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Variations in α-, β-amylase and α-glycosidase activities in two genotypes of wheat under NaCl salinity stress

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Two wheat differing in salt sensivity, was examined for osmolyte contents and activities of α-amylase, β-amylase and α-glucosidase enzymes involved in seeds germination, in absence as well as in presence of 100, 150, 200 and 300 mM NaCl. The inhibitory effects of NaCl differed, depending on the species tested. In wild wheat specie (*Triticum monococcum*), with reduced germination percentage and lower relative water content, the increase in NaCl concentration resulted in the decrease in endogenous level of proline, total soluble carbohydrate and activities of the main enzymes involved in the germination process. In contrast, cultivated wheat specie (*Triticum aestivum*) seed in response to salt stress accumulated higher proline and total soluble carbohydrate concentrations which improved their water status and the enzyme activities involved in the germination process. Differential response of the different species of wheat to salt stress is governed by the accumulation of osmolytes in seeds.

Key words: Salinity, amylases, glucosidases, wheat species.

INTRODUCTION

The effects of salinity on plant growth have extensively been a focus of research because of salt response of plants is a complex phenomenon that involves several physiological and biochemical changes (Pakniyat and Armion, 2007). Ionic imbalance occurs in the cell due to excessive accumulation of Na^+ and Cl^- and reduces the uptake of other mineral nutrients such as K^+ and NO^-_3 (Sultana et al., 2001). It has been suggested that Na^+ and Cl^- accumulation in root over shoot could be useful as indicator of salinity tolerance of plants (Silveira et al., 2001; Collado et al., 2010). The best manifestation of this is exemplified by those cases in which gain in dry mass were associated with decreased accumulation of Na^+ and Cl^- in shoot of some woody plants in the early seedling phase (Viegas et al., 2003; Khosravinjad et al., 2009).

Wheat is one of the world's major cereal crops. Salinity is the key constraint to wheat production in irrigated agriculture in many parts of the world. Salt stress leads to a series of morphological, physiological, biochemical and molecular changes that adversely affect plant growth and productivity. The tolerance to salt stress is accompanied by alterations in the levels of proteins. Salinity causes either decrease or increase in the level of soluble proteins or completely disappears in some proteins when compared to the control treatment (Yildiz and Terzi 2008). In addition, salt stress promotes a complete loss of present proteins and the synthesis of newly formed proteins (Yildiz and Terzi 2008). While some of genes whose expression is activated in response to salt stress encode for protective proteins such osmotin, proteins and

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ion transporters (Souza et al., 2003).

Complex molecular responses including the accumulation of compatible solutes, the production of stress proteins and the expression of different sets of genes are part of the plant signalling and defence system against salinity (Jimenez-Bremont et al., 2006). It is well known that one of the most common responses to salinity is the overproduction and accumulation of praline, glycine-betaine and total carbohydrate. Solute accumulation by cells contributes to stabilization of enzyme/protein and torgor maintenance in growing organs and has been correlated with productivity under stress (Ashraf and Foolad, 2005).

In the present study, two species of wheat with different salinity tolerance were studied to determine factors responsible for failure of germination. To fulfil such an aim, the effects of different NaCl concentrations were evaluated on relevant biochemical process associated with seed germination.

MATERIALS AND METHODS

Plant material and growth conditions

The experiment included two wheat grains *Triticum aestivum* and *Triticum monococcum*. In particular, *T. aestivum* is cultivated commercial wheat specie, whereas *T. monococcum* is a wild local population in Tunisia.

Germination condition

Seven months old well stored (20±1°C and ±5% RU) seeds of each lentil genotype used in the experiment were surface-sterilized for 20 min in 30% (v/v) H_2O_2, rinsed and soaked in distilled water for 1 h. Fifty representative seeds per cultivar were placed on a filter paper in 9 cm Petri dishes containing 3 cm^3 of distilled water (control), or 100, 150, 200 and 300 mM NaCl. The Petri dishes were hermetically sealed with parafilm to prevent evaporation and then care kept in a humidity chamber at a temperature of 25±1°C in the dark. The seeds were considered germinated when there was radicle protrusion through the seed coat. In order to determine the dry weight, twenty-five seeds of each cultivar, were taken out and were dried at 70°C in an oven till there is no decrease in weight.

