

## PLANT SCIENCE

# Agronomic and physiological responses of pearl millet ecotype (*Pennisetum glaucum* (L.) R. Br.) to saline irrigation

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### Abstract

The objective of this study was to identify morphological and physiological traits for salinity tolerance in Tunisian autochthonous ZZ pearl millet ecotype (*Pennisetum glaucum* (L.) R. Br) under local conditions. ZZ ecotype was cultivated under different levels of salinity and growth parameters, water relations and mineral content were measured. Results showed that ZZ pearl millet ecotype was unable to store the large amounts of salt in the leaves, while maintaining high leaf water content and without a grave consequent on panicle yield. This ecotype is fitted for a selective sodium sequestration in the vacuole.

*Key words:* pearl millet, Saline water, Morphological trait, Physiological trait, Leaf water content, Ion content

### Introduction

Pearl millet [*Pennisetum glaucum* (L.) R. Br] is one of the major cereal crops of the semi-arid regions of Africa and Asia and it is certainly the mainstay for millions of people in the Sahel. It's grown as grain and fodder crop (Blummel et al., 2003). In Tunisia, pear millet is not the staple food of rural populations as in the other countries of Africa. Nevertheless, it occupies a very important part of surfaces every year in the centre and in the South of the country. In 2003, local production was about 19150 metric tonnes of pearl millet grains principally produced in Kairouan (50%), Medenine (26%), Nabeul (15.6%) and Mahdia (3.4%) regions (FAO, 2003). All pearl millet production is used for a variety of food products.

Pearl millet is a summer irrigated crop. However, Tunisia, as in the majority of the arid areas, is classified among the countries threatened by dryness and salinity (Qadir et al., 2006). In fact, water availability is below the threshold of 1000 m<sup>3</sup>/person/year (Paranychianakis and Chartzoulakis, 2005). In order to overcome water scarcity, many countries have adopted the use of marginal water for irrigation (Oron et al., 2002).

However, the salinity of those water sources typically exceeds the limit tolerated by conventional crop plants which are for the majority sensitive glycophytes (Hu et al., 2005).

Plants, whether glycophyte or halophyte, cannot tolerate large amounts of salt in the cytoplasm, so, they develop a plethora of mechanisms to cope with salt stress and to facilitate their metabolic functions (Zhu, 2003). In fact, salt stress affects all the major processes such as growth, photosynthesis, protein synthesis, and energy and lipid metabolism (Parida and Das, 2005). However, some moderately or highly salt tolerant plants can survive in salty environments. These species are able to avoid ion toxicity and maintain water uptake in the presence of high salt concentrations (Munns, 2002).

Pearl millet (*Pennisetum glaucum* (L.) R. Br.) is rated to be fairly tolerant to salinity (Krishnamurthy et al., 2007). Moreover, availability of high levels of tolerance in other species of *Pennisetum* (Muscolo et al. 2003) and within the *P. glaucum* (Krishnamurthy et al., 2007) offers a scope for understanding the traits related to tolerance and to integrate these tolerant crop species/genotypes into appropriate management programs to improve the productivity of the saline soils (Baisakh et al., 2008).

Identifying autochthonous ecotypes growing under local agricultural conditions with significant levels of beneficial factors may promote the value-added cultivation and enhancing the agricultural economy. The effects of salt stress on plant growth and physiology have been well documented in other

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cereals (Lopez et al., 2010; Mehta et al., 2010). However data on specific effects of salt stress in autochthonous pearl millet are still fragmentary especially effects on pearl millet physiology. The research objective was to identify morphological and physiological traits for salinity tolerance in Tunisian autochthonous ZZ pearl millet ecotype under local conditions.

## Materials and Methods

### Plant material

Autochthonous ZZ pearl millet ecotype whose salt tolerance characteristics were determined in our previous study (Radhouane, 2008) was used. It is tall stature (> 2m) and has an intermediate duration of cycle (about 80 days). It was collected in Zarzis. The site is located at 33°30' latitude and 11°07' longitude. It has a Mediterranean climate.

