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Screening for Salt Tolerance in *Chenopodium quinoa* Genotype Seedlings through Germination in a Hydroponic System

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Authors' contributions

This work was carried out in collaboration among all authors. Conceptualization, experimental design, writing of the original draft and preparation were done by authors SJ and ZY. Review, editing and supervision of the laboratory were done by authors ZY and SS. Biostatistical analyses and literature research were conducted by authors SJ and SS. All authors read and approved the final manuscript.

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ABSTRACT

Quinoa (*Chenopodium quinoa* Willd.) is poised to be a global life changer with its ability to adapt to a wide range of abiotic stresses and as a highly nutritious and sustainable food source. A trial on screening of salt tolerance was conducted at the germination and seedling stages of 69 quinoa

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genotypes in different concentrations of NaCl 0 (CK), 100, 200, 300, 400, and 500 mM for 21 days in the MS/2 mixture. This results in 16 genotypes with >50% germination at 400 mM NaCl. These were reassessed in germination indices and relative growth. Results indicated that *Chadmo* had the highest germinability of 97% and 32.76% relative height among the 16 genotypes. Considering the germination indices, *Chadmo* had significantly different values (3.05±0.19 day^{-1}) in mean germination time, coefficient of variation of the germination time (38.76±1.97%), the velocity of germination (0.23±0.01 day^{-1}), the uncertainty of germination (0.54±0.08 bit), synchrony of germination (0.42±0.05 and Timson's index (48.89) with significant differences (P<0.05) among the genotypes. Moreover, *Chadmo* had the highest membrane stability index (MSI) (60.03±11.84) at 400 mM NaCl and the least relative change between the CK and 400 mM NaCl with 30.87±2.01%. Assessing the stress inhibitory effect of the 16 genotypes, *Chadmo* had the least relative difference between the CK and 400 mM NaCl with shoot length of 34.34%, root length of 25.57%, fresh weight of 22.05%, dry weight of 3.62% and moisture content of 1.99% with Tukey analyses identifying significant differences (p<0.05). To select the salt-sensitive genotype, an assessment was done on five genotypes that exhibited the least germination at 200 mM NaCl. *Kankolla* had the least germinability with 12 and 4% at 100 and 200 mM NaCl, respectively. Considering all these parameters, *Chadmo* and *Kankolla* were selected as salt-tolerant and salt-sensitive for further analyses.

Keywords: Propagation; quinoa; moisture; NaCl; salt-sensitive; salt-tolerant; salinity.

1. INTRODUCTION

Germination is a critical stage in a plant's life cycle for propagation, continuation and inevitable for the survival of humans, as it forms the source of indispensable food and other necessities [1,2]. Bewley [3] defined germination as the outgrowth of a radicle through the endosperm and episperm of a seed. Germination is a dynamic process in which water plays an important role, hence any condition that limits the availability of water will result in delay or hindrance. Salinity and osmotic stress, temperature, light, and pH all influence the germinability of seeds [4-9]. While some seeds may be susceptible to slight variation, others have evolved to adapt to higher tolerance levels of various conditions. Salinity influences germination based on varieties, species and salt content of the soil and what mechanism is adopted by the plant for its protection and defence [5,8,10,11].

While some studies have indicated that salt tolerance during germination is independent of other growth phases in *Triticum aestivum* L. varieties [12] and *Solanum lycopersicum* L. [13]. Prado and Boero [14] outlined that once sprouting and rooting have occurred, then the seedlings have a high probability of proliferating successfully in their life cycle. Germination techniques and indices were also employed to identify salt-tolerant and salt-sensitive varieties of rice [15-18]. The screening was successful with 182 varieties of quinoa to identify the salt-sensitive and salt-tolerant during germination [19]. This method of screening was also supported by Nasir and Qureshi [20] and Cha-Um and Chuencharoen [21], they concluded that the seedling stage of sugarcane provides an effective strategy for screening salt-tolerant and susceptible varieties.

Salinity prevents or delays the germination of seeds or seedling growth and development. Germination indices can be used to select tolerant variety at an early stage. Even though many plants have a differential response at various stages to salinity, germination indices can be used as a precursor of selection, while some have posted that germination is independent of further growth and development. Also, some seeds that showed tolerance during germination do not necessarily transcend seedling growth and further development [12,13,22-25]. However, others have posited that germination and sprouting at the seedling stage are reliable and effective methods to categorize plants as salt-tolerant and sensitive varieties [14,19,20]. For selecting the tolerant variety for this study, germinability (G), mean germination time (GMT), coefficient of velocity of germination (CV_t), uncertainty of germination (U) and synchrony of germination (Z) indices were employed.

2. MATERIALS AND METHODS

2.1 Plant Materials and Growth Conditions

Salt-tolerant and salt-sensitive varieties were selected through rigorous screening from a

collection of 97 seeds obtained from the United States Department of Agriculture (USDA). The collected seeds were sown and propagated for seed proliferation and enhanced quality control. These plants were grown at the Fujian Agriculture and Forestry University glasshouse in ambient light in the temperature-controlled environment at about 24 - 26 °C and average relative humidity of ~ 65-70 % with 16/8 h light/dark photoperiod [26-28].

After harvest, seeds were stored at 4 °C until the experiment commenced [26]. Seeds were vapour sterilized with 3 % sodium hypochlorate and HCl in a desiccator placed in a fume hood for 4 ½ h after which they were air blown in a horizontal laminar flow hood for 3 h [26,29-31].

2.2 Treatment and Selection

Seeds were tested at 0, 100, 200, 300, 400, and 500 mM NaCl-induced MS/2 media over 21 days [29, 32-34]. Fifteen seeds were sown in tissue culture bottles containing MS/2 media with the respective salt concentration and control in three technical replicates placed in a culture room at 22 °C and 60 – 65 % RH [1,32-34]. Seed germination was recorded daily for seven days [26]. After seven days, the bottles were moved to another room with 26°C and 65 % RH, for better elongation and growth-related conditions to facilitate better seedling development [1,26]. Seedling height for the technical and biological replicates was measured and was recorded after 21 days. Seed tolerance screening occurred systematically in a three-tier method. Criteria used to identify and select the tolerant varieties were the germination percentage, mean germination time, coefficient of the velocity of germination, the uncertainty of germination, synchrony of germination and relative growth (height) - the ratio of plantlet height (cm) between the CK and the 400 mM NaCl to select the tolerant variety. Additionally, for validation and quality control, the varieties with ≥50% germinability (G_{50}) at 400 mM NaCl were subjected to further testing at 450 and 500 mM NaCl, but they all displayed <30 and 15% germinability, respectively and those that germinated died thereafter from apparent desiccation.

