quercetin, and camphor acids (Table 7).

Results showed the amount of phenolic and flavonoid compounds in different varieties was significantly different. The highest amount of gallic, chlorogenic, paracomaric, quercetin, and camphor acids in leaves and seeds were found by 'NSRCQB' variety, and 'NSRCQE' variety had the lowest average of most of these compounds (Table 7).

Discussion

A set of physiological and biochemical properties of three varieties of quinoa was determined. According to the analysis of variance, the physiological and biochemical properties (DPPH, phenol, anthocyanin, flavonoids, MDA, Na^+, K^+, Na^+/K^+ ratio, seed protein, and saponin) of quinoa were significantly affected by salinity, and there were significant differences between the three varieties. Similarly, [25] reported that the tolerance of the three quinoa cultivars to saline stress varied with the salt type, salt concentration, and tested cultivar, with the 'Vikinga' and 'Puno' cultivars showing the best potential for growing under saline conditions.

Although salinity stress leads to oxidative damage by the production of Reactive Oxygen Species (ROS), antioxidant enzymes have an important protective role in scavenging ROS and protecting plant tissues [4]. The antioxidant activities of the lipophilic compounds in quinoa seeds were assessed using DPPH method. Results showed that the increased levels of salinity significantly enhanced the rate of DPPH radical scavenging activity in leaves and seeds. Similarly, *Sharma* and *Ramawat* (2013) [24] reported that NaCl treatment increases the DPPH radical scavenging activity. These activities may be directly linked to the content of phenols, tannins, and flavonoids and consequently to their free radical scavenging activities [4]. The results of the current experiment showed the DPPH radical scavenging activity was significantly and positively correlated with phenol content, anthocyanin content, and flavonoid content. This was also confirmed by a strong positive correlation between roots and leaves for osmoprotectants, lipid peroxidation, and antioxidant enzymes.

Lipids from quinoa have been reported to be generally stable against oxidation, despite their high-fat content and degree of unsaturation, because of the presence of abundant quantities of antioxidant vitamins [27]. The lipophilic compounds including unsaturated fatty acids, tocopherols, and carotenoids have been discussed as important antioxidants/anti-radical compounds, however, the contribution from fatty acids to the antioxidant activities was insignificant [30]. *Tang et al*. (2015) [27] reported the antioxidant activity was dependent on the degree of seed coat color and genotypes and was significantly different among the three quinoas ($P \leq 0.05$) which is in line with the findings of the present study.

Similarly, the results of this research work indicated that the rate of phenol was significantly higher in NSRCQB and severe salinity stress. Although salinity stress may decrease the level of photosynthesis and the production of different metabolites, other research work has also indicated that the production of phenolic compounds increases under salinity stress, which is a mechanism used by plants to avoid the unfavorable effects of salinity stress [1]. Also, in buckwheat, continuous salinity treatment increased the concentration of total phenol compounds and DPPH radical scavenging capacity [21].

Anthocyanins are known to be involved in several stress responses, acting as antioxidants to alleviate plant oxidative damage. In salinity-tolerant transgenic tobacco plants, anthocyanin accumulation was enhanced in parallel with the greater total polyphenol content and radical scavenging capacity, as compared with wild-type plants [17]. When stressed by salinity, plants can activate the expression of a variety of genes, some of which are involved in the biosynthesis of anthocyanins, such as PAL, CHS, and CHI in many plant species [22]. Salinity increased hydrogen peroxide, total phenol content, anthocyanin content, and radical scavenging capacity [21].

The increasing of MDA content can result in peroxidation and dilapidation of membrane lipids with a concomitant loss of membrane integrity [1]. In this research, increasing NaCl concentration observed an enhancement in the peroxidation membrane lipids and caused increasing MDA content.