### Plant growth and treatments

The experiment was carried out at the farm of the Tunisian Agricultural Research Institute in Tunis during the cropping season of 2008. The site is located at 36°51' latitude and 10° 11' longitude. The soil of the experimental site was clay loam. It was sown at 22 May 2008 into randomized block design with four replications.

The sampling area was 5.5 m<sup>2</sup>, having rows five meters long was used. Sowing was done in hills and row to row distance of 50 cm and hill to hill distance of 30 cm were used. A basal dose of 50 kg N in the form of ammonitrate 33% fertiliser was applied at sowing. Irrigation and all other agronomic practices were carried out uniformly for all the experimental units. Total irrigation volume of 420 mm was applied (one a week) and treatments were initiated at emergency of the fourth leaf.

Three salt levels were applied.

T<sub>1</sub>: water containing 1 g/l NaCl (control no added NaCl)

T<sub>2</sub>: T<sub>0</sub> + 3 g NaCl = 4g/l

T<sub>3</sub>: T<sub>0</sub> + 6 g NaCl = 7g/l

### Technical Analysis

Data were recorded on:

- plant height (PHT) in cm
- flag leaf surface (FLS) in cm<sup>2</sup>
- panicle grain yield (PGY) in g
- flag leaf water content (RWC) in %
  - leaf water potential (LWP) in (MPa)
  - some ions contents (Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup>) in %

Plant height was determined using a graduated ruler (from the neck to the insertion of the panicle, while leaf area was measured with leaf area meter (MK 2) immediately after harvesting. At maturity,

mature panicles were shelled and individually weighed.

Relative water content (RWC) was determined on flag leaf tissues excised in the morning (around 8:00 am). Excised leaves were measured for fresh weight (FW), and then rehydrated in a water-filled Petri dish at room temperature. Turgor weight (TW) was measured by allowing full rehydration (16 h), removing all water on the leaf surface, weighing, and then leaves were dried at 70°C for 48h to determine DW (Hensen, 1982). The relative water content was calculated from the following equation

$$[RWC = 100[(FW - DW)/(TW - DW)].$$

Leaf water potential was measured at the abaxial surface of intact plants with pressure chamber (Scholander et al., 1965).

For determining ion content, mature flag leaves were taken and oven dried for 72h at 70°C. After desiccation, samples were minced and incubated overnight in a 0.1N HNO<sub>3</sub>. After filtering, 0.5ml of the solution was used for determination ion contents (Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup>) by flame photometry (Model 410, Corning, England) (Gulati and Jaiwal, 1992).

### Statistical analysis

Data regarding plant height, flag surface leaf, panicle grain yield, relative water content and ions content were recorded on 50 plants at time of maturity.

Data were statistically analyzed using analysis of variance techniques appropriate for randomized complete block design. Main and interaction effects were separated by LSD test at 0.05 level of probability, if the F-values were significant.

## Results and Discussion

The detrimental effects of high salinity on plants can be observed at the whole-plant level as the death of plants and/or decreases in productivity (Parida and Das, 2005).

Salinity is known to affect also various facets of plant metabolism. In fact, the various concentrations of NaCl had a significant effect on ZZ pearl millet ecotype behaviour.

### Plant height

The statistical analysis of the data indicated that salinity had significant effect on plant height of autochthonous ZZ pearl millet ecotype (Fig.1). PHT value decreased significantly by salt treatments. Maximum plant height of 207cm was attained by control (T<sub>1</sub>) and plants treated with T<sub>3</sub> had the lowest mean PHT of about 169 cm. Plant

height for T<sub>3</sub> was 18% significantly lower ( $P \leq 0.01$ ) than T<sub>1</sub> and 13% than T<sub>2</sub>.

