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SALINITY TOLERANCE OF QUINOA (*CHENOPODIUM QUINOA* WILLD.) GENOTYPES TO ELEVATED NACL CONCENTRATIONS AT GERMINATION AND SEEDLING STAGES

A.M.M. AL-NAGGAR[1*], R.M. ABD EL-SALAM[1], A.I.A. HASSAN[2], M.M.A. EL-MOGHAZI[2], and A.A. AHMED[3]

[1]Department of Agronomy, Faculty of Agriculture, Cairo University, Egypt
[2]Plant Breeding Unit, Plant Genetic Resources, Desert Research Center, Cairo, Egypt
[3]Seed Technology Research, Field Crops Research Institute, Agric. Res. Center, Giza, Egypt
*Corresponding author's email: medhatalnaggar@agr.cu.edu.eg
Email addresses of co-authors: rabiehamed@agr.cu.edu.eg, Dr.ahmedebrahim1958@gmail.com, maimoghazi3@gmail.com, abeerabdelaty2015@gmail.com

SUMMARY

Evaluating quinoa genotypes for salinity tolerance at germination and seedling stages is a prerequisite for plant breeders. Thus, the scrutiny of 19 quinoa genotypes at different salinity levels under controlled laboratory and greenhouse conditions occurred at the germination and seedling stages. This study aimed to identify the most tolerant genotypes to elevated salinity levels at germination and seedling stages and to determine the traits of a robust association with salinity tolerance using a factorial experiment based on a randomized complete block design in three replications. The four salinity solutions used were zero (control), 3000, 6000, and 9000 ppm NaCl. Increasing concentrations of NaCl caused a gradual and significant decrease for all studied traits except mean germination time, which significantly increased. At all salinity-stress levels (3000, 6000, and 9000 ppm NaCl), the studied 19 genotypes underwent classification based on their salinity tolerance index (STI) into three categories, i.e., tolerant, moderately tolerant, and sensitive. The four most salinity-tolerant quinoa genotypes under all studied salinity-stress conditions were Rainbow-2, QI3, RH, and KvlSRA2. The strongest correlations were between STI and each of seedling length, root length, seedling fresh weight, seedling vigor index I, and seedling vigor index II under 3000 ppm; germination percentage, speed germination index, seedling extent, root length, seedling fresh weight, and seedling vigor index II under 6000 ppm; and shoot length and seedling vigor index I under 9000 ppm salinity concentration level. Traits showing sturdy correlations with STI, high heritability estimates, high expected genetic advance, and wide phenotypic and genotypic variability were seedling dry weight, seedling fresh weight, seedling vigor index II, and speed germination index at all salinity stress concentrations; they are recommendable as selection criteria for salinity tolerance in quinoa at germination and seedling stages.

Keywords: Quinoa, salinity tolerance, germination, seedling stage, selection criteria, heritability

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Key findings: The results will help quinoa breeders identify salinity-tolerant genotypes and the selection criteria for salinity tolerance in quinoa at the germination and seedling stages.

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INTRODUCTION

About 33% of Egypt’s arable land has already become salinized (Ghassemi *et al*., 1995). The chief causes of this salinization are a lack of precipitation (less than 25 mm annually), high temperatures (summer temperatures range from 35 °C to 45 °C), high surface evaporation (1500–2400 mm/year), a poor drainage system, a rising water table (less than one meter below the soil surface), and poor irrigation water (up to 4.5 dSm^{-1} salinity) (Amer *et al.* 1989). The soil productivity reduction is roughly 30% due to the impact of salinity, endangering the livelihoods of subsistence farmers and substantially impacting Egypt's overall food production (Al-Naggar *et al*., 2015). Hence, dealing with salinity issues in the Egyptian agriculture sector has become urgent. Therefore, new approaches are necessary to cope with these problems. One option is halophytic crop species, such as, quinoa, which can tolerate high salinity levels in soil (Hinojosa *et al*., 2018).

The annual seed crop, quinoa (*Chenopodium quinoa* Willd.), is indigenous to the Andean area of South America. With its high seed value, notable environmental adaptability, and beneficial effects on human nutrition and health, it has gained popularity in recent years worldwide (Hinojosa *et al*., 2019). According to Jacobsen *et al*. (2003) and Hinojosa *et al*. (2018), it is a "facultative" halophyte that is highly adaptable to withstanding abiotic stresses like salinity and drought. It can tolerate salinity levels that are closer to those of the seawater. The majority of the studies explaining the mechanisms behind quinoa's high salinity tolerance (Shabala *et al*., 2012; Adolf *et al*., 2013; Iqbal *et al*., 2020) credit the plant's efficient sodium (Na+) sequestration in leaf vacuoles, oxidative stress defense, and potassium (K+) retention. Salinity tolerance, however, has been found to vary significantly among quinoa cultivars/genotypes (Adolf *et al*., 2012; Peterson and Murphy, 2015; Chaganti and Ganjegunte, 2022), as well as, between various growth phases (Maleki *et al*., 2018; Derbali *et al*., 2020). For instance, Maleki *et al*. (2018) found that quinoa is more sensitive to salinity at the germination and seedling stages than at higher growth stages. Although tolerance at the germination stage may (or may not) relate totally to salinity tolerance at the adult plant stage, determining the germination potential of seeds in saline circumstances gives simple and relevant parameters (Arzani, 2008). In any case, Maleki *et al*. (2018) demonstrated that establishing seedlings and seedling populations under saline conditions depends on the success of these processes.

