



Effects of osmotic stress on *Chenopodium quinoa* seed germination.

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Abstract

Quinoa is a pseudo-cereal native to the Andes known for its exceptional adaptation to extreme soil and climate conditions. The study of the response of *Chenopodium quinoa* to osmotic stress and the contribution to improving its yield aims to ensure production in arid and semi-arid regions as an alternative crop. The aim of this study is to test the germination capacity of 5 selected varieties; CQ-57, UDEC-2; ICBA-4, ICBA-5 and 115-R subjected to varying osmotic pressures using solutions of PEG-6000 and NaCl. Seeds are incubated at 25 °C for 10 days. Results showed that osmotic stress increases the lag phase but does not affect the germination rate at low concentrations of PEG and NaCl. While for high concentrations the germination rates decrease as compared to controls. Radicle and hypocotyl lengths decreased with increasing the degree of osmotic stress. The effects of osmotic stress are comparable in the 5 varieties. At high concentration of PEG or NaCl, ICBA-5 and CQ-57 exhibited the highest value of germination rate showing the capacity of these varieties to establish itself in regions suffering from salinity or water deficit.

1. INTRODUCTION

Seed germination constitutes an essential stage in the life cycle of plants, it dependent on the genetic characteristics of the species and the biotic and abiotic conditions of the environment to which the seeds are exposed. In arid and semi-arid regions plants are subjected to salinity and to water shortage. Water uptake by the seed during the germination process is hampered by the negativity of the soil matrix potential (Santos et al., 2016). Excessive amounts of salts and soil water deficiency are abiotic factors that directly interfere with seed germination and full crop development limiting maximum yield performance (Torres et al., 2000). In addition to the restriction of water uptake due to reduced soil potential toxic effects on seeds undergoing the germination process have been observed. These toxic effects can lead to alterations in cell metabolism, a reduction in the percentage and speed of germination and changes in the

development and growth of seedlings (Pelegrini et al., 2016).

Water movement from the medium to seed is necessary to initiate the germination process. This movement is closely related to the presence of water potential gradient which is limited by the presence of salts in soil solution consequently reduces the water absorption of the seeds (Pereira et al., 2014). Besides the negative interference caused by the toxicity of plants, the presence of salts in the solution can act as an inducer of water stress in plants (Souza and Cardoso. 2000). In this sense the evaluation of the germination process and the development of seedlings under conditions of salinity and water shortage are important since they can be related to the sensitivity or tolerance of crops to later stages of development (Liu et al., 2018). Quinoa (*Chenopodium quinoa* Willd.) is a plant that belongs to the Amaranthaceae family. This plant is native to the Andes of South America (Ceccato et al 2011). Quinoa exhibits high yield

potential under adverse climatic and soil conditions. In addition, the grains present enhanced nutritional properties, arising as a complement in human and animal diets, besides being used as a forage and cover crop (Strenskeet al., 2017). Effects of osmotic stress induced by NaCl and PEG has been reported by Barbieri et al 2019 on *Chenopodium quinoa* at germinative stage, the used concentrations are low indeed osmotic potential of the solution didn't exceed -0.4 MPas. The effects of a high concentration of PEG and salt on seed quinoa germination was poorly investigated thus this study aims to evaluate the variability of response of five quinoa varieties to osmotic stress induced by NaCl and by PEG (osmotic potential ranged between 0 and -2.5 MPas) on germination process and parameters.

2. MATERIALS AND METHODS

2.1. Plant material

Five varieties of quinoa were used in this study CQ-57, UDEC-2, ICBA-4, ICBA-5 and 115-R. These varieties are commercial and they differ in their morphological aspects, color of the seeds, height of the plant, length of the development cycle. CQ57 and UDEC2 are from Bolivia. ICBA-4, ICBA (4 and 5) and 115-R are from the International Center for Biosaline Agriculture. Seeds were provided by the United States, Department of Agriculture (USDA) and the seedbank of International Center for Biosaline Agriculture (ICBA). All studied varieties are commercial.

2.2 Germination test

The experiment consists on studying the effect of osmotic stress on the germination seed capacity of 5 varieties of quinoa. For this purpose, the seeds are soaked for two hours in distilled water then they are germinated in boxes of 14 cm in diameter lined with a double layer of paper filter

soaked in a solution of sodium chloride (NaCl) at different concentrations (0, 100, 200, 300, 400 and 500 mM), with a solution of PEG (175, 225, 275 and 305 g/l) corresponding to (-0.5, -1, -1.5, -2, -2.5 MPas respectively) and a combined treatment of 100 mM NaCl and 225 g/l PEG or 200 mM NaCl and 175 g/l PEG. The boxes containing 20 seeds are placed in an air-conditioned chamber at a constant temperature (25°C) and under an illumination of (10 μmol m⁻²s⁻¹). The Petri dishes are examined every 2 hours to follow the germination of the seeds. The number of seeds that have germinated is noted and the germination percentage is thus calculated.

