Effect of NaCl on germination, growth, and soluble sugar content in *Chenopodium quinoa* Willd. seeds

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**Abstract.** The influence of NaCl on germination rate, growth, and soluble sugar content in quinoa (*Chenopodium quinoa* Willd.) seeds and seedling components (cotyledons and embryonic axes) during early germination was investigated. Under saline conditions germination decreased markedly. The delay in germination rate was not linear. In presence of 0.4 M NaCl, the percentage of germination was only 14% after 14 h, whereas the control at the same time reached maximum germination (87%). The percentage of aborted seeds after 14 h in NaCl was lower than in distilled water (7% and 16%, respectively). A high percentage (67%) of the ungerminated seeds from saline treatment germinated after washing with distilled water. Fresh weight was reduced by salinity in both embryonic axes and cotyledons. The highest increase in fresh weight was observed in embryonic axes, where an increase up to 3-fold higher occurred in distilled water than in saline conditions after 10 h of development. The water content of embryonic axes in distilled water increased considerably for the first 10 h, then remained constant thereafter. In saline conditions, no significant changes were observed in water content. NaCl did not strongly inhibit dry mass production in either embryonic axes or cotyledons. Total soluble sugar content increased markedly in distilled water, peaking after 6 h for both embryonic axes and cotyledons. Reduced glucose and fructose contents were found in embryonic axes in the presence of NaCl. However, in the cotyledons, the glucose and fructose contents did not differ significantly. Levels of sucrose were higher in NaCl-treated cotyledons than in control seeds. The relationships between germination, water content, seedling growth, and soluble sugar content in relation to salt stress are discussed.

**Keywords:** *Chenopodium quinoa*; Germination; Growth; Saline stress; Seeds; Soluble sugar content.

**Introduction**

Quinoa (*Chenopodium quinoa* Willd.) is a dicotyledoneous species native to the Andean Region of South America, where it is used as a very important nutritional resource (Risi and Galwey, 1984). This species is among the most important grain crops in terms of protein content and amino acid balance for human nutrition because of its high lysine and methionine levels (González et al., 1989; Burnouf-Radosevich, 1988; Jacobsen, 1993). A large spectrum of nutritious and other useful products is being made from quinoa for human or animal consumption (Jacobsen and Stalen, 1993). Quinoa and other halophytic members of the Chenopodiaceae have long been known for their extraordinary salt and drought tolerances (Reimann and Breckle, 1993). These characteristics make it an attractive alternative crop for the arid and semiarid regions, where water deficiency and salinity have been recognized as major agricultural problems (Aronson, 1985). In halophytes adaptation to salinity is associated with metabolic adjustments that lead to the accumulation of several organic solutes, such as sugars, polyols, betaines, and free proline (Flowers et al., 1977; Briens and Larher, 1982; Gorham et al., 1981).

The sensitivity of plants to salinity may depend on their developmental stage (Adam, 1990). Different responses to salinity have been reported between germinating and growing seedlings of a number of halophytes (Mayer and Poljakoff-Mayber, 1975). Ungar (1996) observed that seeds of *Atriplex patula* were less affected by salinity than the growing plants. Nevertheless, very little information is available regarding the relative salt tolerance of plants at different stages of development (Ungar, 1991). The results obtained to date are not clear, even though plant response to salinity has been one of the most widely researched subjects (Munns, 1993).

Carbohydrates, especially starch, represent the major reserve substance in most seeds (Bewley and Black, 1994). During early germination, mobilisation of storage carbohydrates occurs, especially after radicle emergence. However, in the growing regions, i.e., embryonic axes, some mobilisation can occur before germination is completed (Bewley and Black, 1994). Once the high molecular weight carbohydrates are mobilised, they are converted into soluble forms, i.e., sucrose, glucose and fructose, that are readily transportable to sites where they are required for growth (Mayer and Poljakoff-Mayber, 1975). The

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soluble carbohydrates also seem to play an important role in osmotic regulation of cells during germination (Gorham et al., 1981; Bolarin et al., 1995). There have been few studies on carbohydrate status in seeds germinated under stress conditions, mainly because the metabolism of these compounds can be affected by a number of environmental factors such as irradiance, temperature, CO₂ concentration, salinity, and type of ions present (Bohnert et al., 1995). Thus, the variations that occur in soluble carbohydrates during germination under saline conditions are poorly understood and information on physiological events involved in this process is scarce.

