Screening for salt tolerance in *Chenopodium quinoa*genotypeseedlings through germination in a hydroponic system

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 genotypes in different concentrations of NaCl0 (CK), 100, 300, 400, and 500 mM for 21 days in 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

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 (Joosen et al., 2010; Larson et al., 2015). Bewley (1997) 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 (Bai et al., 2018; Dekker & Gilbert, 2014; Hanif et al., 2017; Javaid et al., 2018; Mahmood et al., 2016; Tanveer et al., 2013). 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 (Batlla & Benech-Arnold, 2014; Florentine et al., 2016; Hanif et al., 2017; Javaid et al., 2018).

While some studies have indicated that salt tolerance during germination is independent of other growth phases in *Triticum aestivum* L. varieties (Mano & Takeda, 2001) and *Solanum lycopersicum* L.(Foolad & Lin, 1997). Prado et al. (2000) 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 (Mondal & Pramanik, 1995; Shi et al., 2017; Wang et al., 2010; Wang et al., 2011). The screening was successful with 182 varieties of quinoa to identify the salt-sensitive and salt-tolerant during germination (Gómez-Pando et al., 2010). This method of screening was also supported by Nasir et al. (2000) and Cha-Um et al. (2012), 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 to seedling growth and further development (Aflaki et al., 2017; Bybordi, 2010; Foolad &Lin, 1997; Houle et al., 2001; Mano & Takeda, 2001; Sanchez et al., 2014). 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 (Gómez-Pando et al., 2010; Nasir et al., 2000; Prado et al., 2000). 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. Material and Methods

2.2 (i) 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 ^{o}C and average relative humidityof ~65-70 % with 16/8 h light/dark photoperiod (Goyal et al., 2016; Morales et al., 2011; Panuccio et al., 2014).

After harvest, seeds were stored at 4 ^{o}C until the experiment commenced (Panuccio et al., 2014). 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 (Burrieza et al., 2012; Lindsey et al., 2017; Panuccio et al., 2014; Ruiz-Carrasco et al., 2011).

2.2 (ii) Treatment and Selection

Seeds were tested at 0, 100, 200, 300, 400, and 500 mM NaCl-induced MS/2 media over 21 days(Lindsey et al., 2017; Murashige & Skoog, 1962; Postnikova et al., 2013; Wang et al., 2009). 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 ^{o}C and 60 – 65 % RH (Joosen et al., 2010; Murashige & Skoog, 1962; Postnikova et al., 2013; Wang et al., 2009). Seed

germination was recorded daily for seven days (Panuccio et al., 2014). After seven days, the bottles were moved to another room with 26 ^{o}C and 65 % RH, for better elongation and growth-related conditions to facilitate better seedling development (Joosen et al., 2010; Panuccio et al., 2014). 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, andsynchrony of germination at 400 mM NaCl and the relative growth (height) between the control and maximum treatment (Aflaki et al., 2017; Kader, 2005; Kader & Jutzi, 2004; Maguire, 1962; Panuccio et al., 2014; Ranal et al., 2009; Scott et al., 1984). 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 (Lindsey et al., 2017; Murashige & Skoog, 1962; Postnikova et al., 2013; Wang et al., 2009). 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:

2.31 Germinability:

Equation 1

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

Where Si: germinated seeds per time (day), Di represents seed numbers from the start of the experiment to the ith, ni: number of seeds germinated in the ith day (Bai et al., 2018; Maguire, 1962; Panuccio et al., 2014; Ranal et al., 2009).

2.32 Mean germination time:

Equation 2

$$\bar{t} = \frac{\sum_{i=1}^{k} n_i t_i}{\sum_{i=1}^{k} n_i}$$

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 (Aflaki et al., 2017; Kader, 2005; Panuccio et al., 2014; Scott et al., 1984).

2.33 Coefficient of variation of the germination time:

Equation 3 (i)

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

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 timein the subsequent formula:

Equation 3 (ii)

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

Where s_t: the standard deviation of the germination time and t is: mean germination time (Kader & Jutzi, 2004; Maguire, 1962; Panuccio et al., 2014; Ranal et al., 2009).