2.3 Statistical analysis

Data were analysed using the statistical software STATISTICA 5.0 (Dell Software). Significant differences between means were separated using the Tukey test (P = 0.05).

3. RESULTS

3.1. Effect of osmotic stress on germination capacity

As shown in Fig. 1 a large variability was observed in the response of the five varieties of quinoa to osmotic stress. This response is dependent on different agents used in the culture medium and their concentrations. Under control conditions, germination rate is about 100% in all studied varieties with the exception of ICBA 4 (66%). The application of moderate salinity (100 mM NaCl) did not have a considerable effect on the germination rate in the three varieties CQ-57, ICBA-5 and UDEC-2, however, it decreased slightly in the two other varieties. At 200 mM NaCl final germination rate decreased significantly by 35% in ICBA 4. However, when subjected to high doses of salt (400 and 500 mM NaCl) a significant decrease in

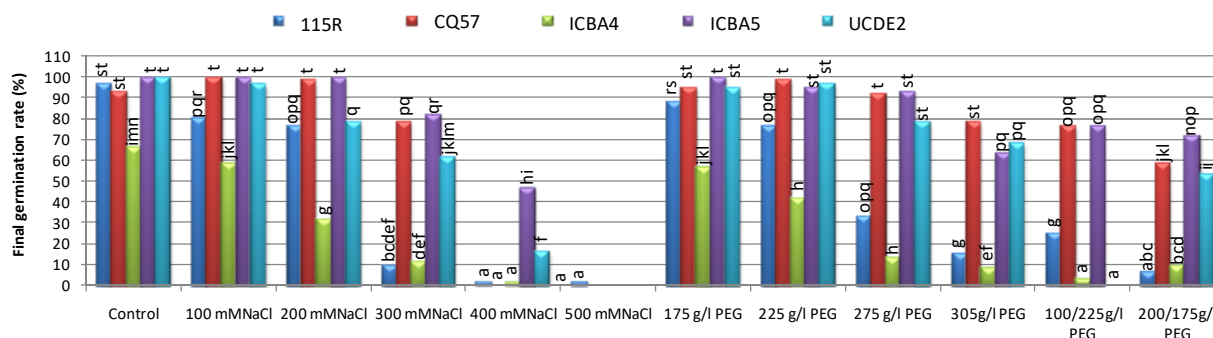


Fig. 1. Final germination rate (%) in 5 varieties of *Chenopodium quinoa* subjected to different doses of NaCl (0, 100, 200, 300, 400 and 500 mM), of PEG (175, 225, 275 and 305 g / l) and of the combined treatment (200/175 and 100 mM /225 g / l).

the germination rate was recorded in all studied varieties. For example in the presence of 400 mM NaCl germination rate was about 46, 0, 16, 1 and 1% for varieties ICBA-5, CQ-57, UDEC-2, 115-R and ICBA-4 respectively. When the salt concentration in the medium exceeds 500 mM NaCl, almost of the varieties are unable to germinate. PEG supply at different concentrations (175, 225, 275 and 305 g/l) has the same effects on the final germination rate of the different studied varieties as compared to that of NaCl. Indeed for low concentrations (175 and 225 g / l) the final germination rate is very important in different varieties. High doses of PEG decreased significantly the germination rate essentially for the varieties UDEC-2, 115-R and ICBA-4 in the presence of 275 g/l by 22, 63 and 43% respectively as compared to the control. At 305 g/l PEG a reduction in the germination rate was recorded for all varieties ranging from 29% for ICBA-5 to 86% for 115-R. The application of the combined doses of NaCl and PEG (200/175 and 100/225) causes a decrease in the germination rate especially for varieties 115-R (25%) and ICBA-4 (10%). It is also observed that there is no germination for the UDEC-2 variety for the treatment (100 mM NaCl/275 g / l PEG) for the other varieties the germination rate is between 76 and 56% for the two treatments.