Salt-tolerant varieties were selected based on the mean germination time, mean germination rate, coefficient of variation of germination time, uncertainty of germination frequency, and synchrony of germination at 400 mM NaCl and the relative growth (height) between the control and maximum treatment [22,26,35-39]. The salt-sensitive varieties were selected based on the least germination percentage at the minimum treatment (100 mM NaCl) but with over maximum per cent germination at the control, credence to seed viability.

The first-tier screening resulted in 20 salt-tolerant and 5 salt-sensitive varieties. These were reassessed following the above procedure. The results did not indicate a significant difference from the initial screening. The most salt-tolerant varieties were then subjected to salt conditions at 400 and 500 mM NaCl-induced MS/2 [29,32-34]. This yielded similar results, as with the previous trials, at 400 mM NaCl but germination was poor (6 %) at 500 mM NaCl and with the few (3 seeds) that germinated, no elongation occurred, sprouting followed by death.

2.3 Germination Analysis

In determining the seed for the tolerant variety, the following germination parameters were used with the respective formulas:

2.3.1 Germinability

$$\sum_{i=1}^{n} Si / Di \qquad \text{Equation 1}$$

Where Si: germinated seeds per time (day), Di represents seed numbers from the start of the experiment to the i^{th}, ni: number of seeds germinated in the i^{th} day [9,26,35,36].

2.3.2 Mean germination time

$$\bar{t} = \frac{\sum_{i=1}^{k} n_i t_i}{\sum_{i=1}^{k} n_i} \qquad \text{Equation 2}$$

Where t_i: time (day) from the start of the experiment to the i^{th}, n_i: number of seeds germinated in the i^{th} day, and k: last time of germination [22,26,37,38].

2.3.3 Coefficient of variation of the germination time

$$s_t^2 = \frac{\sum_{i=1}^{k} n_i (t_i - \bar{t})^2}{\sum_{i=1}^{k} n_i - 1} \qquad \text{Equation 3 (i)}$$

Where t: mean time; t_i: the time between the start of the experiment and the i^{th} day; *ni*: number of seeds germinated in the i^{th} day, and *k*: last day of germination. The variance value will be used to calculate the coefficient of variation of the germination time in the subsequent formula:

$$CV_t = \frac{s_t}{\bar{t}} 100$$

Equation 3 (ii)

Where s_t: the standard deviation of the germination time and t is: mean germination time [26,35,36,39].

2.3.4 Uncertainty of germination

$$U = -\sum_{i=1}^{k} f_i \log_2 f_i, \text{ being } f_i = \frac{n_i}{\sum_{i=1}^{k} n_i}$$

Equation 4

Where $n_{i\ is}$: number of seeds germinated on the i^{th} time, and *k is*: last day of observation [36].

2.3.5 Synchrony of germination (Z)

$$(x + a)^n = \sum_{k=0}^{K} C^k a^{n-k}$$

Equation 5

$$Z = \frac{\sum_{i=1}^{k} C_{n_i,2}}{C_{\sum n_i,2}}, \text{ being } C_{n_i,2} = n_i(n_i\text{-}1)/2$$

Equation 6

Where: $C_{ni,2}$ combinations of the seeds germinated in the i^{th} time, two by two, and *ni*: number of seeds germinated in the i^{th} time. *Z* is the quotient between the sums of the partial combinations of the number of seeds germinated in each *ti*, two by two combinations of the total number of seeds germinated at the end of the experiment [36].

2.3.6 Membrane stability index and stress inhibitory effect

Additionally, the '*stress inhibitory effect*' was calculated as a percentage of at the level of inhibition between the CK and 400 mM NaCl [40]. These 16 genotypes were assessed on their membrane stability index (MSI) for selecting the most salt-tolerant genotype at the control (CK), 200 mM NaCl, 300 mM NaCl and 400 mM NaCl). The salt-sensitive genotypes were selected based on the least germination percentage at the minimum treatment (200 mM NaCl) but with over maximum germination percentage at the CK to ensure seed viability.

3. RESULTS

3.1 Selecting Salt-Tolerant Variety

Even though 20 genotypes have shown >50 germinations at the 400 mM NaCl, only 16 genotypes exhibited growth in shoot and root elongation. Interestingly, they germinated, but further plumule and radicle elongations ceased and hence, these 4 genotypes were not considered as candidates for further screening. Additionally, for validation and quality CK, the genotypes with ≥50% germinability (G_{50}) at 400 mM NaCl were subjected to further testing at 450 and 500 mM NaCl, but they all displayed <32 and 17% germinability, respectively and those that germinated died from apparent desiccation. For the highest germinability, genotypes *Chadmo* and PI 587173 had 97 and 93%, respectively. For the relative growth, they also exhibited the least between the and 400 mM NaCl with *Chadmo* at 32.76% and PI 614884 at 45.89%. ANOVA and Tukey analyses identified varied significant differences (p>0.05) among the genotypes in both germinability and relative growth (height) (Fig. 1).

3.1.1 Selecting salt-tolerant genotype: Germination indices

Even though germinability was not the highest at 400 mM NaCl among the genotypes for *Chadmo*, germinability (between the CK and 400 mM NaCl was recorded as the highest (97.77±2.22). The MGT for Ames 13723 (5.04±0.15), Ames 13735 (5.04 ±0.08) and Ames 13736 (5.60±0.07) were higher than that of *Chadmo* (3.05±0.07) their coefficients of variation in germination time were lower with Ames 13723 (15.41±1.14), Ames 13735 (14.65 ±0.83) and Ames 13736 (9.13±0.51) than *Chadmo* with 38.76±1.97. ANOVA identified the significant difference and a strong correlation between the CK and 400 mM NaCl for all the indices and the genotypes at P<0.05. Additionally, Timson's germination index showed that *Chadmo* (48.89) had the highest value among the genotypes (Table 1).

