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REGULAR ARTICLE

Comparative effect of NaCl and seawater on germination of quinoa seed (*Chenopodium quinoa* willd)

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Abstract

In this study the effect of NaCl and seawater, compared to a control with distilled water, on the seed germination of *Chenopodium quinoa* were investigated. All seeds germinated in all treatments: control and both NaCl and seawater treatments. However saline treatments delayed germination compared to the control seawater causing more delay than NaCl. The hypocotyls and radicle lengths and dry weights were affected more by NaCl than by seawater. The radicles were the most affected by salt stress compared to hypocotyls.

Key words: *Chenopodium quinoa*, Germination, NaCl, Seawater

Introduction

About 97.5% of the world's water is saline (Adolf et al., 2013) and 5% of 1.5 Billion hectares of cultivated land is affected by salt (Ayala-Astrorga and Alcaraz-Melendez, 2010). Furthermore the increasing population amplifies the pressure on freshwater resources and makes it more and more difficult to ensure water supply. In addition, in the context of climate change, higher temperatures and longer droughts are factors that incite researchers to investigate alternatives to freshwater resources.

In Morocco, the salinity problem is widespread, especially in coastal areas extending from Casablanca to Agadir (6 Anonymous, El Iklil, 2001). Nationally, about 160,000 ha of land are affected by the problem of salinity (Ftouhi 1981; Badraoui and Debbarh, 2003). For more than 414.543 ha of irrigated areas, soil salinity and water affects 156.34 ha or 36.75% of the land (Lahlou, 1999). Thus this salinization constitutes a hindrance for agriculture, an important pillar of Moroccan economy. Covering an area of nearly 8.7 million hectares, cereals are by far the main crop in the agricultural production system in Morocco. However, studies conducted on the most consumed cereals in Morocco (barley, wheat and durum wheat and triticale) showed that salinity in the range of 0.74 to 11.69 mS/cm caused a reduction estimated at 30% to 50% in the yield of these cereals (Lahlou, 1999).

Under salt stress, plants have to face two major problems: osmotic effects and ion toxicity (Läuchli and Epstien, 1970). Salt sensitivity or tolerance of plants is related to the stage of growth development (Läuchli and Grattan, 2007). Germination and seedling growth are crucial and at the same time salt-sensitive stages in the development process of most plants. Germination is a critical and a complex phenomenon connecting many physiological and biochemical changes and any stress including salt stress may compromise their process (Wahid et al., 1999). Indeed, seeds subjected to salinity show variations in germination, some fail to germinate, while others tolerate salinity even at high concentrations (Duan et al., 2007).

In response to the growing demand for food and the continued expansion of soils affected by salinity, research on plant responses against salinity has developed rapidly in recent decades (Rao et al., 2006). Among the halophytes of agronomic and nutritional interest stands the pseudo cereal and the facultative halophyte *Chenopodium quinoa*; a species with a potential that could match or even exceed the cultivation of cereals around the world including Morocco.

Chenopodium quinoa, commonly known as quinoa, belongs to the *Chenopodiaceae* family. It is a plant native to the Andes, mainly grown for

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human consumption. Quinoa has a great plasticity and flexibility. Jacobson et al. (2007) reported that it can grow in extremely diverse conditions (drought, salinity, cold, wind). In the present study the effect of NaCl and seawater on the germination of *C. quinoa* is investigated.

Materials and Methods

Quinoa seeds obtained from Prof. Koyro (Justus Liebig Institute of Plant Ecology, Giessen, Germany) were hand sorted to eliminate broken and small ones before the beginning of the experiment. They were then surface sterilized with 5% sodium hypochlorite solution during 10 min. After sterilization the seeds were washed with distilled water several times and transferred into sterilized 9 cm Petri dishes containing filter paper. The saline treatments used were 50, 100, 150 and 200 mM NaCl solutions and 10, 20, 30 and 40% seawater (SW) dilutions. Petri dishes were soaked by 5 ml of the saline treatments compared to a control treated with distilled water. Ten seeds were used for each treatment with tree replicates. The Petri dishes were put in an incubator at 25°C. The number of seeds germinated was recorded daily for 3 days. Seeds were considered to have germinated with the emergence of the radicle. The parameters studied were:

- The rate of germination (in %)
- Germination velocity (GV) calculated as follows:

$GV = 100x\ [\sum Ni/\sum NiTi]$

Where N is the number of germinated seeds on day i and T is the number of days from sowing (Scott et al., 1984; Ghadiri and Bagherani Torshiz, 2000).