3.2. Effect of osmotic stress on the on the latency time of quinoa varieties

The application of the saline treatment caused a gradual increase in the duration of the lag phase in all varieties (Table 1). This increase is proportional to the dose of NaCl and PEG with a more accentuated effect observed in medium supplemented with 400 and 500 mM NaCl and

Table 1. Effects of the different doses of NaCl. PEG and NaCl / PEG on the latency time in 5 varieties of *Chenopodium quinoa*

Variety	115 R	CQ-57	ICBA-4	ICBA-5	UDEC-2	
Control	23.33 bc	40.00 ef	52.67 f	38.00 bcde	38.00 bcde	
NaCl mM	100	38.67 bcd	48.00 f	64.67 g	40.67 cde	42.00 f
	200	50.67 f	49.33 f	76.67 i	50.00 f	70.00 hi
	300	91.33 i	65.33 h	107.33 k	61.33 g	92.67 j
	400	0.00 a	0.00 a	126 kl	99 k	96.00 jk
	500	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a
PEG g/l	175	49.33 f	50.67 f	78.00 i	48.00 f	40.00 f
	225	44.00 f	54.67 g	95.33 j	48.67 f	41.33 f
	275	56.67 g	59.33 g	96.00 j	56.00 gh	63.33 gh
	305	99.33 k	70.67 h	116.67 k	68.67 h	68.00 hi
	100/225	61.33 g	67.33 hi	88.00 ij	68.00 h	0.00 a
	200/175	36.00 bcd	93.33 j	137.33 l	71.33 hi	76.67 hi

305 g/l PEG and more particularly in varieties 115-R and ICBA-4. Even more both varieties 115-R and ICBA-4 exhibit the longest lag phase duration. This duration varies from 23, 40, 52 and 38 on control medium (0 mM) to 91, 65, 107, 61 and 92 h on medium supplemented with NaCl (300 mM) respectively for the varieties 115-R, CQ-57, ICBA- 4, ICBA-5 and UDEC-2 and to 99, 70, 116 and 68 h on medium added with 305 g / l PEG.

3.3. Effect of osmotic stress on germination rate expressed by T50

Analysis of data in Table 2 illustrating the effect of increasing concentrations of NaCl and PEG on the germination rate expressed as the mean germination time (T50 time required for 50% germination) shows that the mean germination time (T50) increases gradually with increasing the dose of NaCl in all varieties. It varies from 41, 46, 41, 49 and 93 h on control medium to 77, 90, and 131 h on medium supplemented with NaCl (300 mM) respectively in the varieties ICBA-5, CQ-57 and UDEC-2 for the other varieties (115-R and ICBA-4) the number of germinated seeds is less than half of the total number of seeds. This gradual decrease in the germination rate as a function of increasing salinity is more marked in the two varieties 115-R and ICBA-4 as compared to the others. The same effect is observed for the seeds of the different varieties germinated on media supplemented with different doses of PEG in fact, the germination rate (T50) gradually increases with the increase in the concentration of PEG used to reach 103, 87, and 99 h respectively for varieties ICBA-5, CQ-57 and UDEC-2.

Table 2: Effects of the different doses of NaCl, PEG and NaCl / PEG on the germination rate expressed by T50 in 5 varieties of *Chenopodium quinoa*

Variety	ICBA-5	CQ-57	UDEC-2	115 R	ICBA-4	
Control	41.33b	46.00bc	41.33b	49.33bcd	93.00ijk	
NaCl mM	100	52.00 bcd	50.00 bcd	60.00 de	80.00 fg	112.00 lm
	200	54.33 bcd	58.33 cde	88.33 ghij	99.00 jkl	0.00 a
	300	77.33 fgh	90 ghijk	131.67 jkl	0.00 a	0.00 a
	400	13.008n	0.00 a	0.00 a	0.00 a	0.00 a
	500	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a
PEG g/l	175	49.67 bcd	54.67 bcd	52.67 bcd	80.33 fg	135.67 n
	225	60.00 de	58.33 cde	59.33 cde	88.67 ghij	0.00 a
	275	70.67 ef	76.67 fg	86.00 ghij	0.00 a	0.00 a
	305	103.33 ke	87.67 ghij	99 jkl	0.00 a	0.00 a
	100/225	94.33 jk	96.33 jkl	0.00 a	0.00 a	0.00 a
	200/175	92.33 ijk	117.00 m	109.00 m	0.00 a	0.00 a