2.34 Uncertainty of germination:

Equation 4

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

Where $n_{i\ is}$: number of seeds germinated on the i^{th} time, and *k is*: last day of observation (Ranal et al., 2009).

2.35 Synchrony of germination (Z)

Equation 6

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

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

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 (Ranal et al., 2009).

2.36 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 (Mickky & Aldesuquy, 2017). 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 with 45.89%. ANOVA and Tukey analyses identified varied significant differences (p>0.05) among the genotypes in both germinability and relative growth (height) (Figure 1).

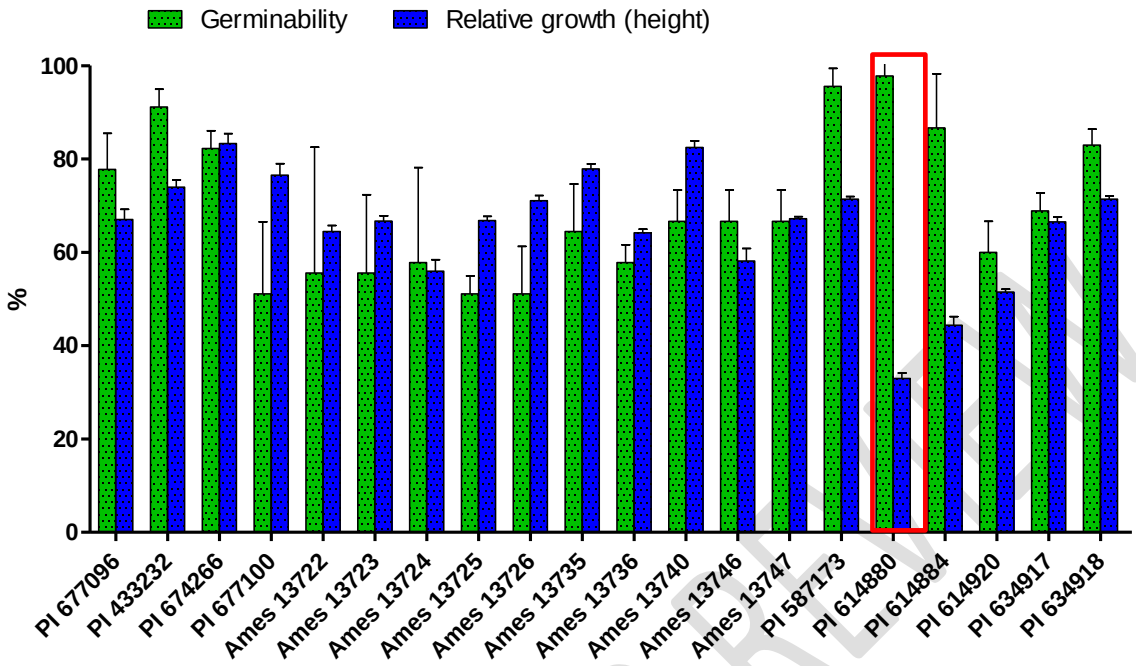

Figure 1 Germinability and relative growth between control and 400 mM NaCl of the salt-tolerant varieties (G_{50}). Data represents means±SD of fifteen biological and three technical replicates.

3.1 (a) 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).

Table 1.Germination indices at 400 NaCl treatment. 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).

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

3.1 (b)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 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 at 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 with 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, and more particularly between the CK and 400 mM NaCl at $p<0.05$.

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

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***

3.1 (c)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 (**Error! Reference source not found.**). 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 variable for each

genotype.