- Hypocotyl and radicle length and dry weight. The length and dry weight of hypocotyl and radicle were measured and recorded 7 days after sowing.
- Efficiency of mobilization of seed reserves. This parameter was obtained by drying the remaining portion of the seed in the oven at 70°C for 48 h. The sensibility of this parameter to salt stress is appreciated by the weight of the dried residual part of the seed at the end of germination (El Iklik, 2001).

Statistical analysis

Data were analyzed by STATISTICA V.6. One way analysis of variance (ANOVA) and the LSD test was used to determine the significance of differences between the mean values at $p<0.05$

Results and Discussion

Germination rate and velocity

Seeds germination started second day after sowing. The germination percentage reached its maximum in the 3th day after sowing. All seeds germinated in the control and both NaCl and seawater treatments. The results revealed that the germination of *C. quinoa* was not affected by salinity in the medium (Figure 1). The germination curve in Figure 1 shows a sigmoid shape with three phases:

- A lag phase corresponding to seed imbibition and metabolism activation;
- A linear phase which corresponds to a rapid increase in the rate of germination and the emergence of the embryo;
- A bearing phase when the final rate of germination was reached.

The germination velocity (GV) decreased in response to the increase of salt concentration in the medium and it was slower in seawater treatments compared to NaCl solutions (Figure 2).

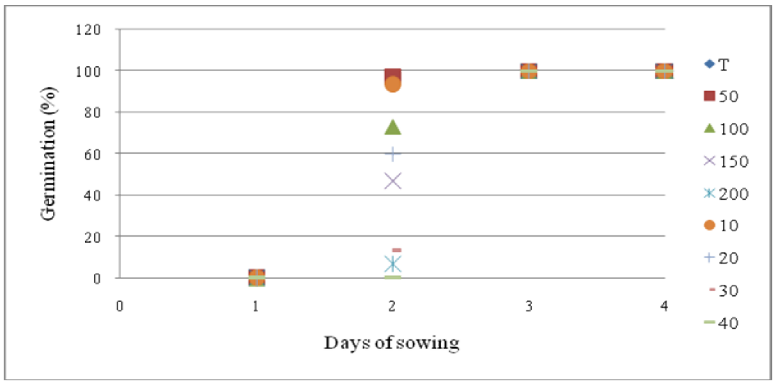

Figure 1. Germination of *C. quinoa* under NaCl (50, 100, 150 and 200 mM) and seawater (10, 20, 30 and 40%) treatments.

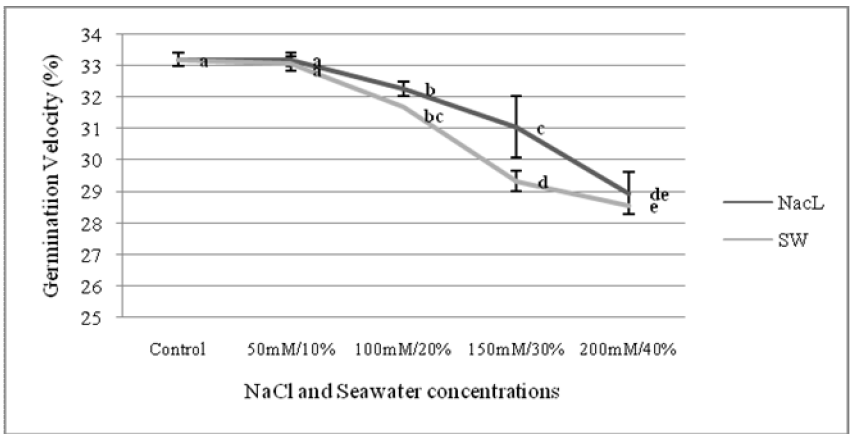

Figure 2. Velocity of germination of *C. quinoa* under NaCl (50, 100, 150 and 200 mM) and seawater (SW) (10, 20, 30 and 40%) treatments. Values with the same letter do not differ significantly at $p < 0.05$.

There was no significant difference between control, 50 mM NaCl and 10% seawater. Above these concentrations the GV decreased significantly in both NaCl and seawater treatments. In fact, all the ten seeds from control, 50 mM NaCl and 10% seawater germinated all in the second day after sowing. In the third day, all seeds from the other saline treatments reached 100%. High salinity level in this study delayed the onset of germination in *C. quinoa* but did not affect the final germination percentage. Our results are consistent with those obtained by Prado et al. (2000). These authors found that final germination rate of *Chenopodium quinoa* seeds was maximum in control and was equal to that of 0.1 M and 0.2 M of NaCl. They also found that germination was delayed slightly by 0.1 M of NaCl and strongly by 0.2 M, 0.3 M and 0.4 M of NaCl.