Enzyme assays

Alpha-, beta-amylase and alpha-glucosidase activities in the crude extracts of each species were determined. The samples seeds of each wheat seeds, in deionised water (control) and treated with different concentrations of NaCl (100, 150, 200, 300 mM) were homogenised in a chilled mortar with distilled water 1:4 (w/v) and centrifuged at 14000 g for 30 min. The supernatants were filtered through a single layer of muslin cloth and were used for α-amylase (EC 3.2.1.1) (Coombe et al., 1967), β-amylase (EC 3.2.1.2) (Bergmeyer et al., 1983), α-glucosidase (EC 3.2.1.20) (Bergmeyer et al., 1983) estimation.

Proline and total soluble carbohydrate dosage

For determination of proline contents seeds were hand-homogenized in 3% of sulfosalicylic acid and centrifuged at 3000 g at 4°C for 10 min. The supernatants were used for proline estimation (Bates et al., 1973). The total soluble carbohydrate was determined with the anthrone method (Hansen and Moller, 1975).

Experimental design and statistical analysis

Data were analyzed separately for each species by one way procedure of ANOVA ($p \leq 0.05$) according to a completely randomized design with five replicates. Treatment means were compared using the Student-Newman-Keul test ($p \leq 0.05$) (Sokal and Rohlf, 1969).

RESULTS

Germination of two wheat species began from 24 h after sowing, reaching germination percentage higher than 98%. Salinity caused a delay in germination that was different among the cultivars. At 48 and 72 h the germination percentage of *T. monococcum* under salinity was significantly lower compared to *T. aestivum*. However, at 300 mM NaCl, final germination percentage was significantly reduced in *T. monococcum* (10%), while under control condition 100 and 98% germination occurred for both species, respectively. The cultivar *T. aestivum* germinated more than the other ones (82%), exhibiting a fair degree of salt tolerance. Increasing salinity a gradual decrease in the relative water content (RWC) in two cultivars was observed. The lower water content was detected in *T. monococcum* (data not shown).

The level of total soluble carbohydrate increased to a smaller extent over stressed seeds of T. *monococcum* specie (Figure 1). On the contrary, as a consequence of stress, in *T. aestivum* specie, increasing salinity a gradual increase in total soluble carbohydrate was observed (Figure 1).

The proline content of *T. estivum* specie was enhanced at different salt concentrations (Figure 2). The highest amount of proline was observed at 200 and 300 mM NaCl for both species. Interestingly, *T. aestivum* accumulated two times more proline than *T. monococcum* (Figure 2). Salinity had pronounced effects on α-amylase, β-amylase and α-glucosidase activities. The activity of these enzymes decreased in a dose-dependent manner, differing among the wheat species (Figures 3 to 5). The effect of salinity was more pronounced in seeds of *T. monococcum*. β-amylase and α-glucosidase activities showed also a decreasing trend with the increase in NaCl concentration. The activities of β-amylase and α-glucosidase in stressed seeds of *T. aestivum* were higher compared to those detected in *T. monococcum* (Figures 4 and 5).