The nutritional imbalance is another negative effect of NaCl stress on plants. Salinity induced significant increases in tissue Na^+ contents; while this effect was more pronounced in the seeds. While K^+ contents of all plant organs were dropped in response to elevated salinity. As a consequence, the Na^+/K^+ ratio was sharply decreased. Similar results have been observed previously and interpreted as a result of competition between K^+ and Na^+ at the level of absorption sites or due to the changes in the membrane integrity caused by the displacement of Ca_2^+ by Na^+ [8]. Similarly, *Cai* and *Gao* (2020) [5] reported that the K^+/Na^+ ratio decreased in both the leaves and roots of quinoa. Under salinity stress conditions, a relatively high K^+/Na^+ ratio and K^+ content can reduce salt damage to plants, which is necessary for the normal activities of organisms [1]. K^+ is not only a key ion relative to salt tolerance but also the cation in most higher plants and can regulate physiological functions, such as osmosis, ion balance, synthesis of protein, turgor pressure, and photosynthesis. However, salt-induced reduction in K^+ contents does not necessarily mean that there was a potassium deficiency because in many dicots the osmotic function of potassium, magnesium, or calcium in the vacuole can be substituted by sodium without any growth depression so that the substances mentioned above can be increasingly used for specific functions in the cytoplasm. Salinity tolerance has been reported to be related to the ability of the plant to maintain an appropriate K^+/Na^+ ratio rather than simply maintaining low Na^+ concentrations [1]. Here, the K^+/Na^+ ratio was significantly lowered with elevating water salinity as previously observed in many chenopodiaceous species. Interestingly, quinoa was able to maintain favorable K^+/Na^+ relations in its seeds and leaves at least in part under salinity conditions [11]. Furthermore, results indicated that the accumulation of Na^+ in the leaves was higher than in the seeds. In this regard, it is noted that quinoa can store Na ion in its shoot part to prevent the transfer of salts to seeds and reduce seed quality [16]. Scientists reported that one of the reasons for quinoa survival in saline conditions is the ability of this plant to regulate the leaf potential, which is partly due to the accumulation of Na and sucrose in roots and leaves. On the other hand, in saline conditions, the surface cover of the seed prevents the transfer of NaCl into the seed [16]. Quinoa plants accumulate more K^+ in leaves under salt stress [13] which was confirmed in this study.

Quinoa is known as a super-food because of its exceptional nutritional profile as its grains contain more protein, and minerals than common cereals. This crop has gained attention to secure future food and nutritional security, especially in marginal environments [13]. Results of the current study depict that seed quality (protein and saponin contents) was not affected under saline conditions, and even improved significantly in some cases, mainly protein and saponin contents were not decreased due to salinity/sodicity, only genotypic differences were found for seed mineral contents. The protein content of quinoa seeds ranged from 12.67% to 20.5% of DW in agreement with data reported by *Pulvento et al.* [19] for different quinoa varieties grown in saline and non-saline conditions. Protein content in quinoa (mean 16.3% of DW) is generally higher than those reported for cereal grains barley (10.8%), maize (10.2%), rice (7.6%), and wheat (14.2%) [19]. In general, it was observed that the severe salinity stress determined a reduction in the accumulation of sapogenins in quinoa seeds, while the medium salinity level (10 dS/m) showed the highest saponin content. These results are in agreement with data reported by *Pulvento et al*.[19] that showed that saponin content for two quinoa varieties, 'Sajama' and 'Chucara', is affected by soil water deficit; seeds from treatment with the highest soil water deficit showed the lowest saponin content during the cycle for both cultivars.

The important aspect of this research work is that salinity improves the phenolic and flavonoid acids compound and hence the nutritional and pharmaceutical properties of quinoa. Furthermore, the present study not only reports for the first time of the phenolic and flavonoid acids composition and content but shows these phytochemicals vary significantly among different varieties. The metabolic basis for the increased production of secondary materials under stress conditions is according to the following. Under salinity or water-deficient conditions, due to the closure of stomata, CO_2 uptake significantly decreases. Accordingly, the consumption of reducing $NADPH^+H^+$ for the fixation of CO_2 by the Calvin cycle significantly decreases and results in the accumulation of $NADPH^+H^+$. Consequently, the production of highly reduced products including isoprenoids, phenols, and terpenoids increases [18]. The authors indicated that although the severe level of salinity can markedly increase the production of secondary metabolites in industrial-medicinal plants, treating the plants with a high

concentration of CO_2 (700 ppm) can also have similar effects. Accordingly, it is possible to adjust the rate of secondary metabolites and enhance the quality of plants by imposing the plants salinity stress [18].

CONCLUSIONS

Quinoa is an emerging pseudo-cereal grain, and the present study focuses on the physiological and biochemical responses of different quinoa varieties to salinity stress. Results showed that salinity significantly affected physiological and biochemical parameters. Salinity was caused by increasing DPPH radical scavenging activity, phenol, anthocyanin, flavonoids, and MDA contents as well as Na^+ accumulation in leaves and seeds while decreasing K+ accumulation and Na^+/K^+ ratio. With increasing salt levels, the accumulation of organic (protein and saponin) and inorganic (K^+, Na^+, phenol, etc.) substances in quinoa plants might be a reflection of the energetic cost associated with osmotic adjustment. The variety which showed the best potential under salinity conditions was 'NSRCQB'. Overall, salt tolerance of quinoa grown on salt-affected soils of Yazd, Iran was linked with better crop stand establishment, low Na^+ accumulation in leaves as well as increased activities of enzymatic and non-enzymatic antioxidants.