In this report we present details on germination and status of soluble carbohydrates in embryonic axes and cotyledons during the first phases of germination and plant development of quinoa seeds under severe salinity stress.

**Materials and Methods**

**Plant Material**

The quinoa (*Chenopodium quinoa* Willd. ev. Sajama) seeds were obtained from the Experimental Station of Patacamaya in Bolivia. Prior to experimentation they were selected for uniformity of size and mass.

**Germination Test**

Seeds were sterilized by immersing in 2% sodium hypochlorite for 7 min and rinsing repeatedly with distilled water. They were then germinated in Petri dishes (5 cm) containing a sheet of filter paper (Whatman 1), moistened with 1 ml of distilled water or saline solution (0.1; 0.2; 0.3, and 0.4 M NaCl). Following a germination study, the 0.4 M concentration was selected for detailed analyses. The highest salt concentration used corresponds to the upper limits of salinity encountered in diverse places of quinoa cultivation in the highland regions of Bolivia and Perú. Each Petri dish contained fifty seeds, and each treatment was performed five times. Germination was carried out at 25 ± 1°C under dark conditions. Germination percentages were estimated after 4; 6; 8; 10; 12; 14; 16; 18; 20; 22, and 24 h using radicle protrusion (≥ 2 mm) as a criterion. The percentage of abnormal germination, i.e. proportion of seeds with cotyledons without radicle protrusion (aborted seeds), was also determined. After 14 h non-germinated seeds under salt stress, were rinsed in distilled water for 3 min and placed on filter paper moistened with distilled water, and incubated again.

**Seedling Analysis**

Germinated seeds in distilled water and in a 0.4 M NaCl solution were sampled according to their developmental stages at 4, 6, 10, and 14 h from the beginning of incubation and then placed at -20°C to stop the germination process. Embryonic axes and cotyledons of uniform size were separated under a binocular microscope. A part of these tissues were weighed to obtain the fresh weight (FW). The dry weight (DW) was obtained after drying the plant material for 48 h at 75°C. Tissue water content was obtained from the (FW-DW)/DW ratio.

**Extraction and Analysis of Sugars**

Sugars were extracted from the embryonic axes and cotyledon as follows: 500 mg (FW) of sample were homogenised with 2 ml of 80% ethanol solution in a mortar and pestle. After heating the homogenate in a water bath at 75°C for 10 min, the insoluble residue was removed by centrifuging at 5,000 g for 10 min. The precipitate was re-extracted with 2 ml of 80% ethanol at 75°C and re-centrifuged. The supernatants were pooled and dried under a stream of hot air, and the residue was resuspended in 1 ml of water and desalted through a column of ion-exchange resin (Amberlite MB3). The filtrate was used for soluble sugar determinations. Total soluble sugars were determined by the phenol-sulphuric acid method (Dubois et al., 1956), glucose by the glucose oxidase-peroxidase coupled reaction (Jorgensen and Andersen, 1973), fructose by the Roe and Papadopoulos (1954) method, and sucrose by the Cardini et al. (1955) procedure.

**Statistics**

Data of germination, growth and soluble sugar content were analyzed by the Student’s t-test. Means were compared between treatments by LSD (least significant difference) at the 0.05 confidence level.

**Results**

**Germination and FW, DW Changes**

Germination of *C. quinoa* seeds in distilled water reached the maximum in 12–14 h (Figure 1). In NaCl maximum germination occurred later than in the control, the extent of delay depending on the salt concentration used. A salt concentration of 0.1 M delayed germination slightly, and higher concentrations caused progressively strong

![Figure 1. Effect of NaCl salinity on seed germination of *Chenopodium quinoa*.](image-url)
inhibition, with maximum effect resulting at 0.4 M. Extending the germinative process to 24 h resulted in germination rates equal to the control in 0.1 and 0.2 M concentrations. The 0.3 and 0.4 M levels of NaCl enabled only 67% and 26% germination, respectively.