DISCUSSION

Genetic variability within a species offers a valuable tool

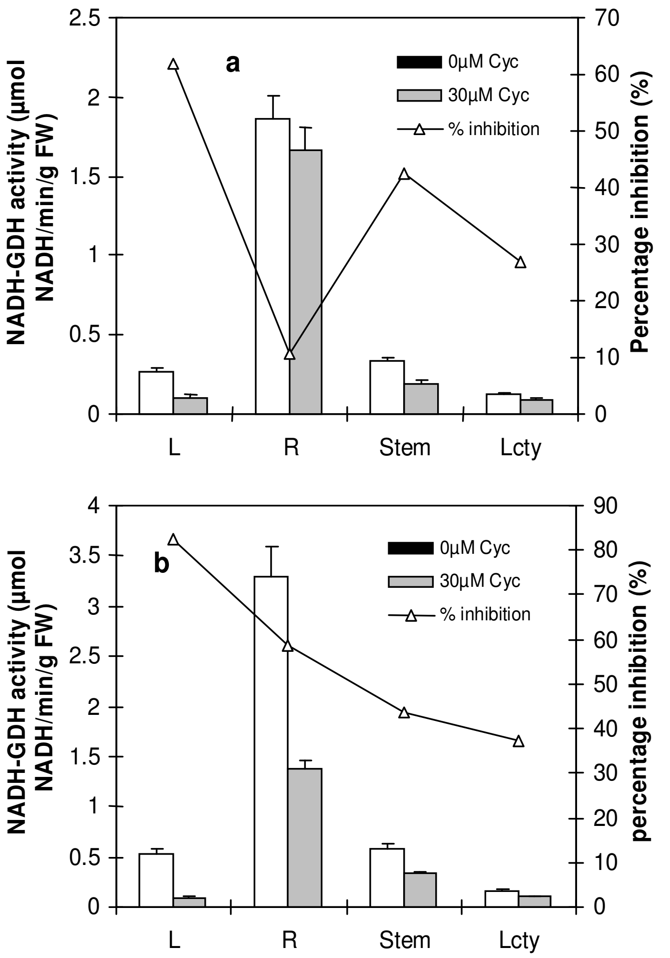

Figure 1. Cycloheximide effects on NADH-GDH activity in leaves (L), roots (R), stem (T+P) and cotyledolnal leaves (Lcoty), in absence (a) or in presence (b) of 20 µM cadmium. Results were expressed as percent (%) of control plants. L: 0.2650 ± 0.02, R: 1.863 ± 0.15, T+P: 0.336 ± 0.025, Fcoty: 0.126 ± 0.012 µmol NADH ox/min/g FW.

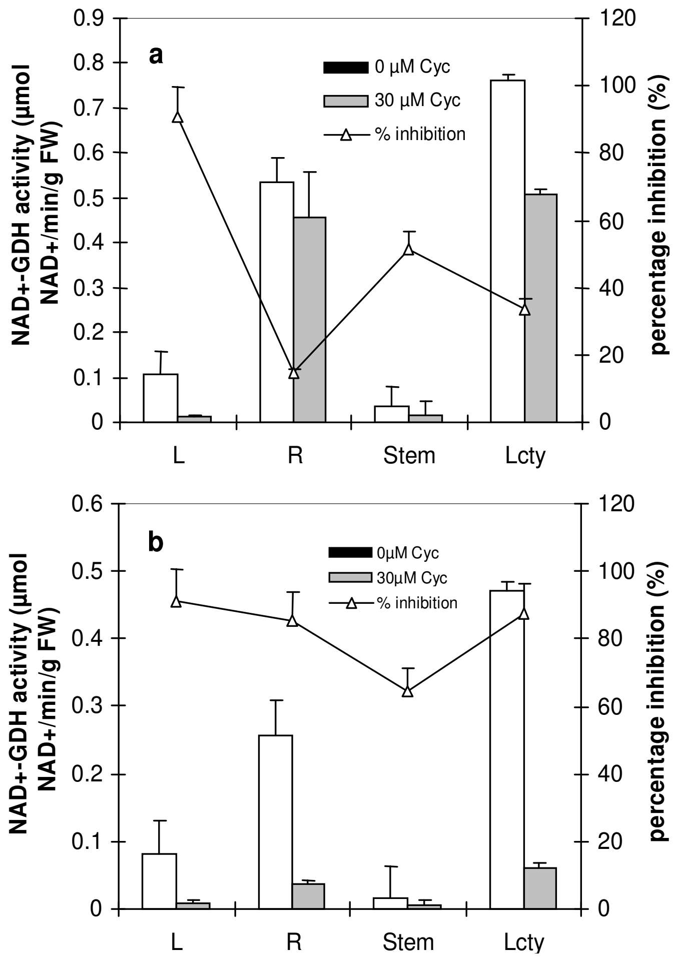

Figure 2. Cycloheximide effects on NAD$^+$-GDH activity in leaves (L), roots (R), stem and cotyledolnal leaves (Lcoty), in absence (a) or in presence (b) of 20 µM cadmium. Results were expressed as percent (%) of control plants. L: 0.273 ± 0.02, R: 2.63 ± 0.15, T+P: 1.26 ± 0.025, Lcoty: 0.26 ±0.012 µmol NADH red/min/g FW.