3.4. Effect of osmotic stress on radicle length

The result reported in Fig. 2 represents the variation in the length of the radicle of the different varieties as a function of different doses of NaCl and PEG, in fact it appears that for all the varieties the length of the radicle is affected by the increase in concentrations of salt or PEG in a significant way. For the control treatment the length of the radicle is of the order of 4.2, 3.5, 3.6, 3.9 and 2.5 cm respectively for the varieties CQ-57, 115-R, ICBA-5, UDEC-2 and ICBA-4 whereas it is of the order of 1 cm for the variety CQ-57 and which does not exceed 0.5 cm for the other varieties for the 300 mM saline treatment, for the doses 400 and 500 mM NaCl radicle emergence was inhibited

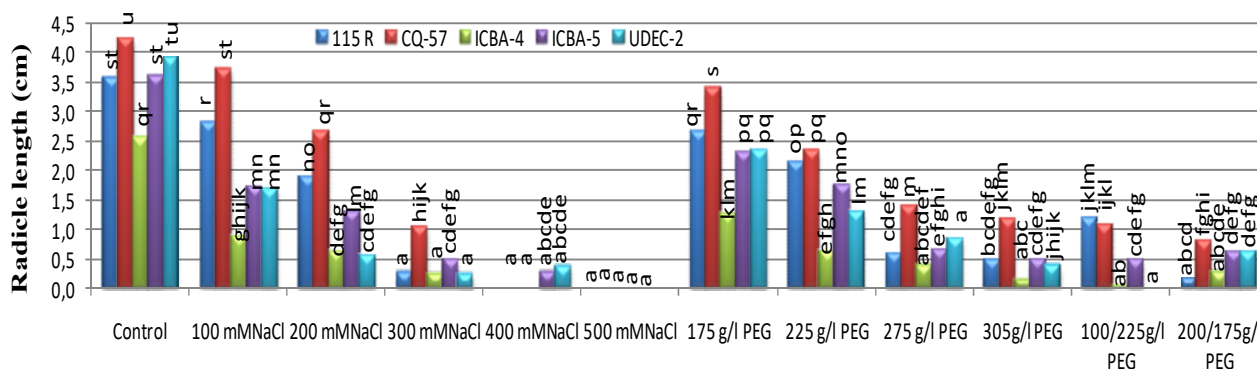


Fig. 2. Effect of different doses of NaCl and PEG on the average radicle length in 5 varieties of *Chenopodium quinoa*.

3.5. Effect of osmotic stress on hypocotyl length

Fig. 3 shows that the variation in the hypocotyl length dependent on the applied doses of NaCl and PEG. Analysis of the results shows that the gradual increase in the concentrations of the agents used induces an inhibitory effect on the length of the hypocotyl in all varieties. In control condition the varieties CQ-57 and ICBA-5 show

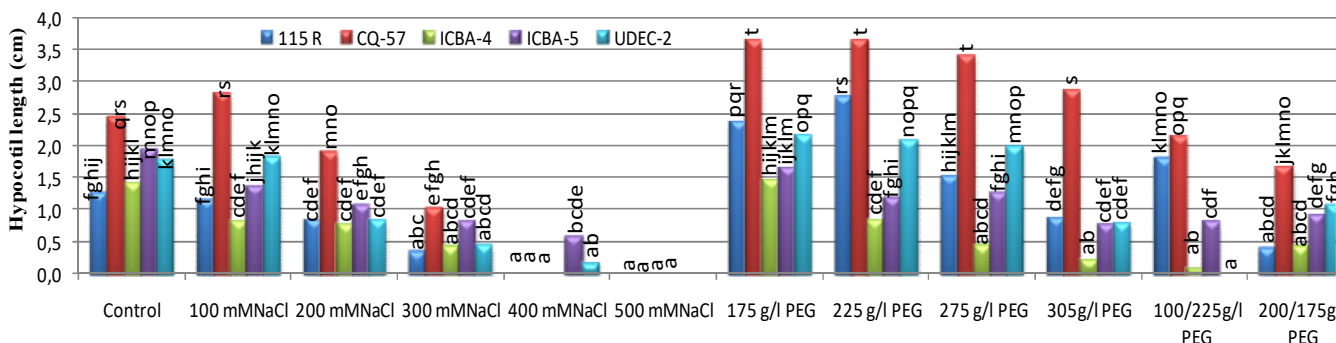


Fig. 3. Effects of different doses of NaCl and PEG on the average hypocotyl length in 5 varieties of *Chenopodium quinoa*.

the longest hypocotyl (respectively 2.4 and 1.9 cm). Moderate salinity (100 and 200 mM) did not have a significant effect on hypocotyl length compared to the control in all varieties. The application of high concentrations of NaCl causes a decrease in hypocotyl length. Regarding the treatment with PEG we notice that at low doses (175 and 225 g / l) increases the length of the hypocotyl as compared to control, it exceeds 3.6 and 2.1 cm respectively for the varieties CQ-57 and UDEC-2 (the increase was about 1.2 and 0.3 cm). At a high dose of PEG (305 g/l) hypocotyl length of the variety CQ-57 remains greater than the control, while there is a decrease for the other varieties. The application of the combined treatment between salt and PEG induces a decrease in the hypocotyl length in all varieties.