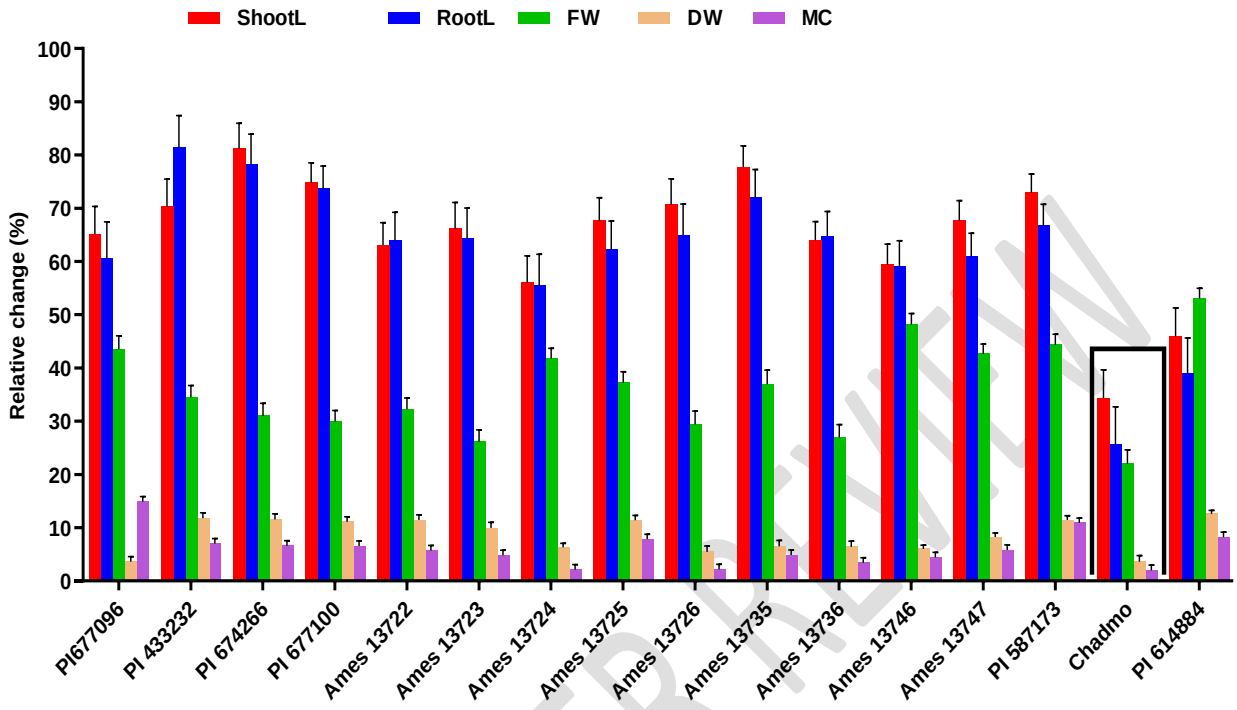

Figure 0 Effect of salinity on the relative change between CK and 400 mM NaCl on the different genotypes. Means±SD for three biological replicates.

3.4 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 they all indicated low germination rate at 200 mM NaCl and they sprouted but with significantly low relative growth (height) (Figure 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 $P<0.05$.