For analyses of effects on carbohydrate levels only the highest salt concentration, 0.4 M was employed. Furthermore, only the first 14 h of the germination process was examined, when the controls in distilled water reached the maximum germination rate. The percentage of aborted seeds in 0.4 M NaCl after 14 h was lower than in distilled water (7% and 16%, respectively). A high percentage (67%) of the ungerminated seeds under saline conditions germinated after washing with distilled water.

The relationship between FW and DW in control and salt-stressed embryonic axes and cotyledons is summarized in Table 1. During early germination, from 4 to 10 h the FW values of embryonic axes, but not of cotyledons, in distilled water increased notably. The highest FW value of embryonic axes was observed at 10 h of development in distilled water, which was 3-fold larger than in saline conditions. In cotyledons the highest value was observed at 14 h, but the cotyledons did not have differences in FW values as great as those in embryonic axes. Under saline conditions a distinct pattern was observed. The FW values of embryonic axes peaked at 10 h, then decreased by 14 h. In the cotyledons FW decreased after the first 4 h and remained low thereafter. In fact, the FW values under NaCl stress did not show significant differences during germination in either embryonic axes or cotyledons. However, our previous results (Prado et al., 1995) showed that water absorption by seeds in distilled water and 0.4 M NaCl had a similar pattern, but the water uptake was slower in salt solution than in the control. The DW displayed no significant differences between the control and salt solution in embryonic axes or cotyledons.

The water content ((FW-DW)/DW ratio), a measure of expansion growth, of embryonic axes in distilled water showed a substantial increase the first 10 h, then levelled off. In cotyledons the water content varied only slightly during the period studied. Under saline conditions, no marked variations in size of embryonic axes or cotyledons were observed over the experimental period.

**Carbohydrate Content**

Total soluble carbohydrate content changed under salinity stress in embryonic axes and cotyledons during germination and seedling growth. The influence of 0.4 M NaCl on total soluble sugar content is shown in (Figure 2A and B). Under saline conditions the sugar content in both embryonic axes and cotyledons decreased notably the first 6 h, then increased between 6 and 14 h, reaching maxima at 10 h and 14 h, respectively. In control seeds the total soluble sugars increased markedly in the embryonic axes the first 6 h, then decreased significantly thereafter (Figure 2A). Cotyledons showed a lower increase in soluble sugar the first 6 h, then decreased thereafter, although not as dramatically as observed in embryonic axes (Figure 2B).

Differences were observed in glucose, fructose, and sucrose content changes between the two treatments. The more substantial differences were observed in glucose and fructose contents. Glucose and fructose content in two tissues showed a similar pattern; thus in embryonic axes in distilled water the levels of these two sugars exhibited marked increases from the initial germination stage up to 10 h, with increases of 4.3 and 4.9 fold for glucose and fructose, respectively (Figure 3A). In cotyledons the glucose and fructose contents showed only slight differences between the two tissues. Nevertheless, the highest values were always observed in distilled water (Figure 3B). The content of sucrose in the first stage (4 h) of germina-

| Table 1. Fresh weight (FW), dry weight (DW), and water content in embryonic axes and cotyledons of *Chenopodium quinoa* seeds in distilled water and 0.4 M NaCl. |
|---|---|---|---|---|
| NaCl (M) | 4 | 6 | 10 | 14 |
| Embryonic axes | | | | |
| FW (mg) | 0.0 | 0.95 ± 0.06a | 1.58 ± 0.33a | 2.85 ± 0.20a | 2.00 ± 0.32a |
| DW (mg) | 0.4 | 0.74 ± 0.02b | 0.70 ± 0.04b | 0.95 ± 0.30b | 0.80 ± 0.05b |
| (FW-DW)/DW | 0.0 | 0.34 ± 0.02a | 0.32 ± 0.01a | 0.44 ± 0.02a | 0.31 ± 0.03a |
| Cotyledons | | | | |
| FW (mg) | 0.0 | 1.79 ± 0.20a | 4.10 ± 0.70a | 5.48 ± 0.30a | 5.45 ± 0.80a |
| DW (mg) | 0.4 | 1.11 ± 0.08b | 1.41 ± 0.20b | 1.88 ± 0.70b | 1.29 ± 0.20b |
| (FW-DW)/DW | 0.0 | 1.11 ± 0.08b | 1.41 ± 0.20b | 1.88 ± 0.70b | 1.29 ± 0.20b |