4. DISCUSSION

The present study aimed at investigating the effects of osmotic stress induced by NaCl and PEG during the germination stage of five varieties of quinoa in order to determine their potential for osmotic stress tolerance and to recognize the most tolerant and sensitive variety.

Our results showed a large variability in the

response of quinoa to osmotic stress. This response is dependent on different agents used in the culture medium and their concentrations. In our case, moderate osmotic stress did not have a considerable effect on the germination rate in the most of studied varieties. However, a high doses of NaCl or decreases significantly the germination capacity and essentially in the two varieties 115-R and ICBA-4. Our results are in agreement with several studies which have shown the reducing effect of NaCl and PEG on most of the germination parameters (Dehnavi et al., 2020; Herrera and Pinto 2009). The decrease in the germination rate recorded can be explained by the accumulation of toxic ions inside the cells of the seeds or the decrease in the water potential in the culture medium caused by the addition of NaCl or PEG. As a result water availability decreases inside the cells of the seed thus preventing the phenomenon of germination and all the mechanisms which trigger it such as imbibitions (by the reduction of all the enzymatic synthesis pathways of aquaporins which have a main role in the mechanism of water penetration inside the seed) (Kshetrimayum et al. 2017) and the resumption of metabolic activities. It has been reported by Almansouri et al., 2001 that in durum wheat. PEG didn't have any effects on the mobilization of reserves. The main effect of PEG occurred via an inhibition of water uptake while detrimental effects of NaCl may be linked to long-term effects of accumulated toxic ions.

Regarding the germination kinetics and according to our data. sodium chloride affects the initiation of germination particularly under the high concentration 400 and 500 mM and that for the 5 varieties. Salinity stress may have much effect on delayed germination time than on final germination percentage for most crops (Almansouri et al., 2001; Hajlaoui et al., 2007). Liu et al., 2018 reported that a delay of water uptake and a decrease in α -amylase activity with an increase in the concentration of NaCl could be implied in delaying of the germination time in rice. Authors showed that the decrease in the α -amylase activity was higher in the salt-sensitive genotypes than in the salt-tolerant genotypes. This reduction in the α -amylase activity results with a significant reduction in the translocation of sugars, essential for the developing embryo. Besides, decreasing sugar concentrations also change the osmotic potential of growing cells, resulting in a decrease in water uptake. Our study also shows an increase in the germination time observed in all treatments

with PEG or NaCl (for all varieties) in comparison with those of the control is directly related with a decrease in the germination rate expressed by T50 (the time means necessary for the germination of 50% of the seeds of the same Petri dish) which may be the consequence of an alteration of the enzymes or / and hormones which are involved in the process of seed germination (Ghrib et al 2011) or the result of delayed seed imbibitions (Jaouadi et al., 2010) or even the total death of the embryo by excess of toxic ion (Na^+ and Cl^-) inside the seed (Hajlaoui et al., 2007).

Radicle and hypocotyl lengths are also affected by osmotic stress in all varieties (except variety CQ-57, hypocotyl length stimulation is recorded for treatments 100 Mm NaCl, 175, 225, 275 and 305 g / l PEG) this indicates that osmotic stress exerts a reducing effect on the length of the aerial parts of the plant. These results are in agreement with those of (Akram et al. 2002) who demonstrated that osmotic stress and essentially the use of increasing concentrations of NaCl induces a reduction in the medullar and cortical parenchyma in wheat. Other studies have shown that the decrease in the length of the roots can be explained by the inhibition of the division of the cells and their elongation which stops the tubular formation and the lignification leading to the formation of the conductive tubes of the radicle and the lignification hypocotyl (Fraser et al. 1990).

5. CONCLUSION

Our study showed that Osmotic stress led to a decrease in the germination rate and in the length of the hypocotyl and to an increase in lag phase of different studied varieties. The effects of salt at high concentration (400 and 500 mM) are more pronounced when compared to its iso-osmotic concentration of PEG (275 and 300 g/l). The 2 varieties ICBA-5 and CQ-57 are the most tolerant to osmotic stress at the germinative stage however ICBA-4 is the most sensitive one.

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