A significant difference was found in the germination rate of 200 quinoa genotypes grown in saline environments (Gómez-Pando *et al*., 2010). The germination of quinoa seeds declined by about 60% in heavily salted water (0.4 M NaCl), with the fresh weight of germinating seeds reduced by about 60%, but the dry weight was unaffected (Prado *et al*., 2000). In quinoa seedlings, NaCl decreases fructose and glucose concentrations while increasing the contents of sucrose. Although particular quinoa accessions may withstand salinities of more than saltwater at the seedling stage, several can endure salinities of up to 150 mM NaCl (Ruiz-Carrasco *et al*., 2011).

Since salt harms crop plants' ability to grow, develop, and produce, it is a problem for agriculture (Anshori *et al*., 2018; Saenghachai *et al*., 2018). Usually, a plant's growth slows linearly after reaching a certain salinity threshold. Salinity inhibits both root and shoot growth; however, the drop in root growth is smaller than that in top maturation (Fageria, 1992). According to several studies (Shannon and Noble, 1990; Flowers and Yeo, 1995), physiological features better predict success in controlled salinity tolerance selection than yield in salt-affected fields. For a plant to grow, germination is an imperative stage (Song *et al*., 2008). During germination or seedling growth, many plants are vulnerable to ion stress (Carvaja *et al*., 1998; Majeed *et al*., 2022). Early germination and seedling stages also reflect plant growth responses to salinity (Cuartero *et al*., 2006). Therefore, investigating quinoa genotypes for salinity tolerance is essential at both phases (Mano and Takeda, 1997; Munir *et al.,* 2013).

This research's goals were: (1) to study the genetic variation among 19 quinoa genotypes in salinity tolerance at germination and seedling stages; (2) to identify the differential response of quinoa genotypes at germination and seedling stages under elevated concentrations of NaCl; and (3) to determine the germination and seedling traits of robust association with salinity tolerance and of high heritability for recommendation as selection criteria for such tolerance in quinoa.

MATERIALS AND METHODS

Plant material

Seeds of 19 quinoa genotypes used in this study came from different research centers and universities, nationally and internationally. Name, origin, source and seed color are presented in Table 1.

Table 1. Name, origin, source, and seed color of quinoa genotypes used in this study.

Genotype	Name	Origin	Source	Seed color
1	Giza-1	Peru	ARC-Egypt	Brown
2	R-103	Peru	FAO	Beige
3	ICBA-Q3	Peru	DRC-Egypt	Dark cream
4	Chipaya	Salares, Bolivia	DRC-Egypt	Beige
5	CICA-17	Peru	DRC-Egypt	Yellow
6	Ollague	Salares, Bolivia	DRC-Egypt	Brown
7	ICBA-Q5	Peru	DRC-Egypt	Dark cream
8	Ku-2	Argentina	DRC-Egypt	Beige
9	RH	Bolivia	USA	Red
10	KvlSRA2	Denmark	USDA	White
11	CO-407	Colorado-USA	DRC-Egypt	Yellow
12	Regalona	Chile	Peru	Orange
13	Misr-1	Peru	ARC-Egypt	Cream
14	Rainbow-2	Chile	USDA	Orange
15	Ql37	Peru	DRC- Egypt	Beige
16	Di-17	Bolivia	DRC-Egypt	White
17	G-13	Bolivia	DRC-Egypt	Yellow
18	Ql3	Bolivia	DRC- Egypt	Beige
19	Line- Q3	Peru	ICBA-UAE	Dark cream

ARC = Agricultural Research Center, DRC = Desert Research Center, USDA = United States Department of Agriculture, USA = United States of America, ICBA = International Center for Biosaline Agriculture, UAE = United Arab Emirates.

Laboratory and greenhouse experiments

The experiments transpired at the Seed Preservation Laboratory of the Seed Technology Department and the Greenhouse of Field Crops Research Institute, Agricultural Research Center, Giza, Egypt. Quinoa seeds' disinfection consisted of a 0.1% $HgCl_2$ solution for 5 min and 3–4 times washing with distilled water to remove its traces, done by dipping the 25 viable seeds of each replicate selected randomly into each salinity level and control.

Sowing method

Planting three replicates of 75 seeds (25 seeds per replicate) from each salinity level and control continued in seedling trays covered with peat moss moistened with distilled water only for the check while distilled water with the salinity concentrations (3000, 6000, and 9000 ppm NaCl) for salinity stress treatments. Seedling trays' incubation in a growth chamber had a temperature of 20 °C ± 2 °C and twice-a-week irrigation by the four solutions. The experimental design included a completely randomized factorial design with water salinity and quinoa genotype as independent factors. Three replicates comprised each salinity and genotype combination.