for studying mechanism of salt tolerance. Our results show a decrease in germination and vigour of seeds in all studied genotypes, with increasing salinity. In the cultivars *T. monococcum* and, in minor extent, the magnitudes of such decrease were more as compared to that of cultivars *T. aestivum*, in particular way when high salinity concentrations were used. A threshold level of hydration is required for the synthesis of hydrolitic enzymes which are responsible for the hydrolysis of stored substrates. The hydrolyzed products are utilized in seedling tissue synthesis and radicle elongation (Canas et al., 2006). In lentil seeds, the role of providing utilizable substrates is taken over mainly by amylases. Inhibition of germination due to salinity as suggested in previous reports (Ates and Tekeli, 2007) is attributed to a decrease water content, that affect the synthesis of hydrolitic enzymes limiting the hydrolysis of food reserves from storage tissues as well as to impaired translocation of food reserves from storage tissue to developing embryo axis (Ben Dkhil and Denden, 2010; Misic et al., 2009). It can be also hypothesized that the presence of NaCl at low concentrations, which is penetrating ions, could have contributed to a decrease in the internal osmotic potential of germinating structures, as suggested by Dodd and Donovan (1999) and Almasouri et al. (2001) leading to water uptake and initiation of germination processes. Our results indicate a lower content of total soluble carbohydrate and proline in presence of the highest salt concentration in *T. monococcum* compared to *T. aestivum* cultivars, suggesting that salt tolerance ability of these two last landraces appears to be associated to the accumulation of osmolytes which improved their water status. Salt stress has been reported to limit the mobilization of starchy endosperm reserves in several species, as a result of inhibition of different enzymatic activities (Ashraf and Foolad, 2005; Besma and Mounir, 2010). Starch mobilization results from simultaneous

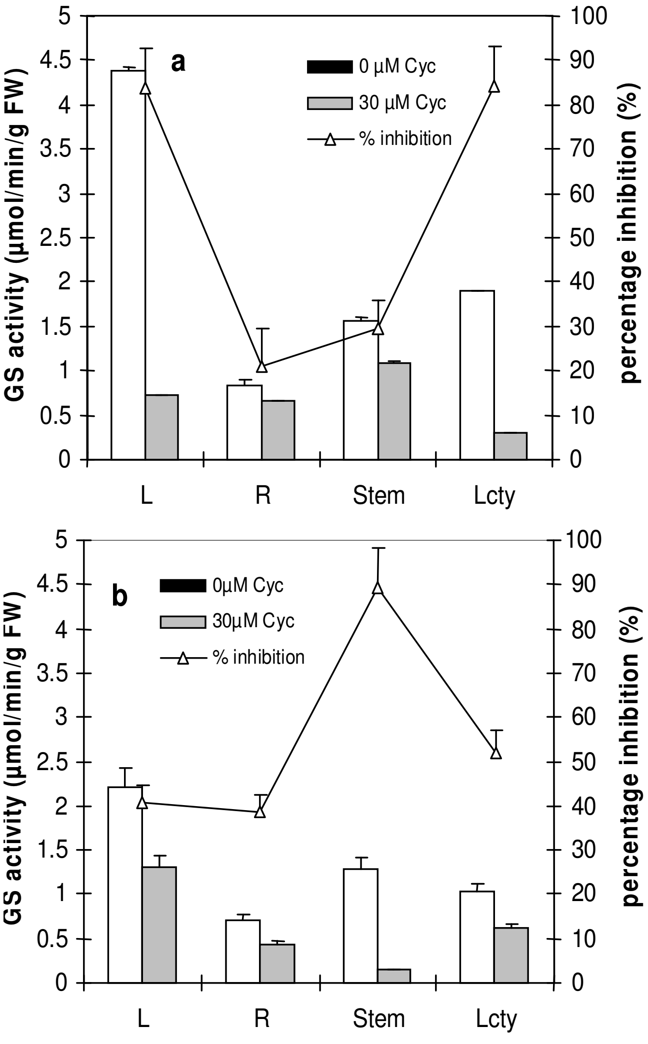

Figure 3. Cycloheximide effects on glutamine synthetase (GS) activity in leaves (L), roots (R), stem (T+P) and cotyledolnal leaves (Lcoty), in absence (a) on in presence (b) of 20 µM cadmium. Results were expressed as percent (%) of control plants: L: 2.65 ± 0.08 µmol γ-GHM/g FW/min, R: 1.012 ± 0.202 µmol γ-GHM /g FW/min, stem: 1.55 ± 0.1 µmol γ-GHM /g FW/min, Lcoty: 0.66 ± 0.052 µmol γ-GHM /g FW/min.