Generally, salinity stress results in a clear stunting of plants (Takemura et al., 2000). Slower growth is a general adaptive feature for plant survival under stress, allowing re-directing cell resources (e.g., energy and metabolic precursors) towards the defence reactions against stress (Zhu, 2001).

In fact, salt in soil water inhibits plants ability to take up water, and this leads to slower growth (Manchanda and Neera, 2008). Suppression of growth occurs in all plants, but their tolerance levels and rates of growth reduction at lethal concentrations of salt vary widely among different plant species. Processes that regulate growth reduction have not been well documented (Hasegawa et al., 2000).

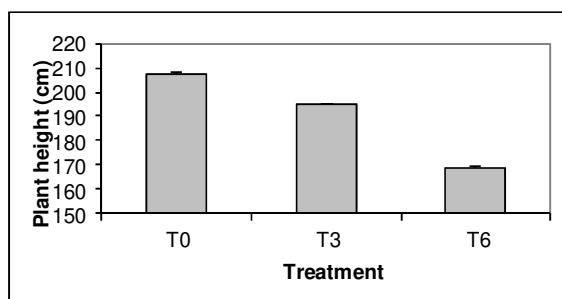


Figure 1. Plant height of ZZ pearl millet ecotype dependent on salinity treatment.

### Flag leaf surface

Increasing NaCl concentration resulted in reduced leaf size for ZZ ecotype (Figure 2). ZZ pearl millet ecotype had maximum flag leaf area at T<sub>0</sub> treatment. Leaf area for T<sub>3</sub> was 6.5 % significantly lower ( $P \leq 0.01$ ) than T<sub>1</sub> and 2.5% than T<sub>2</sub>.

Muscolo et al. (2003) reported that *Panicum clandestinum* growth and leaf length decreased with increase in salinity.

The decreased rate of leaf growth after an increase in soil salinity is primarily due to the osmotic effect of the salt around the roots (Passioura and Munns, 2000). Salt stress initially inhibits leaf expansion through reduced turgor and may in fact eventually result in increased cell wall extensibility, which counteracts the negative effects of low turgor. In the presence of salt, cell wall extensibility of the growing region may decrease (Nonami et al., 1995).

The reduction in leaf growth must be regulated by long distance signals in the form of hormones

or their precursors. It's independent of carbohydrate supply (Munns et al., 2000) and water status (Frickle and Peters, 2002).

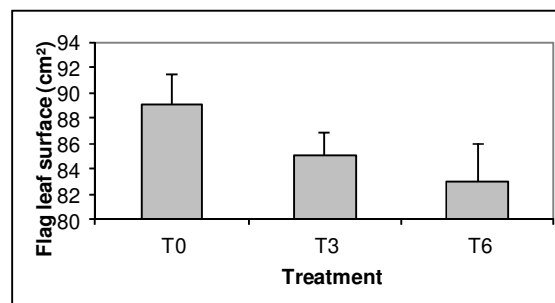


Figure 2. Flag leaf surface of ZZ pearl millet ecotype dependent on salinity treatment.

### Panicle grain yield

Grain head yield of ZZ ecotype continuously decreased with increasing salinity (Figure 3). The lowest head yield for ZZ ecotype occurred with T<sub>3</sub> treatment. Panicle grain yield declined by 8.7 % as planting was effectuated with high salinity. Nevertheless, RGC reduction was about 1.8% when T<sub>2</sub> treatment was applied.

Salinity is the major environmental factor limiting plant growth and productivity (Allakhverdiev et al., 2000). The altered water status leads to initial growth reduction and limitation of plant productivity (Parida and Das, 2005).

Salt stress affects uptake, transport and utilization of different nutrients (Grattan and Grieve, 1999), which may results in excessive accumulation of Na<sup>+</sup> and Cl in tissue (Saqib et al., 2005) and ultimately reduction in crop yield.

Pearl millet grain yields were slightly affected by moderate saline irrigation. This result is corroborated by Hussain et al. (2008). Depressed photosynthesis has been suggested to be responsible for at least part of the growth and yield reduction (Munns, 2002).