3.1.2 Selecting salt-tolerant genotype: MSI

MSI indicates the damage done to the cell membrane under stressful conditions. The higher the MSI, the more adaptable the plant is to that

Table 1. Germination indices at 400 NaCl treatment

No.	Accession #	MGT	CV_t (%)	v (day^{-1})	U (bit)	Z	Timson's index
1	PI 677096	3.97±0.25*c,d*	26.52±3.62*b,c,d*	0.25±0.02*a,b,c,d*	1.70±0.06*a,b*	0.31±0.04*c,d,e*	38.89
2	PI 433232	3.80±0.11*c,d*	27.76±0.00*a,b,c*	0.26±0.01*a,b,c,d*	1.91±0.01*a,b*	0.22±0.00*d,e,f*	45.56
3	PI 674266	3.78±0.21*c,d*	36.98±1.92*a,b,c*	0.26±0.01*a,b,c*	1.22±0.06*b,c*	0.09±0.01*e,f*	41.11
4	PI 677100	3.17±0.15*e*	21.61±4.54*c,d*	0.31±0.01*a,b*	1.31±0.28*b,c*	0.33±0.06*c,d,e*	25.56
5	Ames 13722	4.61±0.42*b*	7.74 ±5.16*f*	0.21±0.02*c,d,e*	0.54±0.35*d*	0.75±0.17*a*	27.78
6	Ames 13723	5.04±0.15*b*	15.41±1.14*e,f*	0.19±0.01*e*	1.41±0.08*b,c*	0.32±0.06*c,d,e*	27.78
7	Ames 13724	3.47±0.21*d,e*	21.06±3.83*d,e*	0.28±0.01*a*	0.98±0.06*c,d*	0.50±0.02*b*	26.67
8	Ames 13725	4.60±0.08*b*	23.48±2.45*c,d,e*	0.21±0.00*c,d,e*	1.62±0.14*a,b*	0.28±0.06*d,e,f*	25.56
9	Ames 13726	4.68±0.19*b*	23.95±4.61*c,d,e*	0.21±0.01*d,e*	1.60±0.28*a,b*	0.28±0.09*d,e,f*	24.44
10	Ames 13735	5.04±0.08*b*	14.65±0.83*e,f*	0.19±0.00*e*	1.44±0.06*b,c*	0.32±0.04*c,d,e*	32.22
11	Ames 13736	5.60±0.07*a*	9.13±0.51*f*	0.17±0.00*e*	0.95±0.06*c,d*	0.47±0.05*b,c*	33.33
12	Ames 13740	3.46±0.17*d,e*	26.74±4.54*b,c,d*	0.29±0.01*a*	1.42±0.29*b,c*	0.36±0.12*b,c,d*	28.89
13	Ames 13747	4.01±0.06*c*	26.58±2.78*b,c,d*	0.24±0.00*a,b,c,d*	1.61±0.23*a,b*	0.30±0.08*c,d,e*	33.33
14	PI 587173	3.59±0.19*b*	35.90±4.89*a,b*	0.21±0.01*c,d,e*	1.91±0.08*a,b*	0.24±0.02*d,e,f*	47.78
15	*Chadmo*	3.05±0.16*f*	38.76±1.97*a*	0.23±0.01*b,c,d*	0.54±0.08*a*	0.42±0.05*f*	48.89
16	PI 614884	3.64±0.19*c,d,e*	32.22±1.60*a,b,c*	0.27±0.01*a,b*	1.89±0.34*a,b*	0.23±0.06*e,f*	46.67

Means±SD (n=45). Different letters indicate a significant difference at P<0.05 (Fisher pairwise grouping comparison) among the different genotypes for each index. (MT = t: mean germination time; v: mean germination rate; CVt: coefficient of variation of the germination time; U: uncertainty of the germination frequency; Z: synchrony of the germination process)

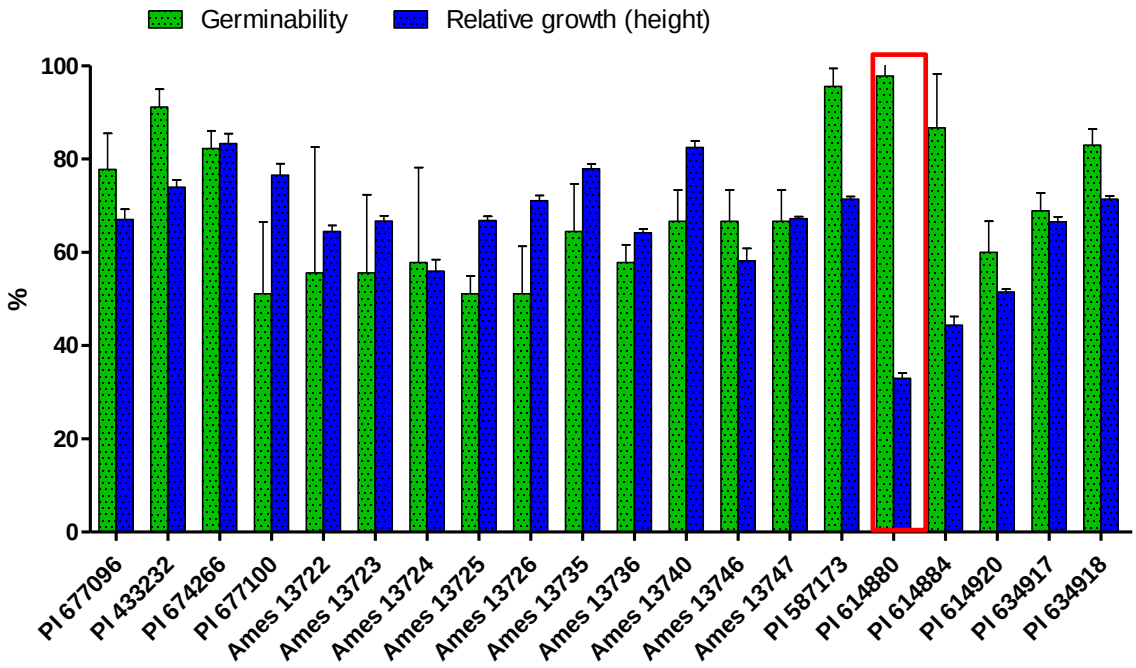

Fig. 1. Germinability and relative growth between control and 400 mM NaCl of the salt-tolerant varieties (G_{50})

Data represents means±SD of fifteen biological and three technical replicates

Table 2. Effect of salinity regimes on the membrane stability index of the genotypes