Data recording

Germination traits

The performed germination tests were according to ISTA (2020). Total numbers of seeds germinated counted daily had their percentages calculated on the 14th day from sowing. Data recording were on: 1) germination percentage (GP) as follows: GP = 100 × (Number of germinated seeds/Total number of seeds); 2) germination rate (GR): GR = a + (a + b) + (a + b + c) …… (a + b + c + m)/ n (a + b + c + m). Where a, b, c is the number of seedlings in the first, second and third count, m is the number of seedlings in the final count, and n is the number of counts; 3) speed germination index (SGI) as described by the Association of Official Seed Analysis (AOSA, 2005), as follows: SGI = (Number of germinated seeds/day of the first count) + (……. /…….) + (Number of germinated seed/day of the final count); 4) mean germination time (MGT) calculated based on the following equation of Kader (2005): MGT = Σ Dn/ Σn, where (n) is the number of seeds, which were germinated for the day, (D) is number of days counted from the beginning of germination.

Seedling traits

Each replicate had data collected from 10 seedlings for seedling traits. Harvesting took place 14 days after the seed sowing date. Data recording included the following seedling attributes: 1) seedling length (SL), 2) shoot length, 3) root length (RL), 4) seedling fresh weight (SFW), 5) seedling dry weight (SDW), 6) seedling vigor index I (SVI) as SVI = germination (%) × seedling dry weight, and 7) seedling vigor index II (SVII) as SVII = germination (%) × seedling length.

Salinity Tolerance Index (STI)

The salinity tolerance trait index (STTI) modified from the grain yield efficiency index suggested by Fageria (1992), served to classify genotypes for tolerance to salinity. The formula used is as follows: STTI = $(Y_1/AY_1) \times (Y_2/AY_2)$, where Y_1 = trait mean at no salinity level (control), AY_1 = average trait of genotypes at no salinity level (control), Y_2 = trait mean at high salinity level (3000, 6000, or 9000 ppm), and AY_2 = average trait of genotypes at high salinity level (3000, 6000, or 9000 ppm). The salinity tolerance index (STI) estimation was the average of the STTI of all studied germination and seedling traits, according to Munir *et al*. (2013), as follows:

$$STI = (STT_1I + STT_2I + \ldots\ldots + STT_nI)/n$$

where, $STTI_1$, $STTI_2$ …… $STTI_n$ = Trait No. 1, Trait No. 2 ……. Trait No. n, and n = number of measured traits. According to this study scale, when STI is ≥1.2, it indicates that the genotype is tolerant (T); if STI is ≤0.8, it specifies that the genotype is sensitive (S); and if STI is <1.2 and >0.8, it indicates that the genotype is moderately tolerant (MT), based on this research's classification.

Biometrical analysis

After verifying the normality of the data, they were subjected to a factorial analysis of variance (ANOVA) using the CoStat system for Windows, Version 6.311 (CoHort software, Berkeley, CA 94701), testing the effects of irrigation water salinity, genotypes, and their respective interactions on studied traits. Mean comparisons used the least significant difference (LSD) test and coefficients of simple correlations between either salinity tolerance trait index (STTI) or salinity tolerance index (STI), calculating all studied traits according to Steel *et al*. (1997). In determining genetic parameters, separate analyses of each salinity treatment ensued on a randomized complete block design (RCBD). Expected mean squares estimates from the ANOVA table of RCBD were according to Hallauer *et al*. (2010), which estimated the genotypic (σ^2_g), phenotypic (σ^2_p), and error (σ^2_e) variances. Calculations of the broad-sense heritability (h^2_b) and expected genetic advance (GA%) from direct selection for all studied traits under each salinity treatment followed the method of Singh and Chaudhary (2000). The GA calculations were as follows: $GA\% = 100\ Kh^2_b\ \sigma_p / \bar{x}$ where $\bar{x}$ = the general mean of the appropriate salinity treatment, σ_p = the square root of the phenotypic variance estimates, and K = the selection differential (K = 1.76 for 10% selection intensity in this study).

RESULTS AND DISCUSSION

Analysis of variance

An analysis of the variance of the studied germination and seedling traits of 19 quinoa genotypes under four NaCl levels using a factorial experiment based on randomized complete block design under laboratory and greenhouse conditions is available in Table 2. Results showed that mean squares due to salinity levels (S) were significant ($P \le 0.001$) for all studied traits. It indicates salinity stress significantly affected all studied quinoa germination and seedling attributes. Results also exhibited that mean squares due to quinoa genotypes (G) were noteworthy ($P \le 0.001$) for all studied traits (Table 2). It suggests that quinoa genotypes used in this study notably differed for all studied germination and seedling growth traits (Chaganti and Ganjegunte, 2022).

Moreover, mean squares due to genotypes × salinity levels, *i.e.,* G × S, were significant ($P \le 0.01$ or $P \le 0.001$) for all studied traits, indicating that quinoa genotypes in this study behaved differently under different salinity levels. This conclusion agrees with other investigators (Munir *et al*., 2013; Chaganti and Ganjegunte, 2022). Mean squares due to salinity levels were much higher in magnitude than those due to quinoa genotypes for all studied germination and seedling growth traits, specifying that salinity effects were more pronounced than genotypic effects for such features.

Effect of salinity level

Increasing the NaCl salinity level affected the germination and seedling growth traits of the 19 quinoa genotypes unfavorably (Table 3). A significant ($P \le 0.01$) gradual reduction was noticeable due to increasing NaCl salinity levels from 0 to 3000, 6000, and 9000 ppm for all studied traits, except mean germination time (MGT), where salt stress at 3000, 6000, and 9000 ppm caused a small but significant ($P \le 0.01$) increase (delay) in mean germination time by 3.88%, 6.98%, and 10.31%, respectively.