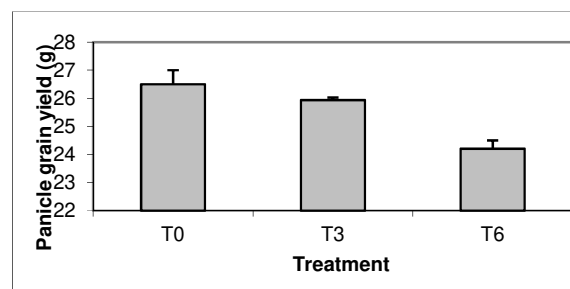


Figure 3. Panicle grain yield of ZZ pearl millet ecotype dependent on salinity treatment.

### Leaf water content

Within a salinity level, differences in RWC were not significant ( $P \leq 0.01$ ) with a range of 84-87% of saturated water content (Figure 4). Relative water content was statistically similar to that of the control. Similar result was found in RWC of many plants (Rivelli, 2002).

Lu et al. (2002) showed that RWC remained relatively unchanged under salinity for *Sueda salsa*. Maintenance of favourable plant water status contributes to salinity tolerance of the salt tolerant ecotypes (Oweis, 2009).

Maintaining a high water content in the growing leaves and in leaves expansion in the presence of stress, indicates osmotic adjustment effectiveness (Meloni et al., 2004). The osmotic adjustment (if any) results in a slower decrease of RWC when the leaf water potential continues to decline as observed by some authors on *T. durum* and *T. polonicum* (Al Hakimi et al., 1995).

ZZ pearl millet ecotype was able to balance the low external water potential and may potentially generated turgor and growth by many mechanisms to protect sensitive cellular sites of the salt adverse effects. This ZZ ecotype performance suggests a tolerance to salinity.

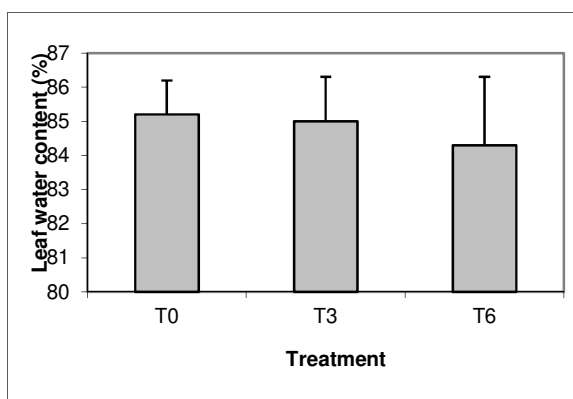


Figure 4. Leaf water content of ZZ pearl millet ecotype dependent on salinity treatment.

### Leaf water potential

The leaf water potential ( $\psi$  flag leaf) of ZZ pearl millet ecotype was higher in control plants as compared to the two different treatments (Figure 5). Brackish water irrigation has reduced water potential of 28% and 37% respectively for moderate treatment ( $T_2$ ) and severe stress ( $T_3$ ). Water potential becomes more negative with an increase in salinity (Gulzar et al., 2003). Decrease of the leaf water potential under salt stress has been reported by many authors, especially in  $C_4$  (Poaceae) (Maricle et al., 2006).

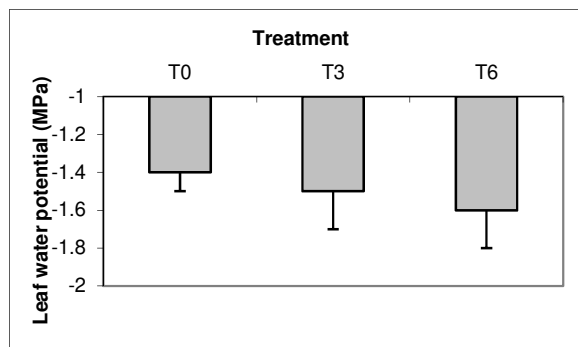


Figure 5. Leaf water potential of ZZ pearl millet ecotype dependent on salinity treatment.