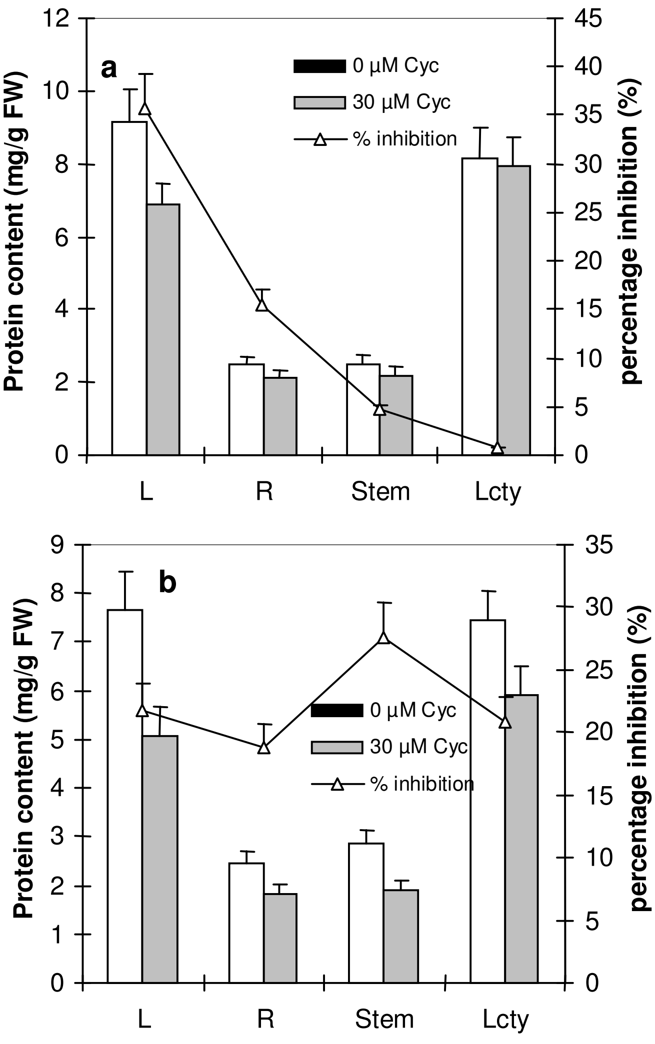

Figure 4. Cycloheximide effects on total soluble protein content in leaves (L), roots (R), stem and cotyledonal leaves (Lcoty), in absence (a) or in presence (b) of 20 µM cadmium. Results were expressed as percent (%) of control plants. L: 9.126 ± 0.92, R: 2.475 ± 0.15, stem: 2.376 ± 0.25, Lcoty: 8.164 ± 0.8 mg/g FW.

activities of α-amylase, α-amylase and α-glucosidase. In germinating seeds, starch degradation is initiated by α-amylase (Kaur et al., 2005), that produces soluble oligosaccharides from starch and these are then hydrolyzed by β-amylase to liberate maltose. Finally, α-glucosidase breaks down maltose into glucose, the main respiratory substrate (Sticklen, 2008). Numerous works correlated germination performance with α-amylase, but in a study of the comparative importance of α and β-amylase in determining germination ability, Yamasaki (2003) demonstrated greater importance of β-amylase compared to α-amylase, during the early hours of germination in wheat scutella. In addition, Nandi et al. (1995) showed that β-amylase activity becomes detectable immediately before visible germination becomes evident, whereas α-amylase activity is initiated at later stage of germination, suggesting that α-amylase affects rate of seedling growth while β-amylase activity is associated with initiation of germination. Therefore, β-amylase is a crucial and essential enzyme for germination. Our results show that high salt concentrations adversely affected each enzyme in all genotypes examined. Nevertheless in this work β-amylase activity was always significantly lower in *T. monococcum* compared to their own control and salt treated *T. aestivum* at every concentration used. In addition also when *T. monococcum* was treated with low salt concentration (150 mM), β-amylase activity at 72 h was lower than that detected at 24 h in their own control or in *T.aestivum* salt treated seeds at every concentration