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REFERENCES

[1] Aghighi Shahverdi M., Omidi H., Tabatabaei S.J. Stevia (Stevia Rebaudiana Bertoni) Responses to NaCl Stress: Growth, Photosynthetic Pigments, Diterpene Glycosides and Ion Content in Root and Shoot, *J. Sau. Soc. Agri. Sci.*, **18(4):** 355-360 (2019).

[2] Ahmed A.R., Gabr M., AL-Sayed H., Smetanska I. Effect of Drought and Salinity Stress on Total Phenolic, Flavonoids and Flavonols Contents and Antioxidant Activity *in Vitro* Sprout Cultures of Garden Cress (Lepidium Sativum), *J. of Applied Sciences Research*, **8(8):** 34-42 (2012).

[3] Bazile D., Martínez E.A., Fuentes F., Diversity of Quinoa in a Bio-Geographical Island: A Review of Constraints and Potential from Arid to Temperate Regions of Chile, *Not. Bot. Hor. Agro. Cl.-Nap.*, **42(2):** 289-298 (2014).

[4] Bettaieb I., Knioua S., Hamrouni I., Limam F., Marzouk B. Water-Deficit Impact on Fatty acid and Essential Oil Composition and Antioxidant Activities of Cumin (Cuminum Cyminum L.) Aerial Parts, *J. of Agriculture Food Chemistry.*, 59(1):328-34 (2011).

[5] Cai Z.Q., Gao Q., Comparative Physiological and Biochemical Mechanisms of Salt Tolerance in Five Contrasting Highland Quinoa Cultivars, *BMC Plant Biology.*, **20(1):** 1-15 (2020).

[6] Derbali W., Goussi R., Koyro H.W., Abdelly C., Manaa A., Physiological and Biochemical Markers for Screening Salt Tolerant Quinoa Genotypes at Early Seedling Stage, *J. Plan. Inter.*, **15(1):** 27–38 (2020).

[7] Dhindsa R.S., Plumb-Dhindsa P., Thorpe T.A., Correlated with Increased Levels of Membrane Permeability and Lipid Peroxidation, and Decreased Levels of Superoxide Dismutase and Catalase, *J. of Experimental Botany.*, **32(1):** 93-101 (1981).

[8] Eisa S., Hussin S., Geissler N., Koyro H.W., Effect of NaCl Salinity on Water Relations, Photosynthesis and Chemical Composition of Quinoa (Chenopodium Quinoa Willd.) as a Potential Cash Crop Halophyte, *Australian J. of Crop Science*, **6(2):** 357-68 (2012).

[9] Farooq M., Hussain M., Wakeel A., Siddique K., Salt Stress in Maize: Effects, Resistance Mechanisms, and Management, A review, A*gronomy for Sustainable Development.*, **35(2):** 461-81 (2015).

[10] Gonzalez J.A., Bruno M., Valoy M., Prado F.E., Genotypic Variation of Gas Exchange Parameters and Leaf Stable Carbon and Nitrogen Isotopes in Ten Quinoa Cultivars Grown under Drought, *J. of Agronomy Crop Science.*, **197(2):** 81-93 (2011).

[11] Hariadi Y., Marandon K., Tian Y., Jacobsen S.E. Shabala S., Ionic and Osmotic Relations in Quinoa (Chenopodium Quinoa Willd.) Plants Grown at Various Salinity Levels, *J. of Experimental Botany*, **62(1):** 185-193 (2012).

[12] Hatano T., Kagawa H., Yasuhara T., Okuda T., Two New Flavonoids and other Constituents in Licorice Root: their Relative Astringency and Radical Scavenging Effects, *Chemical and Pharmaceutical Bulletin.*, **36(6):** 2090-2097 (1988).

[13] Iqbal S., Basra S., Afzal I., Wahid A., Saddiq M.S., Hafeez M.B., Yield Potential and Salt Tolerance of Quinoa on Salt-Degraded Soils of Pakistan, *J. of Agronomy and Crop Science.*, **205(1):** 13-21 (2019).

[14] Koyro H.W., Geissler N., Seenivasan R., Huchzermeyer B., "Plant Stress Physiology; Physiological and Biochemical Strategies Allowing to Thrieve under Ionic Stress", CRC Press, Taylor & Francis Group, 1051-1094 (2011).

[15] Maleki P., Saadat S., Bahrami H.A., Rezaei H., Esmaeelnejad L., Accumulation of Ions in Shoot and Seed of Quinoa (Chenopodium Quinoa Willd.) under Salinity Stress, *Communications in Soil Science and Plant Analysi.*, **50(6):** 782-793 (2019).