Each value corresponds to weight of an embryonic axis and a pair of cotyledons. For each seedling component, values within each column followed by a different letter are significantly different at the 0.05 confidence level. (n=5)
tion was higher in the embryonic axes as well as cotyle-
dons of seeds in NaCl than in non-stressed seeds (Figure 
3A and B). Subsequently, the sucrose content of embry-
onic axes in saline conditions was lower than in the con-
trol seeds (Figure 3A). In cotyledons the sucrose content 
in salt-stressed seeds was higher than in the control in 
distilled water after 6 h of germination. However, in dis-
tilled water the sucrose content in cotyledons decreased 
markedly after 6 h (Figure 3B).

The total soluble sugar/dry weight ratio in embryonic 
axes and cotyledons during the first 6 h of seed germina-
tion increased dramatically in controls, then decreased 
rapidly (Figure 4A and B). Under salt stress, both tissues 
showed an initial decrease in total soluble sugar/dry 
weight ratios, followed by recovery in later stages.

![Figure 2](image)

**Figure 2.** Changes in total soluble sugars content in embry-
onic axes (A) and cotyledons (B) of quinoa seeds germinating in ⊗, 0.0 M and ●, 0.4 M NaCl. The values correspond to an 
embryonic axis and a pair of cotyledons. Values are means of 4 replicates and each sample was measured 4 times. Bars on data 
points are ± SE of the mean (not shown when smaller than the symbol).

![Figure 3](image)

**Figure 3.** Content of fructose (top), glucose (middle) and su-
crose (bottom) in embryonic axes (A) and cotyledons (B) of 
quinoa seeds germinating in ◆, 0.0 M and ●, 0.4 M NaCl. The 
values correspond to an embryonic axis and a pair of cotyledons. 
Values are means of 4 replicates and each sample was measured 
4 times. Bars on data points are ± SE of the mean (not shown 
when smaller than the symbol).

**Discussion**

This investigation monitored changes caused by salt 
stress in germination rate, growth, and carbohydrate lev-
els of *Chenopodium quinoa* Wild. seeds. Chenopodiaceae 
and other halophytic taxa are well known for their extraor-
dinary salt and drought tolerance (Reimann and Breckle, 
1993; Storey and Wyn Jones, 1979). In spite of the over-
whelming importance of the Chenopodiaceae in saline 
environments, most studies of their ion relations have 
been restricted to salt tolerant, natrophilic species (Naidoo 
and Rughunan, 1990; Yeo and Flowers, 1986). Growth 
stimulation by salt has been reported for several 
alophytes, but it is less clear from the available data 
whether this occurs at the germination stage of 
development. In this regard our results are in agreement 
with those of Mayer and Poljakoff-Mayber (1975) and 
Ungar (1996), who found that the seeds and seedlings of 
several halophytes were less tolerant to salinity than grow-
ing plants. However, Uchiyama (1987) determined that 
seeds of *Atriplex nummularia* (Chenopodiaceae) were less 
sensitive to salinity than mature plants.
quinoa seeds, where the final percentage of germination, at 24 h, was severely reduced (-70%) by high levels of salt. With regard to the second consideration, our investigation showed that inhibition of quinoa seed germination was almost totally reversed in distilled water, with a high percentage of recovery (67%). Similar declines and recoveries in seed germination have been reported for several other halophytes (Khan and Ungar, 1984; Woodell, 1985; Ungar, 1996; González and Prado, 1992). Consequently, and in coincidence with data obtained for other members of the Chenopodiaceae by Ungar (1996), we can assume that salinity treatments did not induce a specific salt toxicity in *C. quinoa*. Nevertheless, the quinoa seeds according to Ungar (1996) seemingly develop an osmotically enforced “dormancy” in the presence of NaCl, which is removed once that seeds are transferred to distilled water. Thus, it may be more appropriate to use the term “seed survival,” instead of “germinability,” as a criterion for success of seeds under saline conditions. In terms of evolution, the ability of halophytes to survive under hypersaline conditions can be considered as a selective advantage, distinguishing them from most glycophyles.