Seawater treatments delayed germination more than NaCl treatments. These results are similar to those of Zia and Khan (2002) where they found that germination in *Limonium stocksii* was inhibited more by seawater than by NaCl treatments. Relatively high salinities affect the germination in 2 ways that can act in combination: Lowering osmotic potential which prevents or delays water uptake and/or by seed intoxication through salt uptake from the medium (Kaymakanova, 2009).

Because all seeds germinated in all treatments, the osmotic potential may be the reason for germination delay (Table 1). Salt stress appears to act through its osmotic effect during the germination phase, *sensu stricto*, and its osmotic and toxic effect during seedling growth phase (Bounakba, 1998). In a previous work (data not shown) the germination of *C. quinoa* seeds (stored for one year) treated by of 150 mM NaCl and 30% seawater was strongly affected by salinity such that only 45% and 50% of seeds respectively germinated. In fact, aging or prolonged storage affect the germination potential of seeds (Wahid et al., 1999). Läuchli and Grattan (2007) reported that plant age is among the factors that influence salt sensitivity of plants. Although most plants are tolerant during germination, salinity stress delays this process and there may be no difference in the percentage of germinated seeds from low to high salinity treatments (Läuchli and Grattan, 2007). Adolf et al. (2013) reported that the tolerance of *C. quinoa* during the germination phase is a result of the distribution of potentially toxic ions (Na^+, Cl^-) and essential ions K^+, $Mg^{2+,}$ Ca^{2+}, and SO^{2-}_4, respectively at the pericarp and embryo.

Table 1. Osmotic potential of different concentrations of NaCl and seawater used for the germination test.

	Osmotic potential (MPa)	
	NaCl	SW
Control	-0.012 ± 0.009	
50 mM/10%	-0.249 ± 0.013	-0.296 ± 0.038
100 mM/20%	-0.46 ± 0.019	-0.533 ± 0.036
150 mM/30%	-0.689 ± 0.026	-0.761 ± 0.045
200 mM/40%	-0.862 ± 0.015	-1.03 ± 0.075

Hypocotyl and radicle length

Increasing salt concentrations affected negatively radicle elongation of *C. quinoa* (Table 2). Seedlings from the control presented a significant longer radicle compared to those of plants in saline treatments. NaCl treatments decreased radicle length more than seawater treatments. The highest saline treatments reduced radicle length by 72% in 200 mM NaCl and only by 16% in 40% seawater. Similar result was reported by Mostafavi (2012) for

sugar beet. Wahid et al. (1999) reported that sodium chloride affects the emergence of young tissues more adversely than other salt species. The reduction of radicle growth under salt stress conditions may be due to the diminution in the turgor of radicle cells (Li, 2008; Bewley and Black, 1994). Moderate salt concentrations stimulated the length of hypocotyl of *C. quinoa*. Hypocotyl length reached longest sizes in 50 mM NaCl and 20% seawater treatments and then decreased with increasing salinity. The results obtained in this study join those of Li (2008) where he found that the hypocotyl length of *Glycine soja* was stimulated by 50 mM of NaCl. Salt stress inhibited radicle growth more than hypocotyls in *C. quinoa*. Chen et al. (2012) have also obtained similar results. They observed that radicle growth of *Chenopodium glaucum* was more adversely affected by salt stress compared to hypocotyl growth. The decrease in radicle length with the increase in salinity might be due to more inhibitory effect of salinity, espacialy NaCl, on radicle growth compared to hypocotyl growth (Hakim et al., 2010).

Hypocotyl and radicle dry weight

High salinity did not affect hypocotyls dry weight, which rather increased with increasing salinity (Table 2). The hypocotyls dry weights were 1.6 and 1.5 fold higher respectively in 200 mM NaCl and 40 % seawater treatments compared to the control plants. The studies done by Bayuelo-Jimenez et al. (2002) on *Phaseolus* species under different NaCl treatments ranging from 0, 60, 120 and 180 mM showed that the hypocotyls of *P. microcarpus* were stimulated by 60 mM NaCl. An explanation for the increase of hypocotyls dry weight was given by Johnson and Cheeseman (1983) and Drew and Läuchli (1987) who worked on corn. These authors asserted that toxic ions are stored in the mesocotyls up to a certain limit as a strategy for salt effects tolerance. Wahid et al. (1998) reported that epicotyls and hypocotyls can use this strategy to avoid salt effects and thus ensure better growth. As for radicle length, salinity decreased significantly radicle dry weight. This parameter was negatively affected more by NaCl than seawater treatments. In fact, while the radicle dry weight decreased approximately by 23% in 40% seawater treatment, it has decreased by 55% in 200 mM NaCl treatment compared to the control. However, we noticed that 10% of seawater had significantly stimulated the radicle dry weight. Several studies have reported similar result (Keshavarzi et al., 2012; Bahrani and Hagh Joo, 2012). Redman et al. (1994) reported that under increasing salinity, reduction of the radicle dry weight is to be expected. They also reported that this reduction maybe due the osmotic effect caused by salinity which limits the water absorption during germination. Regarding the salt kind, Khan and Ungar (1985) reported that NaCl affects adversely the emergence of young tissues more than other kinds of salt species.