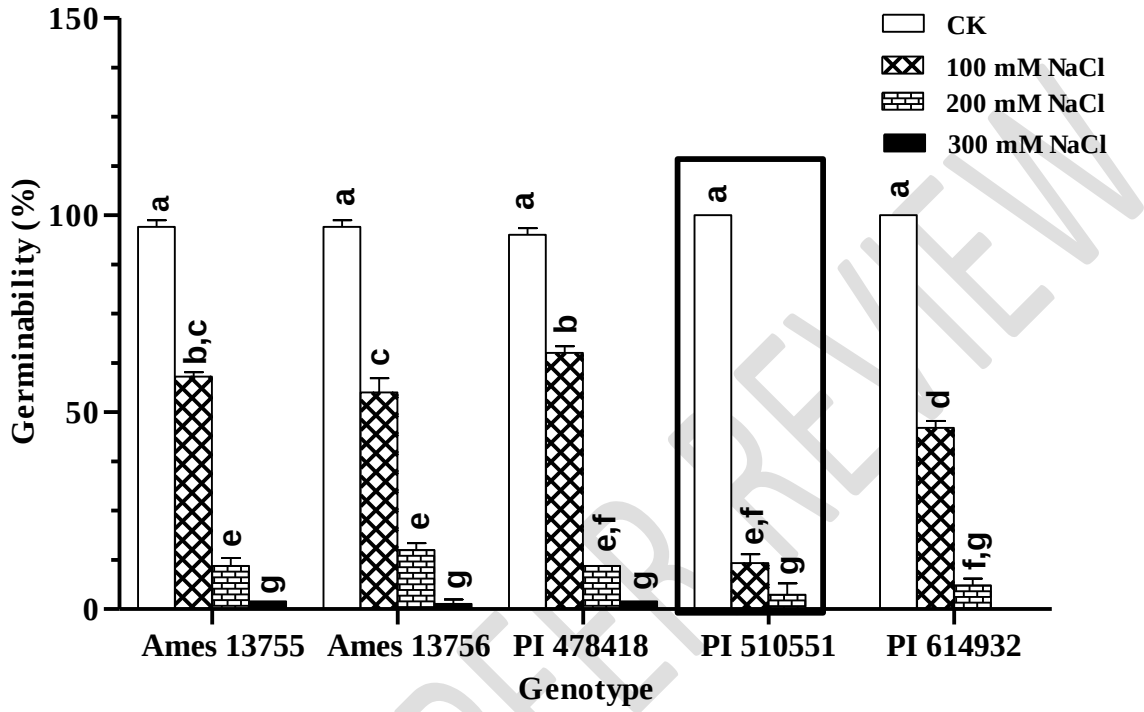

Figure 1 Effect of salinity on the germinability of the salt-sensitive genotypes. Mean ± SD (n=45) Different letters indicate a significant difference at $P<0.05$ among the genotypes and treatment.

Table 3 Selected salt-sensitive varieties through germination and growth. Nd – no data/no 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±1.8**	**1.6±0.67**	**nd**	**nd**	**nd**	**68.21**	**nd**	**nd**

	7							
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

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 (Mickky & Aldesuquy, 2017; Yadav et al., 2011). 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 reproduced and hence, are deemed to be tolerant (Dekker & Gilbert, 2014; Javaid et al., 2018; Mahmood et al., 2016; Tanveer et al., 2013). Despite its halophytic nature, quinoa is rather sensitive to stressful conditions in its vegetative stage, hence, if survived seedling establishment is highly possible (Ruiz et al., 2017; Ruiz et al., 2019). 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(Mickky & Aldesuquy, 2017; Yadav et al., 2011). 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 (Gómez-Pando et al., 2010; Katembe et al., 1998; Munns & Tester, 2008). Some plants are very well adapted to exclude salt at the level of the root by developing salt filtration mechanism through enhancing hydrophobic barrier deposition, which prevents the absorption of non-selective apoplastic ions (Kolattukudy, 1984; Krishnamurthy et al., 2014; Lawton et al., 1981). 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 (Gómez-Pando et al., 2010; Prado et al., 2000).

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 indexor electrolyte leakage (Munns & Tester, 2008; Panta et al., 2014; Sairam & Srivastava, 2002; Shabala & Mackay, 2011). 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 (Shahid et al., 2012). In support, it was also concluded that salt-treated strawberries had a 10% reduction in MSI when treated with 50 mM NaCl (Avestan et al., 2019).

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 (Aflaki et al., 2017; Bybordi, 2010; Houle et al., 2001; Sanchez et al., 2014). Quinoa tolerance to salinity during germination results from the changes in the primary metabolites and enzyme activity in response to salinity (Adolf et al., 2012; González et al., 2015). Many also supported that germination and sprouting at the seedling stage are reliable and effective methods to categorize plants as salt-tolerant and sensitive genotypes (Gómez-Pando et al., 2010; Prado et al., 2000).

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 (Gómez-Pando et al., 2010; Prado et al., 2000). 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 (Kaymakanova, 2009). Furthermore, the responses of *Atriplex prostrataandA. patula*after being exposed to NaCl and PEG were determined with the application of germination rate and percentages were used to assess their susceptibility (Katembe et al., 1998). 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 (Panuccio et al., 2014). 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 select salt-sensitive genotypes because to decide on their sensitivity, it was based on them not germinating and developing into seedlings under saline conditions(Gómez-Pando et al., 2010). 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 (Flowers & Colmer, 2008; Flowers & Yeo, 1995; Sanchez et al., 2003). *Kankolla* based on these criteria was regarded as the most salt-sensitive genotype (Anjum et al., 2014; Bosque-Sanchez et al., 2003; Glenn et al., 2013; Razzaghi et al., 2015).

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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