Maximum reduction due to salinity stress resulted at 9000 ppm NaCl solution concentration in all studied germination and seedling growth stages. Decreases at the 9000 ppm NaCl level ranged from 18.42% for germination rate to 90.04% for seedling vigor index I, followed by 80.40% for seedling dry weight. The mean germination time trait that exhibited a significant increase of 10.31% and root length and germination rate that gave the lowest decrease of 24.71% and 18.42%, respectively, at 9000 as compared with control (no salt stress) could be considered the most tolerant traits to salinity in the presented study. Hussain *et al.* (2013) reported that seedling root length showed the minimum suppression in root elongation at higher salt concentrations in wheat. Munir *et al.* (2013) also concluded that seedling root characters are wheat's most tolerant features to salinity stress. Salinity inhibits both root and shoot growth; however, the drop in root growth is smaller than that at the top (Fageria, 1992).

Table 2. Analysis of variance of studied traits at germination and seedling stages of 19 quinoa genotypes under four NaCl concentrations.

S.O.V.	d.f.	Mean squares					
		Germination %	Germination rate	Mean germination time	Speed germination index	Seedling length	Shoot length
Salinity level (S)	3	23129***	0.0686 ***	11.488 ***	461.25 ***	119.63***	40.43***
Genotype (G)	18	2047.9 ***	0.0037 ***	0.5828 ***	32.65 ***	20.91 ***	3.98***
S×G	54	160.57 ***	4.8518 ***	0.065 ***	3.164 ***	2.343 ***	0.66***
Error	150	61.3609	1.5646	0.01889	0.5916	0.289	0.081
		Root length	Seedling fresh weight	Seedling dry weight	seedling vigor index I	seedling vigor index II	
Salinity level (S)	3	19.053 ***	3399.9 ***	82.512 ***	665819 ***	3796343***	
Genotype (G)	18	9.1498 ***	456.47 ***	4.230 ***	27196 ***	341792 ***	
S×G	54	1.8285 ***	90.38 ***	1.087 **	7519 **	39799 ***	
Error	150	0.1985	44.619	0.616	4219.35	6083.6	

** and *** indicate significant at 0.01 and 0.001 probability levels, respectively.

Table 3. Effect of water salinity levels on germination and seedling traits of quinoa.

Salinity level ppm	Means	Red. %	Mean	Red. %	Mean	Red. %	Mean	Red. %
	Germination %		Germination rate (Germinated seed/day)		Mean germination time (day)		Speed germination index	
Control	78.76 a	-	0.383 a	-	9.02 d	-	8.65 a	-
3000	62.73 b	20.35	0.360 b	5.26	9.37 c	-3.88	6.13 b	29.13
6000	49.82 c	36.74	0.331 c	13.16	9.65 b	-6.98	4.23 c	51.1
9000	37.94 d	51.83	0.311 d	18.42	9.95 a	-10.31	2.86 d	66.94
	Seedling length (cm)		Shoot length (cm)		Root length (cm)		Seedling fresh weight (mg)	
Control	9.92 a	-	5.56 a	-	4.37 a	-	24.0 a	-
3000	8.55 b	13.81	4.59 b	17.45	3.98 b	8.92	15.7 b	34.58
6000	7.7 c	22.38	4.15 c	25.36	3.56 c	18.54	10.2 c	57.5
9000	6.99 d	29.54	3.75 d	32.55	3.29 d	24.71	9.9 d	58.75
	Seedling dry weight (mg)		Seedling vigor index I		Seedling vigor index II			
Control	3.01 a	-	236.0 a	-	791.0 a	-		
3000	2.01 b	33.22	124.4 b	47.29	548.7 b	30.63		
6000	1.14 c	62.13	60.1 c	74.53	398.4 c	49.63		
9000	0.59 d	80.40	23.5 d	90.04	274.8 d	65.26		

Means followed by different letters are significantly different at 0.05 probability level.
Red. = Reduction = 100 × (control - salinity level)/control.

Study results concur with previous studies, which also reported significant reductions in quinoa seed germination with increasing salinity (Gómez-Pando *et al*., 2010; Panuccio *et al*., 2014; Stoleru *et al*., 2019; Chaganti and Ganjegunte, 2022). For example, Gómez-Pando *et al*. (2010) found that seed germination drastically declined at a 300-mM (~30 dSm^{-1}) NaCl concentration when testing 182 Peruvian accessions for their salinity tolerance at the germination stage. In contrast, Maleki *et al.* (2018) found that the salinity threshold value of quinoa at its germination and seedling stages was 8 dSm^{-1}. It reduces germination due to salinity, which can refer to both osmotic and toxic (Na+ and Cl-) effects, resulting in reduced seed imbibition (Munns, 2002; Ma *et al*., 2006). The increased content of salts in the growth medium lowers the osmotic potential of the solution, consequently inhibiting seeds from imbibing water to initiate the first stages of germination and preventing radicle protrusion through the seed coat (Causin *et al*., 2020).