No	Genotype	MSI – mM NaCl			
		CK	200	300	400
1	PI677096	86.88±6.15*a*	66.90±9.13*b*	50.67±9.17*c*	51.25±9.13*c*
2	PI 433232	84.59±9.09*a*	68.27±8.68*b*	59.12±6.32*b,c*	52.85±10.00*c*
3	PI 674266	89.09±1.73*a*	70.11±7.62*b*	58.05±8.26*c*	56.67±10.46*c*
4	PI 677100	85.83±7.30*a*	74.68±8.31*b*	54.39±10.21*c*	55.79±9.35*c*
5	Ames 13722	86.98±7.94*a*	78.19±8.72*a*	58.43±13.77*b*	59.58±10.89*b*
6	Ames 13723	88.35±8.07*a*	79.76±12.39*a,b*	73.44±12.74*b*	47.23±13.79*c*
7	Ames 13724	90.07±6.59*a*	76.24±10.39*b*	70.58±12.90*b*	56.17±11.05*c*
8	Ames 13725	83.15±9.40*a*	74.91±10.85*a*	53.61±12.87*b*	48.53±7.28*b*
9	Ames 13726	85.85±7.64*a*	76.16±7.84*a*	61.31±13.16*b*	55.61±7.81*c*
10	Ames 13735	87.04±7.17*a*	75.48±10.91*b*	56.32±12.95*c*	49.11±7.59*c*
11	Ames 13736	90.67±6.35 *a*	79.04±12.16*b*	51.37±9.07*c*	47.78±9.78*c*
12	Ames 13746	86.26±7.86*a*	77.57±8.79*a.b*	71.62±11.72*b*	48.54±12.70*c*
13	Ames 13747	87.68±7.72*a*	79.09±10.39*a*	51.37±9.07*b*	47.78±9.78*b*
14	PI 587173	86.86±7.87*a*	77.57±8.79*a*	57.95±11.76*b*	49.11±7.59*b*
15	*Chadmo*	86.84±7.03*a*	74.95±12.44*b*	64.76±9.88*b,c*	60.03±11.84*c*
16	PI 614884	88.87±1.72*a*	70.11±7.62*b*	58.05±8.26*b*	56.67±10.46*c*

Means±SD (n=15) with three biological replicates. Different letters indicate a significant difference at P<0.05 (Tukey analyses) between the CK and the different concentrations of the different genotypes

condition. The results indicated that *Chadmo* had the highest (60.03±11.84) MSI among the genotypes at 400 mM NaCl while at 200 mM NaCl and 300 mM NaCl Ames 13723 had the highest with 79.76±12.39 and 73.44±12.74, respectively. It must be noted that between the CK and 400 mM NaCl, *Chadmo* had a 46.99% decrease, representing the least affected/damaged while the most affected genotype was the least affected decrease as

opposed to Ames 13736 which was affected the most with a 47.3% decrease. Analysis of variance identified significant differences among all the treatments and genotypes, more particularly between the CK and 400 mM NaCl at $p<0.05$

3.1.3 Stress inhibitory effect (relative change)

The relative change between the CK and 400 mM NaCl for *Chadmo* for shoot length, root length, fresh weight, dry weight, and moisture content were 34.34±5.31, 25.57±7.12, 22.05±2.56, 3.62±1.16 and 1.99±0.97, respectively. The highest relative change was observed in shoot length, root length, fresh weight, dry weight, and moisture content were PI674266 (81.18±4.74), PI433232 (81.19±5.97), PI614884 (53.07±1.92), PI614884 (12.58±0.69) and PI677096 (14.92±0.90), respectively (Fig. 2). From the *stress inhibitory effect* among the genotypes for the shoot length, root length, fresh weight, dry weight, and moisture content, it is evident the least effect was the one with the lowest values, *Chadmo*, and hence, regarded as the most tolerant in salinity stress at 400 mM NaCl. Analyses of variance and Tukey have identified a significant difference ($P<0.05$) between the variables for each genotype.

3.2 Selection of Salt-Sensitive Variety

The criteria applied for the selection of the salt-sensitive varieties were the lowest germination percentage at the 100 and 200 mM NaCl and the largest height difference between the control and 100 mM NaCl (68.21%) and between control and 200 mM NaCl (72.24%). The highest reduction in relative growth between the CK and 100 was observed with PI510551 (68.21%) while the lowest was Ames 13756 (23.70%). However, between CK and 200 mM NaCl, Ames 13755 (74.94%) had the highest and Ames 13756 had the lowest with 38.94% (Table 3). Germination took precedence for genotypes *Kankolla* (4%) and PI 614932 (6%) for 200 mM NaCl because it marked the threshold for halophytes. Additionally, while *Kankolla* had shown 4% germination at 200 mM NaCl, no plumule or radicle elongation occurred. Ames 13755, Ames 13756 and PI 478418 all indicated low germination rates at 200 mM NaCl and they sprouted but with significantly low relative growth (height) (Table 3), and also showed evidence of germination at 300 mM NaCl which excludes them from being considered as highly salt-sensitive. A significant difference was observed among the treatment and genotypes by ANOVA and Tukey at $P<0.05$.