Efficiency of mobilization of seed reserves (EMSR)

The mobilization of seed reserves results in the maintenance of seedling growth during the early stages of development. Table 2 shows the effect of salinity on the ESRM estimated by weight of the remaining portion of the grain of *C. quinoa*. In seawater treatments, this parameter decreased from 2.1 mg to 1.6 mg and 1.7 mg respectively in control, 10% and 20% SW, resulting in an increase of the ESRM. Above these concentrations, the weight of the remaining portion of the grain increased compared with control resulting in a decrease of the ESRM. With NaCl treatments, the weight of the remaining portion of the grain increased significantly in all concentrations compared with the control treatment. In fact, the weight of the remaining portion of the grain was 1.4 fold higher in 200 mM NaCl compared with control treatment. Thus NaCl treatment decreased significantly ESRM of *C. quinoa* seeds. These results show that the application of high salinity decreased the mobilization of seed reserves. Moreover, NaCl treatments affected more the ESRM than SW. El Iklil (2001) reported the same results on two species of *Lycopersicon* irrigated with different concentrations of NaCl.

When germinating under saline conditions seeds uptake high ions concentration into their tissues thus resulting in toxicity to a number of physiological and biochemical processes. Rahman et al. (2008) reported that one of the consequences of salinity on germination is to slow or diminish the mobilization of reserves causing the alteration of cell division and injuring hypocotyls. Iyengar and Reddy (1993) noted the reduction of the mobilization of seed reserves as a result to the negative effect of salinity on enzymes responsible of the reserve hydrolysis and/or the translocation of the products, obtained from the reserve hydrolysis, into the embryonic axis.

Table 2. Early seedling growth of *C. quinoa* after 7 days of growth under NaCl and seawater (SW) stress.

	EMSR (mg)		RL (cm)		HL (cm)		RW (mg)		HW (mg)	
	NaCl	SW	NaCl	SW	NaCl	SW	NaCl	SW	NaCl	SW
Control	2.1a		5.625a		2.675a		8.25ab		14a	
50 mM/10%	2.1a	1.6a	4.25b	3.65ac	3.175bcd	3.45de	8.05b	8.9a	17.85e	19.9b
100 mM/20%	2.8b	1.7a	4.1b	4.975bc	3abc	3.625e	6.3c	7.9b	20b	20.9c
150 mM/30%	2.9b	2.0a	3.125d	4.275bc	2.8ab	3.3cde	5.2d	6.7c	22.05f	23d
200 mM/40%	3.1b	3.0b	1.575e	4.725b	2f	2.925abc	3.7e	6.3c	21.15c	23.4d

Values with the same letter do not differ significantly at $p < 0.05$. RL: EMSR: efficiency of mobilization of seed reserves, radicle length, HL: hypocotyls length, RW: radicle dry weight, HW: Hypocotyls dry weight.

Conclusion

Seed germination and growth are key factors in the life of a plant, especially in the presence of some limiting factors such as salt. Relatively high salinities did not devitalize seed germination. All seeds germinated in all NaCl concentrations and seawater treatments. Seawater (under controlled laboratory conditions) clearly delayed seed germination in comparison to NaCl solutions, probably due to an osmotic effect. However, NaCl treatments affected more seedling growth than seawater, most likely due the toxic effect of Na^+ and Cl^-. In general seedling growth parameters showed that radicle growth was more sensitive to salt stress than hypocotyls. Salinity affected negatively the mobilization of the reserves of *C. quinoa* seeds, with a higher effect obtained with NaCl treatment compared with seawater. Nevertheless relatively low concentration of seawater (10 and 20%) enhanced the mobilization of the reserves. This work shows that C. quinoa seeds can germinate and start growing under relatively high salinity in the growing medium (up to 200 mM NaCl and 40% seawater).

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