Effect of genotypes

Generally, the means of 19 quinoa genotypes showed a wide range (the difference between the minimum and maximum value) for all studied germination and seedling traits, except germination rate and mean germination time across all salinity levels (Table 4). Genotype No. 8 (Rainbow-2 from Chile) gave the highest value for nine out of 11 traits, namely, germination percentage, germination rate, speed germination index, seedling length, root length, seedling fresh weight, seedling dry weight, seedling vigor index I, and seedling vigor index II. Maximum shoot length came from genotype No. 3 (ICBA-Q3 from Peruvian origin). Contrarily, minimum values resulted from genotype No. 16 (Di-17 from Bolivia) for four traits, namely, seedling length, seedling fresh weight, seedling dry weight, and seedling vigor index I; genotype No. 2 (R-103) for germination rate and root length; genotype No. 8 (Ku-2) for germination percentage and germination rate; genotype No. 7 (ICBA-Q5) for shoot length; genotype No. 5 (CICA-17) for germination rate and seedling vigor index II; and genotype No. 6 (Ollague) for speed germination index. Genotypic differences in germination and seedling traits of quinoa also came from other investigators' works (Ruiz-Carassco *et al.,* 2011; Adolf *et al*., 2012; Peterson and Murphy, 2015; Chaganti and Ganjegunte, 2022).

Salinity level by genotype interaction

Maximum and minimum values of 19 quinoa genotypes under control and under salinity stress levels for studied germination and seedling traits are in Table 5. Since mean squares due to genotypes × salinity levels were significant ($P \leq 0.01$ or $P \leq 0.001$) for all studied features (Table 2), expect the genotypes' ranking to change from one salinity level to another. For example, maximum germination percentage appeared with genotype No. 14 under control, 3000, and 6000 ppm NaCl levels, but emerged with genotype No. 19 (Line- Q3 from Peru), under 9000 ppm level. Maximum values under control, 3000, 6000, and 9000 ppm resulted in the genotype Nos. 16, 14, 14, and 19 for germination rate, Nos. 1, 8, 5, and 11 for mean germination time, Nos. 14, 14, 14, and 19 for speed germination index, Nos. 14, 14, 18, and 19 for seedling length, Nos. 15, 17, 9, and 3 for shoot length, Nos. 3, 14, 14, and 18 for root length, Nos. 14, 18, 7, and 7 for seedling fresh weight, Nos. 7, 6, 14, and 14 for seedling dry weight, Nos. 7, 14, 14, and 14 for seedling vigor index I, and Nos. 14, 14, 14, and 19 for seedling vigor index II, respectively. Inversely, minimum values under control, 3000, 6000, and 9000 ppm showed for genotype Nos. 6, 8, 8, and 8 for germination percentage, Nos. 1, 8, 8, and 2 for germination rate, Nos. 16, 14, 14, and 19 for mean germination time, Nos. 6, 8, 8, and 8 for speed germination index, Nos. 6, 16, 8, and 8 for seedling length, Nos. 1, 8, 8, and 6 for shoot length, Nos. 6, 6, 15, and 15 for root length, Nos. 12, 8, 2, and 16 for seedling fresh weight, Nos. 12, 16, 16, and 16 for seedling dry weight, Nos. 12, 16, 8, and 8 for seedling vigor index I, Nos. 6, 8, and 8 for seedling vigor index II, respectively. The salinity × genotype interaction for germination and seedling traits

Table 4. Genotypes' numbers showing minimum (Min) and maximum (Max) values for germination and seedling growth traits of quinoa genotypes across all salinity levels.

Parameters	Traits			
	Germination %	Germination rate (Germinated seed/day)	Mean germination time (day)	Speed germination index
Mean	57.31	0.35	9.5	5.47
Min	37.54(8)	0.32(2,5,8)	9.05(15)	3.3(6)
Max	85.05(14)	0.38(14)	9.84(2)	9.31(14)
LSD 5%	6.3	0.01	0.11	0.61
	Seedling length (cm)	Shoot length (cm)	Root length (cm)	Seedling fresh wt. (mg)
Mean	8.29	4.52	3.79	15.26
Min	6.44(16)	3.52(7)	2.55(2)	10(2,16)
Max	10.29(14)	5.26(3)	5.19(14)	30(14)
LSD 5%	0.43	0.23	0.36	5.37
	Seedling dry weight (mg)	Seedling vigor index I	Seedling vigor index II	
Mean	1.69	110.98	503.22	
Min	0.54(16)	44.8(16)	277.2(5)	
Max	2.68(14)	237.7(14)	899.5(14)	
LSD 5%	0.63	52.3	62.8	

Each value is followed by a genotype number (between brackets). For genotype name, see Table 1.

Table 5. Genotypes' numbers showing minimum (Min) and maximum (Max) values for germination and seedling traits of quinoa seeds germinated under different salinity levels.