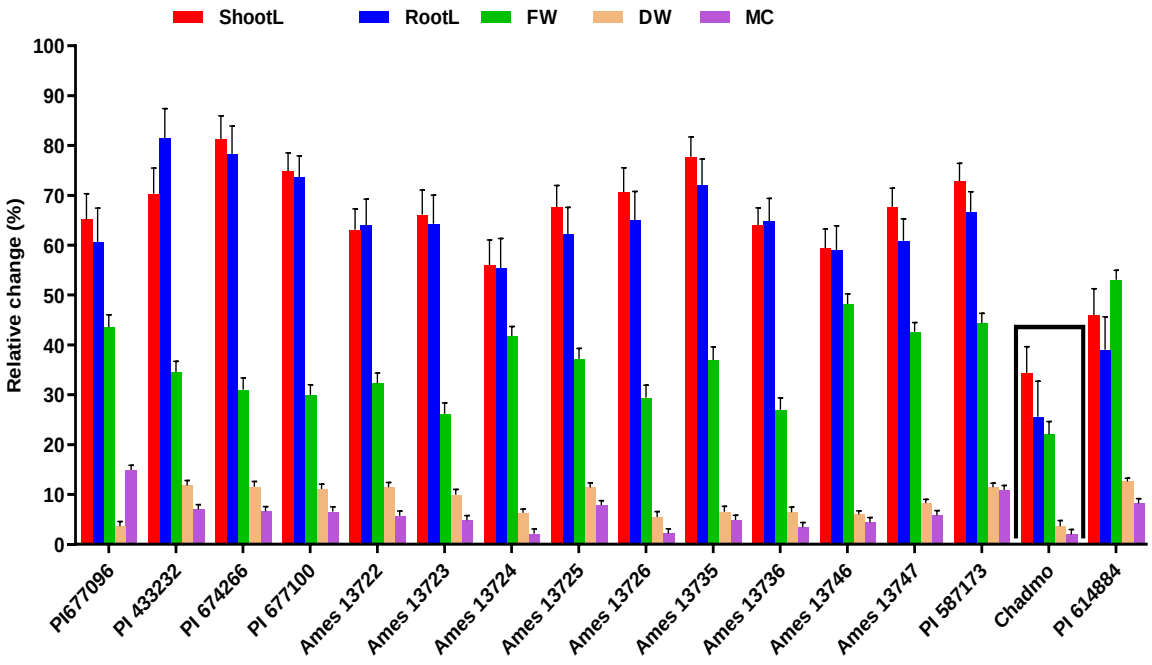

Fig. 0. Effect of salinity on the relative change between CK and 400 mM NaCl on the different genotypes

Means±SD for three biological replicates

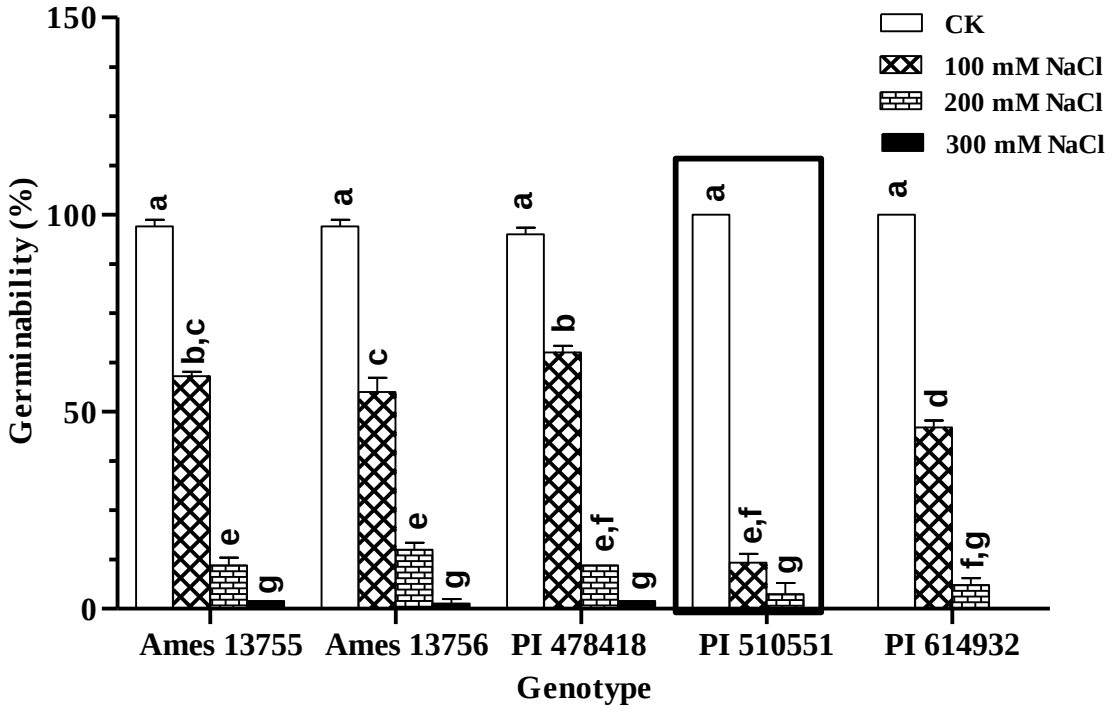

Fig. 1. Effect of salinity on the germinability of the salt-sensitive genotypes

Mean ± SD (n=45) Different letters indicate a significant difference at P<0.05 among the genotypes and treatment

Table 3. Selected salt-sensitive varieties through germination and growth

Genotype	Height (cm)					Relative growth (height)		
	CK	100	200	300	400	100	200	300
Ames 13755	3.99±1.98	1.34±0.87	1±0.00	1.1±0.00	0±0.00	66.41	74.94	72.43
Ames 13756	3.98±0.90	2.35±1.01	2.43±0.68	1.5±0.5	0±0.00	23.70	38.94	62.31
PI 478418	3.64±1.01	2±1.1	2±1.01	1.4±0.3	0±0.00	45.05	45.05	61.54
Kankolla	**5.02±1.87**	**1.6±0.67**	**nd**	**nd**	**nd**	**68.21**	**nd**	**nd**
PI 614932	3.89±1.87	2.4±0.67	1.9±1.01	0±0.00	0±0.00	38.30	51.16	nd

Nd – no data/no growth

4. DISCUSSION

Germination is a key process that catapults plants into a continuous cycle of multiple biological and physiological processes that will determine their ability to survive and reproduce, and in many instances, in conditions that may not be conducive. Seed germination is the most critical stage in the growth of the plant and is the most sensitive when exposed to abiotic stress [40,41]. Salinity is an environmental factor that significantly influences germination and hence, the plant's prospect of continuity. Salinity has shown a delay or prohibition of germination in many genotypes of plants and those that survived, in most cases, proceeded to grow, produce, and reproduce and hence, are deemed to be tolerant [4,6-8]. Despite its halophytic nature, quinoa is rather sensitive to stressful conditions in its vegetative stage, hence, if survives seedling establishment is highly possible [42,43]. Among the 16 most tolerant genotypes assessed, *Chadmo* had the highest percentage germinability, among the highest in germination meantime, and the lowest relative growth is calculated as the difference between the CK and 400 mM NaCl. Having the highest germinability (97%) among the 16 genotypes, and with no fatality, is indicative of the ability to germinate in highly saline conditions and progress to the seedling establishment (400 mM NaCl). Germination is regarded as the most sensitive stage to abiotic stress in the development of a plant and therefore, once they

have survived, the seedling establishment will progress [40,41]. While germination is important in determining tolerance to salinity, if seedling elongation and growth do not proceed then it is irrelevant to the continuity of its life cycle. However, in the assessment of the seedling elongation and growth of the 16 genotypes, *Chadmo* had the lowest relative growth (32.76%), which is interpreted as the least difference between the CK and 400 mM NaCl among the genotypes.