[16] Maleki P., Bahrami H.A., Saadat S., Sharifi F., Dehghany F., Salehi M. Salinity Threshold Value of Quinoa (Chenopodium Quinoa Willd.) at Various Growth Stages and the Appropriate Irrigation Method by Saline Water, *Communications in Soil Science and Plant Analysis*, **49(15):** 1815-25 (2018).

[17] Naing A.H., Park K. I., Ai T.N., Chung M.Y., Han J.S., Kang Y.W., Overexpression of Snapdragon Delila (Del) Gene in Tobacco Enhances Anthocyanin Accumulation and Abiotic Stress Tolerance, *BMC Plant Biology.*, **17(1):** 65 (2017).

[18] Omidi H., Shams H., Seif Sahandi M., Rajabian T. Balangu (Lallemantia sp.) Growth and Physiology under Field Drought Conditions Affecting Plant Medicinal Content, *Plant Physiology and Biochemistry*, **130:** 641-646 (2018).

[19] Pulvento C., Riccardi M., Lavini A., Iafelice G., Marconi E., D'Andria R., Yield and Quality Characteristics of Quinoa Grown in Open Field under Different Saline and Non-Saline Irrigation Regimes, *J. of Agronomy and Crop Science.*, **198(4):** 254-63 (2012).

[20] Rosa M., Hilal M., Gonzalez J.A., Prado F.E., Low-Temperature Effect on Enzyme Activities Involved in Sucrose-Starch Partitioning in Salt-Stressed and Salt-Acclimated Cotyledons of Quinoa (Chenopodium Quinoa Willd.) Seedlings, *Plant Physiology and Biochemistry*, **47(4):** 300-307 (2009).

[21] Sakamoto M., Suzuki T., Methyl Jasmonate and Salinity Increase Anthocyanin Accumulation in Radish Sprouts, *Horticulturae.*, **5(3):** 1-10 (2019).

[22] Sakamoto M., Suzuki T., Synergistic Effects of a Night Temperature Shift and Methyl Jasmonate on the Production of Anthocyanin in Red Leaf Lettuce, *American J. of Plant Sciences.*, **8(7):** 34-49 (2017).

[23] Shahverdi M.A., Omidi H., Tabatabaei S.J., Plant Growth and Steviol Glycosides as Affected by Foliar Application of Selenium, Boron, and Iron under NaCl Stress in Stevia Rebaudiana Bertoni, *Industrial Crops and Products*, **125:** 408-415 (2018).

[24] Sharma V., Ramawat K.G., Salinity-Induced Modulation of Growth and Antioxidant Activity in the Callus Cultures of Miswak (Salvadora Persica), *Biotechnology.*, **3(1):** 11-17 (2013).

[25] Stoleru V., Slabu C., Vitanescu M., Peres C., Cojocaru A., Covasa M., Tolerance of Three Quinoa Cultivars (Chenopodium Quinoa Willd.) to Salinity and Alkalinity Stress during Germination Stage, *Agronomy.*, **9(6):** 1-14 (2019).

[26] Talebnejad R., Sepaskhah A.R. Quinoa: A New Crop for Plant Diversification under Water and Salinity Stress Conditions in Iran, *Acta Horticulture.*, **1190:** 101-6 (2018).

[27] Tang Y., Li X., Chen P.X., Zhang B., Hernandez M., Zhang H., Characterisation of Fatty Acid, Carotenoid, Tocopherol/Tocotrienol Compositions and Antioxidant Activities in Seeds of Three Chenopodium Quinoa Willd. Genotypes, *Food Chemistry*, **174:** 502-508 (2015).

[28] Waqas M., Yaning C., Iqbal H., Shareef M., Rehman H., Yang Y. Paclobutrazol Improves Salt Tolerance in Quinoa: Beyond the Stomatal and Biochemical Interventions, *J. of Agronomy and Crop Science*, **203(4):** 315-322 (2017).

[29] Yang A., Akhtar S.S., Amjad M., Iqbal S. Jacobsen S.E., Growth and Physiological Responses of Quinoa to Drought and Temperature Stress, *J. of Agronomy and Crop Science*, **202(6):** 445-53 (2016).

[30] Zhang B., Deng Z., Tang Y., Chen P., Liu R., Ramdath D.D., Fatty Acid, Carotenoid and Tocopherol Compositions of 20 Canadian Lentil Cultivars and Synergistic Contribution to Antioxidant Activities, *Food Chemistry.*, **161:** 296-304 (2014).