Parameters	Control	3000	6000	9000	Control	3000	6000	9000
	Germination %				Germination Rate (Germinated seed/day)			
Min	50.1(6)	35.07(8)	25(8)	15(8)	0.35(1)	0.32(8)	0.27(8)	0.29(2,5,11)
Max	100 (14)	95.13(14)	80.07(14)	70.03(19)	0.41(16)	0.39(14)	0.39(14)	0.34(15,18,19)
LSD 5%	12.61				0.02			
	Mean germination time (day)				Speed germination index			
Min	8.6(16)	8.8(14)	9.0(14)	9.5(19)	4.88(6)	2.54(8)	1.93(8)	1.37(8)
Max	9.4(1)	9.9(8)	10.1(5)	10.3(11)	11.94(14)	10.83(14)	9.14(14)	6.16(19)
LSD 5%	0.22				1.23			
	Seedling length (cm)				Shoot length (cm)			
Min	6.8(6)	6.55(16)	5.33(8)	5.5(8)	4.48(11)	3(8)	3.17(8)	2.91(6)
Max	12.41(14)	11.39(14)	9.85(18)	8.55(19)	8.29(15)	5.71(17)	4.98(9)	4.76(3)
LSD 5%	0.867				0.46			
	Root length (cm)				Seedling fresh weight (mg)			
Min	2.91(6)	2.35(6)	2.34(15)	2.26(15)	6.0(12)	5.0(8)	2.0(2)	2.0(16)
Max	4.76(3)	6.16(14)	6.28(14)	5.18(18)	49.0(14)	28.0(18)	35.0(7)	29.0(7)
LSD 5%	0.717				0.61			
	Seedling dry weight (mg)				Seedling vigor index I			
Min	0.93(12)	0.44(16)	0.23(16)	0.13(16)	84.1(12)	33.4(16)	6.7(8)	3.0(8)
Max	5.01(7)	3.08(6)	2.41(14)	1.7(14)	429.4(7)	240.6(14)	193.3(14)	109.4(14)
LSD 5%	1.265				104.6			
	Seedling vigor index II							
Min	340.8(6)	232.9(8)	163.3(8)	125.0(8)				
Max	1241.5(14)	1084.4(14)	766.3(14)	598.9(19)				
LSD 5%	125.6							

Each value is followed by a genotype number (between brackets). For genotype name, see Tables 1 or 6.

Table 6. Salinity tolerance index (STI) of quinoa genotypes under different salinity stress levels.

No.	Genotypes	STI		
		3000 ppm	6000 ppm	9000 ppm
1	Giza-1	1.144 (MT)	0.856 (MT)	0.922 (MT)
2	R-103	0.766 (S)	0.474 (S)	0.751 (S)
3	ICBA-Q3	1.006 (MT)	1.032 (MT)	1.232 (T)
4	Chipaya	1.367 (T)	1.091 (MT)	1.214 (T)
5	CICA-17	1.654 (T)	0.926 (MT)	1.166 (MT)
6	Ollague	0.575 (S)	0.506 (S)	0.525 (S)
7	ICBA-Q5	1.248 (T)	1.541 (T)	1.702 (T)
8	Ku-2	0.654 (S)	0.531 (S)	0.924 (MT)
9	RH	1.972 (T)	1.315 (T)	1.64 (T)
10	KvlSRA2	1.792 (T)	1.309 (T)	1.273 (T)
11	CO-407	0.871 (MT)	0.714 (S)	0.642 (S)
12	Regalona	0.775 (S)	0.656 (S)	0.607 (S)
13	Misr-1	0.879 (MT)	0.765 (S)	0.689 (S)
14	Rainbow-2	3.157 (T)	2.795 (T)	2.971 (T)
15	Ql37	0.876 (MT)	0.876 (MT)	0.924 (MT)
16	Di-17	0.791(MT)	0.69 (S)	0.668 (S)
17	G-13	1.452 (T)	1.262 (T)	1.399 (T)
18	Ql3	2.24 (T)	1.841 (T)	1.784 (T)
19	Line- Q3	1.398 (T)	1.153 (MT)	1.388 (T)
	Max.	3.157 (14)	2.795 (14)	2.971 (14)
	Min.	0.575 (6)	0.474 (2)	0.525 (6)
No. of tolerant genotypes		9	6	9
No. of moderately tolerant		6	6	4
No. of sensitive genotypes		4	7	6

T = Tolerant, MT = Moderately tolerant, S = Sensitive.

of quinoa has also been reported by other investigators (Ruiz-Carassco *et al*., 2011; Adolf *et al*., 2012; Peterson and Murphy, 2015; Chaganti and Ganjegunte, 2022).

Salinity tolerance

Based on the salinity tolerance index (STI) calculated across germination and seedling traits, grouping the 19 quinoa genotypes resulted in three categories at each salinity stress level, namely, tolerant, moderately tolerant, and sensitive genotypes (Table 6). The number of tolerant, moderately tolerant, and sensitive genotypes was nine, six, and four under 3000 ppm NaCl conditions, six, six, and seven under 6000 ppm NaCl conditions, and nine, four, and six under 9000 ppm NaCl conditions, respectively, indicating that quinoa genotypes in the presented study had the lowest number of sensitive genotypes at the minimum salinity stress conditions (3000 ppm NaCl).