With these 16 genotypes, root length decreased significantly between the CK and 400 mM NaCl, *Chadmo* exhibited the minimum difference between the CK and 400 mM NaCl, which indicated it is least affected and hence the most tolerant regarding root growth while PI674266 was most affected with 81.19%. The survival of a plant being exposed to salinity mainly depends on how the root system manipulates the intake and distribution of salt as it is the first interface between the plant and that stressful abiotic condition. However, the robust root structure allows them to survive and more so it is sometimes the least affected as compared to shoots, but root elongation is affected by salinity at higher concentrations [19,44,45]. Some plants are very well adapted to exclude salt at the level of the root by developing a salt filtration mechanism through enhancing hydrophobic barrier deposition, which prevents the absorption of non-selective apoplastic ions [46-48]. At all levels of plant growth, height decrease is symptomatic of salinity stress and if a plant can germinate and proceed to growth then it is undoubtedly tolerant of such stressful conditions [14,19].

To infer from a comparative perspective, the MSI index was assessed at CK, 200, 300 and 400 mM NaCl. While the response was differential among the genotypes and the treatments, *Chadmo* recorded the lowest difference (26.81%) between the CK and 400 mM NaCl while the highest relative difference (42.90%) was observed in Ames 13736. Salinity results in significant to plant cells and more particularly on the membrane and these damages are measured through membrane stability index or electrolyte leakage [44,49-51]. In this study, therefore, *Chadmo* (30.87%) had the least relative difference between the CK and 400 mM NaCl among the 16 salt-tolerant genotypes and hence is designated as the most salt-tolerant. Additionally, nine genotypes of pea plants indicated a decrease in MSI under salinity for all the genotypes at different NaCl treatments (25, 50 and 75 mM NaCl) as compared to the CK [52]. In support, it was also concluded that salt-treated strawberries had a 10% reduction in MSI when treated with 50 mM NaCl [53].

Germination indices can be used to select tolerant genotypes at an early stage. Even though many plants have a differential response at various stages to salinity, germination indices can be used as a precursor for selection, while some have posited that germination is independent of further growth and development [22-25]. Quinoa tolerance to salinity during germination results from the changes in the primary metabolites and enzyme activity in response to salinity [54,55]. Many also supported the idea that germination and sprouting at the seedling stage are reliable and effective methods to categorize plants as salt-tolerant and sensitive genotypes [14,19].

Based on these results on the germination indices, *Chadmo* is deemed as the most tolerant genotype among the 16 salt-tolerant genotypes. Hence, the germination process under saline conditions is independent of other biological and physiological processes [14,19]. These germination indices have been used singly or collectively to screen for salt tolerance at the seedling stage in many plants. Germinability and seedling growth were used to assess the responses of three cultivars of bean (*Phaseolus vulgaris* L.) to NaCl and Na_2SO_4 and results showed that both have an inhibitory effect on germination and seedling development [56]. Furthermore, the responses of *Atriplex prostrata and A. patula* after being exposed to NaCl and PEG were determined with the application of germination rate and percentages were used to assess their susceptibility [45]. In support, also worked with quinoa (cv *Titicaca*) to identify germination and seedling tolerance levels to saline water using the germination traits of the coefficient of velocity of germination, germination rate index and mean germination time [26]. They posited that salinity at a lower concentration does not affect germination percentages but rather increases the germination rate.

The germination indices were irrelevant to selecting salt-sensitive genotypes because to decide on their sensitivity, it was based on them not germinating and developing into seedlings under saline conditions [19]. Among the five sensitive genotypes assessed, *Kankolla* had the lowest germinability at 100 mM NaCl and 200

mM NaCl with 12 and 4%, respectively and no germination occurred at 300 mM NaCl. Therefore, upon these observations, *Kankolla* was selected as the most salt-sensitive among the five tested genotypes. Plants that proliferate in about 200 mM NaCl concentration are referred to as halophyte which makes up about only 1% of all other plants [57-59]. *Kankolla* based on these criteria was regarded as the most salt-sensitive genotype [60-63].

5. CONCLUSION

It can be concluded that the two contrasting genotypes of salinity tolerance were *Chadmo* (salt-tolerant) and *Kankolla* (salt-sensitive). Further evidence to support this, was entrenched in the origin and locale of both genotypes and the prevailing environmental conditions; *Chadmo* originated from the coastline of Chile (<10 m from the Southern Pacific Ocean), while *Kankolla* originated deeper, and in the upland area of Arapa District in Peru (387 km from the Southern Pacific Ocean). The coastline is normally inundated with saltwater and marshy areas, therefore, causing the soil to be saline. Hence, if *Chadmo* thrives in this area then would have to be halophytic. Conversely, *Kankolla* predominantly grows in the upland areas thereby becoming more adapted and thrives in non-saline soils and is, therefore, more sensitive to salinity. Additionally, the results of germination in response to the different salinity to select the most salt-tolerant genotype, in the above experiment, have been further corroborated in morpho-physicochemical considerations on the salt-treated and control seedlings in the subsequent experiment.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Joosen RV, Kodde J, Willems LA, Ligterink W, van der Plas LH, Hilhorst HW. GERMINATOR: A software package for high-throughput scoring and curve fitting of Arabidopsis seed germination. Plant J. 2010;62(1):148-59.
2. Larson JE, Sheley RL, Hardegree SP, Doescher PS, James JJ. Seed and seedling traits affecting critical life stage transitions and recruitment outcomes in dryland grasses. J Appl Ecol. 2015; 52(1):199-209.
3. Bewley JD. Seed Germination and Dormancy. Plant Cell. 1997;9(7):1055-66.
4. Dekker J, Gilbert J. Weedy adaptation in *Setaria* spp.: IX. Effects of salinity, temperature, light and seed dormancy on *Setaria faberi* seed germination. arXiv preprint arXiv:14086187. 2014.
5. Hanif Z, Naeem M, Ali HH, Tanveer A, Javaid MM, Peerzada AM, et al. Effect of Environmental Factors on Germination of *Salsola foetida*: Potential Species for Rehabilitation of Degraded Rangelands. Rangeland Ecol Manage. 2017;70(5):638-43.
6. Mahmood AH, Florentine SK, Chauhan BS, McLaren DA, Palmer GC, Wright W. Influence of Various Environmental Factors on Seed Germination and Seedling Emergence of a Noxious Environmental Weed: Green Galenia (*Galenia pubescens*). Weed Sci. 2016;64(3):486-94.
7. Tanveer A, Mumtaz K, Javaid MM, Chaudhry MN, Balal RM, Khaliq A. Effect of ecological factors on germination of horse purslane (*Trianthema portulacastrum*). Planta Daninha. 2013;31 (3):587-97.
8. Javaid MM, Florentine S, Ali HH, Weller S. Effect of environmental factors on the germination and emergence of *Salvia verbenaca* L. cultivars (verbenaca and vernalis): An invasive species in semi-arid and arid rangeland regions. PLoS One. 2018;13(3).
9. Bai J, Yan W, Wang Y, Yin Q, Liu J, Wight C, et al. Screening oat genotypes for tolerance to salinity and alkalinity. Frontiers in plant science. 2018;9.
10. Batlla D, Benech-Arnold RL. Weed seed germination and the light environment: Implications for weed management. Weed Biol Manage. 2014;14(2):77-87.
11. Florentine SK, Weller S, Graz PF, Westbrooke M, Florentine A, Javaid M, et al. Influence of selected environmental factors on seed germination and seedling survival of the arid zone invasive species