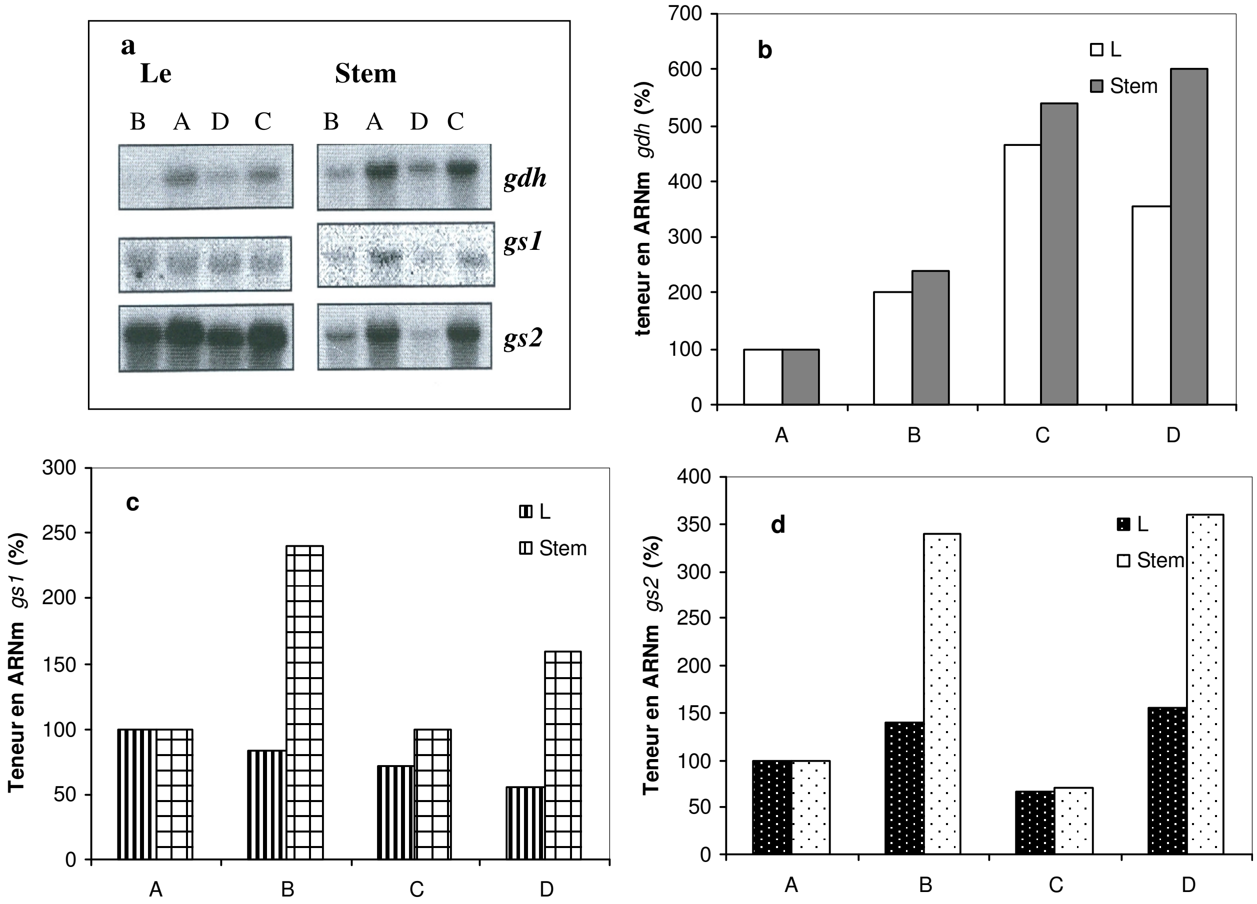

Figure 5. Cycloheximide effects on NADH-GDH activity report, NAD$^+$-GDH activity report and GS activity report: B/A and D/C. A: -Cd/-Cylohéximide; B: -Cd/+Cyclohéximide; C: +Cd/-Cyclohéximide; D: +Cd/+Cyclohéximide.

concentration used, suggesting that this enzyme could be salt sensitive related. The variation in stress sensitivity of contrasting lentil genotypes may be linked to their ability to osmoregulate under stress, which cause a strong decrease in water content affecting the hydrolytic enzyme activities, particularly β-amylase levels. β-amylase is probably synthesized during imbibition, in fact a seed hydratation pre-treatment in rice or other cereals species, that enhances seed vigour was found also to bring about an enhancement in β-amylase activity (Mares and Mrva, 2008). Thus, data presented in all these reports provide support for the view that the higher β-amylase activity in *T. aetivum* might be the cause for the major seed cultured in presence of NaCl salinity.

Some important conclusions can be drawn from the results achieved during this experiment. Although wheat is considered a very sensitive specie to salinity, much more than other legumes such as broad bean and soybean, we have identified *T. aestivum*, found in a southern Tunisian semiarid environment, that could be utilized not only in breeding programs to improve the saline resistance of the species but also to be cultivated in environments where salinity of the soils is a frequent constraint.

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