Generally, quinoa is a salt-tolerant crop (Hinojosa *et al*., 2018). Salinity tolerance, however, has been shown to vary significantly among quinoa cultivars/genotypes (Adolf *et al*., 2012; Peterson and Murphy, 2015; Kiani-Pouya *et al*., 2019; Nguyen *et al*., 2020), as well as, between various growth phases (Maleki *et al*., 2018; Derbali *et al*., 2020). It is worthy to mention that the six genotypes, ICBA-Q5 (G No. 7), RH (G No. 9), KvlSRA2 (G No. 10), Rainbow-2 (G No. 14), G-13 (G No. 17), and Ql3 (G No. 18), are tolerant to all salinity stress levels (3000, 6000, and 9000 ppm NaCl). These genotypes indicated the potential for good plant stand establishment and biomass production at the early seedling stage, essential for high production under high salt stress. The three genotypes, R103 (G No. 2), Ollague (G No. 6), and Regalona (G No. 12), are sensitive to all salinity stress levels. The other seven genotypes had different responses under different salinity stress levels.

Table 7. Correlation coefficients between stability tolerance index (STI) and absolute means for studied traits of 19 quinoa genotypes under different NaCl concentrations.

Trait	3000	6000	9000
Germination %	0.631**	0.709***	0.586**
Germination rate	0.499*	0.659**	0.487*
Speed germination index	0.675**	0.780***	0.546*
Mean germination time	-0.566**	-0.696***	-0.46*
Seedling length	0.884***	0.780***	0.598**
Shoot length	0.676**	0.663**	0.704***
Root length	0.850***	0.779***	0.254
Seedling fresh weight	0.855***	0.725***	0.591**
Seedling dry weight	0.298	0.673**	0.577**
Seedling vigor index I	0.712***	0.863***	0.759***
Seedling vigor index II	0.892***	0.833***	0.639**

*, **, and *** indicate significant at 0.05, 0.01 and 0.001 probability levels, respectively.

In descending order, the four most salinity-tolerant quinoa genotypes under all studied salinity stress conditions were Rainbow-2 (G No. 14), Ql3 (G No. 18), RH (G No. 9), and KvlSRA2 (G No. 10). These genotypes are highly recommendable to quinoa breeding programs aiming at improving salinity tolerance at the germination and seedling stages and to quinoa growers in the saline lands of Egypt. Contrastingly, the three most sensitive genotypes to salinity at 3000, 6000, and 9000 ppm NaCl levels were R103 (G No. 2), Ollague (G No. 6), and Regalona (G No. 12). Genotypic differences in salinity stress tolerance among quinoa genotypes at germination and seedling staged have also released by Maleki *et al*., (2018), Derbali *et al.* (2020), and Chaganti and Ganjegunte (2022).

Correlation analysis

Most studied germination and seedling traits had significant ($P \leq 0.05$, 0.01, or 0.001) and positive correlation coefficients with salinity tolerance index, except mean germination time, which had negative correlation coefficients with STI under elevated salinity stress levels (Table 7). Correlations between salinity tolerance index (STI) and studied germination and seedling traits of 19 quinoa genotypes were generally tougher at 6000 ppm for five features (germination percentage, germination rate, speed germination index, mean germination time, and seedling vigor index I), at 3000 ppm for four traits (seedling length, root length, seedling fresh weight, and seedling vigor index II), and at 9000 ppm for one quality (shoot length). Considering that significant correlation coefficients higher than 0.7 are strong correlations, the strongest correlations occurred with seedling length, root length, seedling fresh weight, seedling vigor index I, and seedling vigor index II under 3000 ppm, germination percentage, speed germination index, seedling length, root length, seedling fresh weight, seedling vigor index I, and seedling vigor index II under 6000 PPM, and shoot length and seedling vigor index I under 9000 ppm salinity concentration level. It suggests that these traits could be optional for quinoa breeders as selection criteria for improving salinity tolerance under the respective salinity concentration levels, especially if the heritability of these traits is high.

Genetic variability, heritability, and genetic advance

Phenotypic (PCV) and genotypic (GCV) coefficients of variation for germination and seedling growth traits of the 19 quinoa genotypes at different salt solution concentrations appear in Table 8. Maximum variability at the phenotypic and genotypic levels was notable in seedling vigor I trait, followed by seedling vigor II, fresh and dry weights, speed germination index, and germination percentage under all salinity concentrations. Such wide variability at both

Table 8. Phenotypic (PCV) and genotypic (GCV) coefficient of variation for germination and seedling traits of 19 quinoa genotypes under different NaCl concentrations.

Traits	Control		3000		6000		9000	
	PCV%	GCV%	PCV%	GCV%	PCV%	GCV%	PCV%	GCV%
Germination %	29.36	28.68	34.37	33.45	52.31	51.87	65.15	64.56
Mean germination time	4.26	4.23	5.18	5.11	5.5	5.45	4.4	4.31
Speed germination index	42.85	42.66	65.8	65.36	75.41	74.9	85.07	84.25
Seedling length	32.12	31.96	32.8	32.65	31.1	30.82	22	21.5
Shoot Length	31.52	31.40	31.22	31.07	23.42	23.04	25.99	25.44
Root length	47.58	47.23	44.64	44.23	43.42	42.8	25.77	24.76
Seedling fresh weight	68.23	66.38	80.86	78.04	133.76	130.50	123.19	112.29
Seedling dry weight	61.43	56.66	74.98	73.39	105.25	102.35	127.59	124.35
Seedling vigor index I	70.44	66.02	84.62	81.76	136.01	134.1	178.13	176.43
Seedling vigor index II	51.57	51.28	68.29	67.63	74.11	73.46	78.74	77.98

phenotypic and genotypic levels is a prerequisite for the successful development of salt-tolerant varieties of quinoa. Consequently, genotypes having vigorous seedlings of more dry and fresh weight might be a good option under salinity stress (Mohammad *et al*., 2008; Munir *et al*., 2013; Hinojosa *et al*., 2018). It is observable that variability at the phenotypic or genotypic level rises by increasing salinity concentration, reaching its maximum at 9000 ppm NaCl, suggesting that selection for more salinity tolerance would be more efficient under the severest salinity stress of 9000 ppm NaCl. Conversely, the lowest PCV and GCV percentage recording was by the mean germination time characteristic.