tobacco bush (*Nicotiana glauca* R. Graham). Rangeland Journal. 2016;38(4):417-25.
12. Mano Y, Takeda K. Genetic resources of salt tolerance at germination and the seedling stage in wheat [*Triticum aestivum*]. Japanese Journal of Crop Science. 2001;70(2):215-20.
13. Foolad M, Lin G. Absence of a genetic relationship between salt tolerance during seed germination and vegetative growth in tomato. Plant Breeding. 1997;116(4):363-7.
14. Prado FE, Boero C, Gallardo M, Gonzalez JA. Effect of NaCl on germination, growth, and soluble sugar content in *Chenopodium quinoa* Willd. seeds. Botanical Bulletin of Academia Sinica. 2000;41.
15. Mondal A, Pramanik S. In vitro studies of salt tolerance of three rice races in Bay Islands. J Indian Soc Coastal Agric Res. 1995;13(2):127-31.
16. Shi YY, Gao LL, Wu ZC, Zhang XJ, Wang MM, Zhang CS, et al. Genome-wide association study of salt tolerance at the seed germination stage in rice. Bmc Plant Biology. 2017;17(1):92.
17. Wang ZF, Wang JF, Bao YM, Wu YY, Zhang HS. Quantitative trait loci controlling rice seed germination under salt stress. Euphytica. 2011;178(3):297-307.
18. Wang ZF, Wang JF, Bao YM, Wu YY, Xuan SU, Zhang HS. Inheritance of rice seed germination ability under salt stress. Rice Science. 2010;17(2):105-10.
19. Gómez-Pando LR, Álvarez-Castro R, Eguiluz-de la Barra A. Short Communication: Effect of Salt Stress on Peruvian Germplasm of *Chenopodium quinoa* Willd.: A Promising Crop. Journal of Agronomy and Crop Science. 2010;196(5):391-6.
20. Nasir N, Qureshi R, Aslam M. A quick screening technique for salt tolerance in sugarcane (*Saccharum officinarum* L.). Pakistan Sugar Journal. 2000;15(1): 2-7.
21. Cha-Um S, Chuencharoen S, Mongkolsiriwatana C, Ashraf M, Kirdmanee C. Screening sugarcane (*Saccharum* sp.) genotypes for salt tolerance using multivariate cluster analysis2012 10/12/2018.
22. Aflaki F, Sedghi M, Pazuki A, Pessarakli M. Investigation of seed germination indices for early selection of salinity tolerant genotypes: A case study in wheat. Emirates Journal of Food and Agriculture. 2017:222-6.
23. Bybordi A. The Influence of Salt Stress on Seed Germination, Growth and Yield of Canola Cultivars. Notulae Botanicae Horti Agrobotanici Cluj-Napoca. 2010;38(1):128-33.
24. Houle G, Morel L, Reynolds C, Siegel J. The effect of salinity on different developmental stages of an endemic annual plant, *Aster laurentianus* (Asteraceae). Am J Bot. 2001;88(1):62-7.
25. Sanchez PL, Chen M-k, Pessarakli M, Hill HJ, Gore MA, Jenks MA. Effects of temperature and salinity on germination of non-pelleted and pelleted guayule (*Parthenium argentatum* A. Gray) seeds. Ind Crops Prod. 2014;55:90-6.
26. Panuccio MR, Jacobsen SE, Akhtar SS, Muscolo A. Effect of saline water on seed germination and early seedling growth of the halophyte quinoa. AoB PLANTS. 2014;6:plu047.
27. Goyal E, Amit SK, Singh RS, Mahato AK, Chand S, Kanika K. Transcriptome profiling of the salt-stress response in Triticum aestivum cv. Kharchia Local. Scientific reports. 2016;6:27752.
28. Morales AJ, Bajgain P, Garver Z, Maughan PJ, Udall JA. Physiological responses of *Chenopodium quinoa* to salt stress. International Journal of Plant Physiology and Biochemistry. 2011;3(13):219-32.
29. Lindsey BE, 3rd, Rivero L, Calhoun CS, Grotewold E, Brkljacic J. Standardized Method for High-throughput Sterilization of Arabidopsis Seeds. J Vis Exp. 2017(128):56587.
30. Burrieza HP, Koyro H-W, Tosar LM, Kobayashi K, Maldonado S. High salinity induces dehydrin accumulation in Chenopodium quinoa Willd. cv. Hualhuas embryos. Plant Soil. 2012;354(1-2):69-79.
31. Ruiz-Carrasco K, Antognoni F, Coulibaly AK, Lizardi S, Covarrubias A, Martínez EA, et al. Variation in salinity tolerance of four lowland genotypes of quinoa (Chenopodium quinoa Willd.) as assessed by growth, physiological traits, and sodium transporter gene expression. Plant Physiol Biochem. 2011;49(11):1333-41.
32. Murashige T, Skoog F. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiologia plantarum. 1962;15(3):473-97.
33. Postnikova OA, Shao J, Nemchinov LG. Analysis of the Alfalfa Root Transcriptome