In relation to seedling growth, the cotyledons and the embryonic axes were suppressed by NaCl. They were smaller than in distilled water because of reduced FW resulting from reduced water absorption (Prado et al., 1995). The reduction was principally in the embryonic axes, where a large increase in FW was observed in distilled water when compared to that in NaCl. The FW increase of embryonic axes in distilled water was mainly due to an increase in tissue water content and was reflected in a (FW-DW)/DW ratio change from nearly 1.80 to 5.50. This increase was not significant in cotyledons, where the (FW-DW)/DW ratio showed no significant variation. The DW did not show significant differences in both embryonic axes and cotyledons, probably because cell expansion was not accompanied by cell division (González, et al., unpublished results). Thus, the FW increase is largely attributable to cell enlargement by water absorption, cell vacuolation, and turgor-driven wall expansion (Dale, 1988). DW increases are associated with cell division and new material synthesis (Sunderland, 1960). Our results suggest that a strong increase in cell size, instead of an increase in cell number, explains the increased growth of embryonic axes in distilled water.

The total soluble sugar/dry weight ratio of both embryonic axes and cotyledons during seed germination and seedling growth of *C. quinoa* under saline conditions decreased initially. This might be attributable to a diminished synthetic activity. The latter was evident in the little variation we observed in DW values. The increase observed at 6 h probably reflected an increase in carbohydrate metabolism, in response to the increased water uptake by germinating seeds. In distilled water, the same relation showed an initially strong increase, probably due to biosynthesis of soluble sugars, but after 6 h in NaCl a pronounced decrease was observed, which is probably attributable to active tissue growth.
According to Gill and Singh (1985) germination, growth, respiration, and other related processes can be affected in seeds that are subjected to salt stress; changes in any one of these processes can affect other metabolic activities, particularly the carbohydrate metabolism that plays an important role in germination. In this context, our results, showing variations that occurred in total soluble sugar content during exposure of *C. quinoa* seeds to salt stress, could be very useful for understanding the physiological events associated with germination of seeds under salt stress. Seed carbohydrate metabolism under stress conditions can be considered a dynamic process involving often concomitantly occurring processes of polysaccharide degradation and synthesis of new compounds. However, this supposition does not apply to quinoa seed germination because the low level of total soluble sugars observed in NaCl produced no increase in DW. Our results differed from that obtained with alfalfa (*Medicago sativa*), where a linear correlation existed between salt stress and dry matter accumulation (Schubert et al., 1995). Nevertheless, an increase in soluble carbohydrate content, principally sucrose in both monocotyledon and dicotyledon plants, has been associated with an adaptation to saline conditions among higher halophytes (Briens and Larher, 1982; Quick et al., 1989; Wang et al., 1996). It might also be mentioned that the adaptive value of sucrose in salt stress is questionable in species exhibiting a weak potential to store carbohydrates (Jefferys et al., 1979). Although some researchers agree that salinity and water stress induce soluble sugar accumulation (Binzel et al., 1989; Wang and Stutte, 1992; Kameli and Lösel, 1995), there are objections to the suggestion that metabolically labile primary metabolites, such as reducing sugars, are “compatible” cytosolutes, since many of them have effects on cytoplasmic enzymes and could be incompatible in high concentrations (Rozema et al., 1978). This differs from other compatible osmotic—such as betaine, polyols, and proline—that have little effect on enzyme activities (Ahmad et al., 1979). Thus, we could assume that the increase in sucrose content we observed in embryonic axes of salt-stressed *C. quinoa* seeds was associated with an active osmotic adjustment. It is also possible that the high sucrose content of salt-stressed *C. quinoa* seeds was due to a low invertase activity under such conditions (Prado et al., 1995). It could also be attributed to a general decrease in total metabolic activity caused by salt stress. In this regard the latter explanation could account for the decreases observed in glucose and fructose of NaCl treated embryonic axes. It is also possible that a more rapid hexose metabolism, supplying compounds involved in water balance is involved. This assertion is supported by studies with a variety of plants that demonstrate a salt or drought-induced conversion of hexoses and other carbohydrates, such as sucrose and starch, into sugar alcohols (polyols) and proline (Pfeiffer and Kutschera, 1996; Wang et al., 1996; Pérez-Alfocea and Larher, 1995). The high level of hexoses observed in embryonic axes in distilled water could also be related to an NaCl inhibition of α-amylase activity, as we observed in another preliminary study of starch perisperm degradation in *C. quinoa* seeds under saline conditions (Kortsarz, personal communication), and supported by other investigations on rice seedling growth (Lin and Kao, 1995). Nevertheless, the above explanation can not be applied to cotyledons, since differences in sugar levels were not as large as in embryonic axes, possibly because of weak development followed by a diminished metabolic activity in cotyledons.