Water potential reduction is the result of a rapid osmotic adjustment and an increase of the concentrations of osmotically (Koyro, 2006).

### Antagonistic interactions

Saline environment, most commonly mediated by high NaCl, results in perturbation of ionic steady state not only for  $\text{Na}^+$  and  $\text{Cl}^-$  but also for  $\text{K}^+$  and  $\text{Ca}^{2+}$  (Niu et al., 1995). Plants showed a change of the mineral composition towards  $\text{Na}^+$  and  $\text{Cl}^-$  uptake especially in the leaf (Koyro, 2006).

Accumulation of  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$  ions in flag leaf of ZZ pearl millet ecotype under three NaCl concentrations were presented in Figures 6, 7 and 8.

$\text{Na}^+$  is the predominate soluble cation in many of the soils of arid and semi-arid areas (Zhu et al., al., 2004). When saline applications were made, there has been a  $\text{Na}^+$  ion increasing in the leaves of ZZ ecotype resulting in positive correlations between leaf Na content and NaCl (Figure 6).

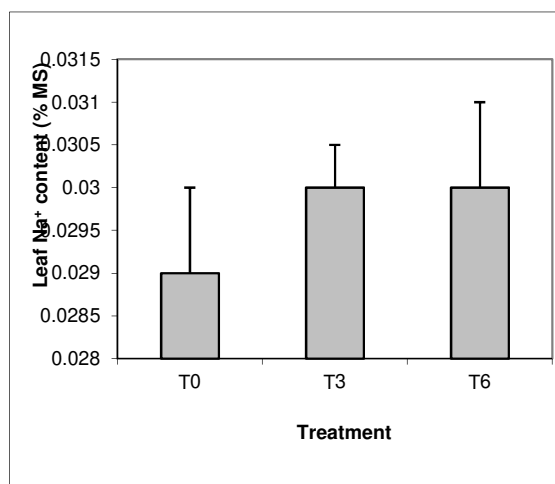


Figure 6. Foliar  $\text{Na}^+$  content of ZZ pearl millet ecotype dependent on salinity treatment.

As in some other plants (Kusvuran et al., 2007), tolerance to salinity is has been related to  $\text{Na}^+$  ion accumulation in plant green matter.

ZZ pear millet ecotype which grows and survives in saline media is fitted for a selective sodium sequestration in the vacuole (Cuin et al., 2003). This system therefore functions as a metabolic regulatory cycle to avoid critical concentrations in the cell. This adaptive mechanism thus has a homeostatic function in supplying metabolism with essential elements as well as detoxifying function (Smekens and Tienderen, 2001).

Sodium sequestration into vacuole appears to constitute the most effective mechanism of plant cells to handle efficiently high concentrations of salts and to prevent their toxic effects on cytoplasm. The compartmentalization of  $\text{Na}^+$  into vacuoles allows plants to use  $\text{Na}^+$  as an osmoticum, maintaining the osmotic potential that increases the water content within the cells (Blumwald et al., 2000).

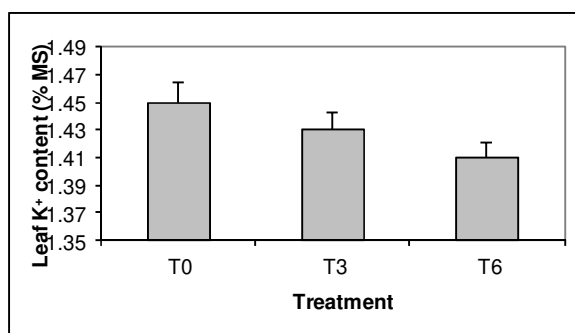


Figure 7. Foliar  $\text{K}^+$  content of ZZ pearl millet ecotype dependent on salinity treatment.