Heritability in the broad-sense (Table 9) was generally high (>83.0%), ranging from 83.08% for seedling fresh weight under 9000 ppm to 99.23% for shoot length under zero ppm salinity concertation level. Generally, the lowest heritability was under the severest salinity stress (9000 ppm) for seven traits (mean germination time, speed germination index, seedling length, shoot length, root length, seedling fresh weight, and seedling vigor index II), under no stress for two traits (seedling dry weight and seedling vigor I), and at 3000 ppm for one (germination percentage). Inversely, the highest heritability estimate showed under no salinity stress (control) for five traits (mean germination time, speed germination index, shoot length, root length, and seedling vigor index II), under 3000 ppm for two traits (seedling length and seedling dry weight), under 6000 ppm for seedling fresh weight, and with 9000 ppm for seedling vigor I feature. High salinity levels had more inhibiting effects on the heritability of seven studied germination and seedling traits. These results align with those on wheat reported by Munir *et al*. (2013) and Al-Naggar *et al*. (2015).

The highest expected genetic advance as a percent from the mean of the respective trait (GA%) from selection (based on 10% selection intensity) was generally noticeable if performing choices under the severest salinity stress (9000 ppm NaCl) for five traits (germination percentage, speed germination index, seedling dry weight, seedling vigor index I, and seedling vigor index II), under control (no salinity) for two traits (shoot and root lengths), at 6000 ppm for two features (mean germination time and seedling fresh weight), and under 3000 ppm for mean germination time and seedling fresh weight characteristics (Table 9).

The highest expected GA% emerged for seedling vigor I, followed by seedling dry and fresh weights, seedling vigor II, and speed germination index. In contrast, the lowest GA% occurred for mean germination time. These traits, with high genetic advance, also showed high heritability, and therefore, they are under the control of additive genetic variance amenable to efficient selection (Al-Naggar *et al*., 2015). Moreover, they are strongly correlated with salinity tolerance (Table 8) and, therefore, highly significant as good selection criteria for salinity tolerance in quinoa. Al-Naggar *et al*. (2015) concluded that germination and emergence rates could serve as selection criteria for wheat salt stress tolerance at early growth stages. Both root and shoot weights positively correlated with all seedling traits studied, indicating that root and shoot dry weights might be good selection criteria to evaluate genotypes under salinity stress (Munir *et al*., 2013; Al-Naggar *et a*l., 2015). Maas (1986) and Ashraf *et al.* (1999) also suggested that root and shoot dry weights could better serve as selection criteria under salinity stress. Meneguzzo *et al*. (2000) expressed that the dry weight can indicate salinity tolerance in controlled conditions.

Table 9. Broad-sense heritability (h^2_b%) and expected genetic advance from selection (GA %) for germination and seedling traits of 19 quinoa genotypes under different NaCl levels.

Traits	h^2_b%				GA%			
	Control	3000	6000	9000	Control	3000	6000	9000
Germination %	95.45	94.75	98.36	98.19	49.32	57.31	90.55	112.59
Mean germination time	98.20	97.03	98.11	95.83	7.37	8.85	9.5	7.43
Speed germination index	99.10	98.67	98.63	98.09	74.74	114.27	130.91	146.86
Seedling length	98.99	99.11	98.24	95.56	55.97	57.21	53.77	36.99
Shoot length	99.23	99.04	96.82	95.82	55.05	54.43	39.9	43.83
Root length	98.54	98.18	97.16	92.31	82.52	77.14	74.25	41.87
Seedling fresh weight	94.65	93.14	95.18	83.08	113.65	132.56	224.08	180.14
Seedling dry weight	85.07	95.79	94.58	94.98	91.97	126.42	175.18	213.28
Seedling vigor index I	87.86	93.36	97.22	98.10	108.92	139.05	232.72	307.55
Seedling vigor index II	98.87	98.08	98.23	98.08	89.74	117.88	128.14	135.93

CONCLUSIONS

Based on the salinity tolerance index calculated across the germination and seedling traits, the 19 quinoa genotypes grouped into three categories comprised the severest salinity stress level (9000 ppm NaCl), namely, tolerant (nine genotypes), moderately tolerant (four genotypes), and sensitive (six genotypes). The salinity-tolerant genotypes could be good recommendations to quinoa breeders and farmers who grow quinoa in the saline lands of Egypt. Quinoa traits showing robust correlations with STI, elevated heritability estimates, and high expected selection gain in this study are seedling vigor I, seedling dry and fresh weights, seedling vigor II, and speed germination index. These traits also showed wide variability at the phenotypic and genotypic levels and, therefore, distinctly recommended as selection criteria for germination and seedling salinity tolerance of quinoa under elevated levels of controlled salinity conditions.

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