As for the low sugar level observed in embryonic axes of salt stressed *C. quinoa* seeds, several questions remain open: Is this due to a low water entry into seeds, or is it due to metabolic alterations, or to both? Vacher et al. (1994) suggested that the salt tolerance of this species might be related to its capacity to adjust osmotically, but they did not provide direct supporting evidence. Their study focused only on growth of plants, without considering the germination process. Consequently, our study is the first contribution to understanding the physiological events that occur during quinoa seed germination and early development. Further investigations are needed, however, to enhance our understanding of the salt stress effect during seed germination.

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**Literature Cited**


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氯化鈉對 *Chenopodium quinoa* Willd. 種子發育、生長及水溶性糖含量的影響

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本研究乃探討氯化鈉對 *quinoa* (*Chenopodium quinoa* Willd.) 種子及發芽的子葉及胚軸在早期發育時的影響。在高鈉的環境下發育速率很明顯地下降。發芽率的延緩與氯化鈉濃度並非呈線性關係。在 0.4 M 氯化鈉浸泡 14 小時後，發芽率只有 14%，然而控制組在同樣的時間下發芽率可達到 87%。在 14 小時的氯化鈉處理後無法發芽的種子百分比比用蒸餾水處理的還要低（分別是 7% 及 16%）。在這些以氯化鈉處理後而無法發芽的種子中，若以蒸餾水清洗則可得到一個很高的發芽率（67%）。在鹽水處理後胚軸與子葉的濕重均下降。最明顯的濕重增加發生在胚軸，經歷 10 小時的發育之後，以蒸餾水處理的種子比以鹽水處理者的胚軸溼重高 3 倍。在蒸餾水處理的環境下，胚軸的水含量在前 10 小時顯著的增加，然後則維持恆定。在鹽水處理的環境下，水含量並沒有顯著的改變。氯化鈉並不會強烈的抑制胚軸或子葉的乾重。胚軸與子葉的總可溶性糖含量，在蒸餾水處理的環境中很明顯的上揚，並在 6 小時後達到顴峰。在有氯化鈉的環境下，胚軸的葡萄糖與果糖含量有降低的情形，然而子葉的葡萄糖與果糖含量則沒有顯著的不同。以氯化鈉處理的種子其子葉的蔗糖含量比控制組高。關於鹽水逆境對發芽、水含量、幼苗的生長及可溶性糖含量彼此關係的影響將在本研究中進行討論。

關鍵詞：*Chenopodium quinoa*：發芽：生長：鹽逆境：種子：可溶性糖量。