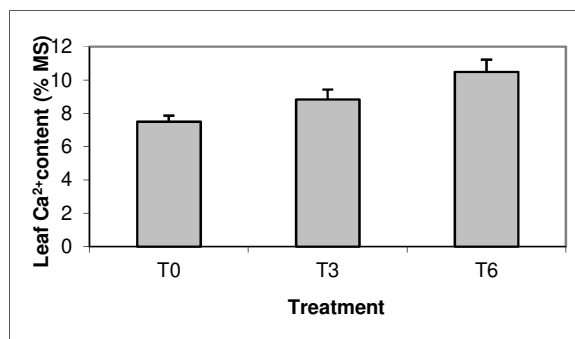


Figure 8. Foliar  $\text{Ca}^{2+}$  content of ZZ pearl millet ecotype dependent on salinity treatment.

$\text{Na}^+$  compartmentation is regulated by  $\text{Na}^+/\text{H}^+$  antiporters (Hasegawa et al., 2000). The overexpression of genes encoding  $\text{Na}^+/\text{H}^+$  antiporters in different plant species induced the

tolerance of plants to salinity (Zhang and Blumwald, 2001).

Potassium ( $\text{K}^+$ ) concentration in mature leaves was significantly ( $p < 0.05$ ) lower in plants grown with salinity (Figure 7). Although it has been found that there were increases in  $\text{Na}^+$  ion intake, there has been a decrease in  $\text{K}^+$  ion intake, External  $\text{Na}^+$  negatively impacts intracellular  $\text{K}^+$  influx, attenuating acquisition of this essential nutrient by cells (Niu and al., 1995). It has been reported that leaf potassium concentration is lowered by increasing  $\text{NaCl}$  concentration (Ozalp et al., 2000). Under saline soils, higher levels of external  $\text{Na}^+$  interfere with  $\text{K}^+$  acquisition limiting plant  $\text{K}$  uptake (Hussain et al., 2008).

Liu et al. (2000) reported that high affinity  $\text{K}^+$  transporters may act as low affinity  $\text{Na}^+$  transporters under salt stress which may reduce  $\text{K}^+$  uptake. In the cytosol, the presence of  $\text{K}^+$  is essential for the activation of many enzymes, for example, those involved in pyruvate synthesis and protein translation. Due to physicochemical similarities between  $\text{Na}^+$  and  $\text{K}^+$ , excess  $\text{Na}^+$  tends to substitute  $\text{K}^+$ , for  $\text{Na}^+$  at these binding sites and hence impair cellular biochemistry (Manchanda and Neera, 2008).

Saline water irrigation has increased Calcium content in the leaf of ZZ ecotype (Fig.8) of 17% and 40% respectively for moderate treatment ( $T_2$ ) and severe stress ( $T_3$ ). Similar result was found by Munns and Tester (2008).

Calcium has been shown to ameliorate the adverse effects of salinity on plants and is well known to have regulatory roles in metabolism (Ehret et al., 1990)

Bush (1995) hypothesized that sodium ions may compete with calcium ions for membrane-binding sites. Therefore, high calcium levels can protect the cell membrane from the adverse effects of salinity. Increase of  $\text{Ca}^{2+}$  uptake is associated with the rise of ABA under salt stress and thus contributes to membrane integrity maintenance, which enables plants to regulate uptake and transport under high levels of external salinity in the longer term (Chen et al., 2001).

## Conclusion

Autochthonous ZZ pearl millet is an ecotype known to be salt tolerant in the south of Tunisia. The ability of this ecotype to cope with severe salt stress is the combined characteristic of many plant features, both morphological and physiological. These mechanisms enable ZZ pearl millet ecotype to store the large amounts of salt in the leaves, while maintaining high leaf water content and

without a grave consequent on panicle yield. ZZ pearl millet ecotype is enabling to be a widely distributed species on all continents especially near the sea and to be utilized for different applied purposes: production of biomass for energy, enzymes or antioxidants and phytoremediation programs of saline soils.

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