in Response to Salinity Stress. Plant and Cell Physiology. 2013;54(7):1041-55.
34. Wang W-B, Kim Y-H, Lee H-S, Kim K-Y, Deng X-P, Kwak S-S. Analysis of antioxidant enzyme activity during germination of alfalfa under salt and drought stresses. Plant Physiol Biochem. 2009;47(7):570-7.
35. Maguire JD. Speed of Germination—Aid In Selection And Evaluation for Seedling Emergence And Vigor 1. Crop science. 1962;2(2):176-7.
36. Ranal MA, Santana DGd, Ferreira WR, Mendes-Rodrigues C. Calculating germination measurements and organizing spreadsheets. Brazilian Journal of Botany. 2009;32(4):849-55.
37. Scott S, Jones R, Williams W. Review of Data Analysis Methods for Seed Germination 1. Crop science. 1984;24(6):1192-9.
38. Kader M. A comparison of seed germination calculation formulae and the associated interpretation of resulting data. Journal and Proceeding of the Royal Society of New South Wales. 2005;138:65-75.
39. Kader M, Jutzi S. Effects of thermal and salt treatments during imbibition on germination and seedling growth of sorghum at 42/19 C. J Agron Crop Sci. 2004;190(1):35-8.
40. Mickky BM, Aldesuquy HS. Impact of osmotic stress on seedling growth observations, membrane characteristics and antioxidant defense system of different wheat genotypes. Egyptian Journal of Basic and Applied Sciences. 2017;4(1):47-54.
41. Yadav PV, Kumari M, Ahmed Z. Seed priming mediated germination improvement and tolerance to subsequent exposure to cold and salt stress in capsicum. Research Journal of Seed Science. 2011;4(3):125-36.
42. Ruiz K, Rapparini F, Bertazza G, Silva H, Torrigiani P, Biondi S. Comparing salt-induced responses at the transcript level in a salares and coastal-lowlands landrace of quinoa (*Chenopodium quinoa* Willd.). 139. 2017:127/42.
43. Ruiz KB, Maldonado J, Biondi S, Silva H. RNA-seq Analysis of Salt-Stressed Versus Non Salt-Stressed Transcriptomes of *Chenopodium quinoa* Landrace R49. Genes. 2019;10(12):1042.
44. Munns R, Tester M. Mechanisms of salinity tolerance. Annu Rev Plant Biol. 2008;59:651-81.
45. Katembe WJ, Ungar IA, Mitchell JP. Effect of Salinity on Germination and Seedling Growth of two Atriplex species (Chenopodiaceae). Ann Bot. 1998;82:167-75.
46. Kolattukudy PE. Biochemistry and function of cutin and suberin. Can J Bot. 1984;62(12):2918-33.
47. Krishnamurthy P, Jyothi-Prakash P, A, Qin L, He J, Lin Q, Loh C-S, et al. Role of root hydrophobic barriers in salt exclusion of a mangrove plant *Avicennia officinalis*. Plant, Cell Environ. 2014;37 (7):1656-71.
48. Lawton JR, Todd A, Naidoo D. Preliminary investigations into the structure of the roots of the mangroves, *Avicennia marina* and *Bruguiera gymnorrhiza*, in relation to ion uptake. New Phytol. 1981;88(4):713-22.
49. Panta S, Flowers T, Lane P, Doyle R, Haros G, Shabala S. Halophyte agriculture: Success stories. Environmental and Experimental Botany. 2014;107:71-83.
50. Sairam RK, Srivastava GC. Changes in antioxidant activity in sub-cellular fractions of tolerant and susceptible wheat genotypes in response to long term salt stress. Plant Sci. 2002;162(6):897-904.
51. Shabala S, Mackay A. Ion transport in halophytes. Advances in botanical research. 2011;57:151-99.
52. Shahid M, Balal R, Pervez M, Abbas T, Ashfaq M, Ghazanfar U, et al. Differential response of pea (*Pisum sativum* L.) Genotypes to salt stress in relation to the growth, physiological attributes antioxidant activity and organic solutes. Australian Journal of Crop Science. 2012;6(5):828.
53. Avestan S, Ghasemnezhad M, Esfahani M, Byrt CS. Application of Nano-Silicon Dioxide Improves Salt Stress Tolerance in Strawberry Plants. Agronomy. 2019;9(5):246.
54. Adolf VI, Shabala S, Andersen MN, Razzaghi F, Jacobsen S-E. Varietal differences of quinoa's tolerance to saline conditions. Plant and Soil. 2012;357(1-2):117-29.
55. González JA, Eisa SS, Hussin S, Prado FE. Quinoa: an Incan crop to face global changes in agriculture. Quinoa: Improvement and sustainable production [Internet]. 2015 26/03/2019:[1-18 pp.].

Available:https://onlinelibrary.wiley.com/doi/abs/10.1002/9781118628041.ch1.
56. Kaymakanova M. Effect of salinity on germination and seed physiology in bean (phaseolus vulgaris l.). Biotechnology & Biotechnological Equipment,. 2009;23(1):326-9.
57. Flowers TJ, Colmer TD. Salinity tolerance in halophytes. New Phytol. 2008;179(4):945-63.
58. Flowers TJ, Yeo AR. Breeding for Salinity Resistance in Crop Plants: Where Next? Functional Plant Biology. 1995;22(6):875.
59. Sanchez HB, Lemeur R, Damme PV, Jacobsen SE. Ecophysiological Analysis Of Drought And Salinity Stress Of Quinoa (*Chenopodium quinoa* Willd.). Food Rev Int. 2003;19(1-2):111-9.
60. Anjum NA, Ahmad I, Válega M, Mohmood I, Gill SS, Tuteja N, et al. Salt marsh halophyte services to metal–metalloid remediation: Assessment of the processes and underlying mechanisms. Crit Rev Environ Sci Technol. 2014;44(18):2038-106.
61. Glenn EP, Anday T, Chaturvedi R, Martinez-Garcia R, Pearlstein S, Soliz D, et al. Three halophytes for saline-water agriculture: An oilseed, a forage and a grain crop. Environmental and Experimental Botany. 2013;92:110-21.
62. Bosque-Sanchez H, Lemeur R, Damme Pv, Jacobsen S-E. Ecophysiological analysis of drought and salinity stress of quinoa (Chenopodium quinoa Willd.). Food Rev Int. 2003;19(1-2):111-9.
63. Razzaghi F, Jacobsen S-E, Jensen CR, Andersen MN. Ionic and photosynthetic homeostasis in quinoa challenged by salinity and drought–mechanisms of tolerance. Functional Plant Biology. 2015;42(2):136-48.

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