Full Length Research Paper

Salinity effect on plant growth at the seedling stage of durum wheat (*Triticum durum* Desf.)

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Soil salinity is a serious threat in many parts of the world in general and of Tunisia in particular, which negatively affects plant production. In this experiment, response of durum wheat to salinity in seven genotypes namely; four local cultivars (Azizi, Jenah Khotifa, Hmira and Swebâa Eljia), two improved varieties (Karim and Om Rabia) and an algerian cultivar (Oued Zeneti)] to salinity (0 and 100 mM of NaCl) was investigated in hydroponic conditions. Salinity induced no effects in all genotypes on the maximal efficiency of PSII (F_v/F_m) photochemistry. The F_v/F_m showed that Jenah Khotifa and Karim were the most tolerant genotypes under saline conditions (100 mM of NaCl). This may be associated with higher chlorophyll (a and b) contents. Results also depicted that all genotypes showed higher content proline as well as lower sodium content as compared with Jenah Khotifa with a reduction of 68.53% under 100 mM NaCl. It was concluded that high chlorophyll content and the proline accumulation in plant will improve the salt tolerance of wheat genotypes in breeding programs.

Key words: Durum wheat, salt stress, genotypic variation, seedling stage.

INTRODUCTION

Salinity stress is one of the most serious abiotic factors limiting the productivity of agricultural crops. The detrimental effects of salt on plants are a consequence of both a water deficit resulting in osmotic stress and the effects of excess salt ions on critical biochemical processes (Wyn-Jones, 1981). Salinity affect more than 25% of worth land (Levigneron et al., 1995), and desertification and salinization are rapidly increasing on a global scale declining average yields for most major crop plants by more than 50% (Bray et al., 2000). Salt stress affects almost all aspects of plant development including germination, vegetative growth and reproductive development. Numerous physiological and biochemical changes occur in response to salt stress in various plant species. Changes in protein expression, accumulation and synthesis have been observed in many plant species as a result of plant exposure to salt stress during growth. Relative water content has been used as one of the

potential water relation parameters for assessing intra-specific variation for salt tolerance in a number of crops (El-Bassiouny and Bekheta, 2005; Yağmur and Kaydan, 2008; Achakzai et al., 2009) such as wheat (Pier and Berkowitz, 1987), maize (Premachandra et al., 1990) and sorghum (Jones et al., 1980). Meanwhile, leaf photosynthetic capacity depends on physiological characteristics such as chlorophyll contents, it has been reported that chlorophyll content decreases in salt susceptible plants such as tomato (Lapina and Popov, 1970) and pea (Hamada and El-Enany, 1994), but that chlorophyll content increased in salt tolerant plants such as pearl millet (Reddy and Vora, 1986) and mustard (Singh et al., 1990).

Chlorophyll fluorescence analysis may also provide a sensitive indicator of stress condition in plants. It can also be used to estimate the activity of the thermal energy dissipation in photosystem II, which protects both plant photosytems from the adverse effect of light and heat stress. The measurement of chlorophyll fluorescence *in situ* is a useful tool to evaluate the tolerance of the photosynthetic apparatus to environmental stress (Maxwell and Johnson, 2000). In salt stressed plants,

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osmotic potential of vacuole decreased by proline accumulation. It was thought that accumulated proline under environmental stress do not inhibit biochemical reactions and plays a role as an osmoprotectant during osmotic stress (Yoshiba et al., 1997). In addition, several possible roles have been attributed to super optimal levels of proline; osmoregulation under drought and salinity conditions, stabilization of proteins, prevention of heat denaturation of enzymes and conservation of nitrogen and energy for a post-stress period (Aloni and Rosenshtein, 1984). It is suggested that the low osmotic potential may cause proline accumulation in tissues (Buhl and Stewart, 1983; Singh et al., 1973).

To improve the capacities of tolerance to the salinity of cereals, it is of primary importance to include/understand the complexity of the mechanisms of tolerance and to propose stable criteria of selection (Monneveux et al., 1993). For this purpose, physiological measurements (chlorophyllian fluorescence, content chlorophyl), hydrous (relative water content, hydratation) as biochemical (proline content) were led in hydroponie on seven durum wheat genotypes at the seedling stage.

MATERIALS AND METHODS

Vegetal material

The experiment was conducted in the Laboratory of Genetic and Plant Breeding, Department of Agronomy and Biotechnology. Seven durum wheat genotypes (*Triticum durum* Desf.) namely, four local cultivars (Azizi, Jenah Khotifa, Hmira and Swebâa Eljia), two improved varieties (Karim and Om Rabia) and an algerian cultivar (Oued Zeneti) were used in this study.

Seed germination test and seedling growth

Seed of the seven durum wheat genotypes were initially sterilized with 12% bleach of sodium hypochlorite solution for 10 min and washed 3 times with sterilized water. Seeds were then transferred to Petri dishes (10 seeds per Petri dish with three replications) containing two filters paper moistened with 10 ml of water or the same solution added with NaCl. Petri dishes placed in a germination chamber in the dark condition for 72 h at 25 ± 2°C. Then the Petri dishes were transferred in same conditions under a 12 h daylight photoperiod. The Petri dishes were controlled in one day intervals for solutions content. Germination percentages were obtained on the 8th days using radical extrusion (2 mm long) criterion.

The seedlings at one leaf stage were placed in vats covered with a black plastic film supporting the root development and containing the nutritive solution of Hoeagland (FAO, 1984) oxygenated by a pump of ventilation and changed once per week. At the three leaves stage, plants were treated with different conditions; (1) control plants were grown in half strength Hoagland solution (FAO, 1984), and (2) salt-treated plants were grown in half-strength Hoagland solution plus 100 mM NaCl.

Measured traits

The relative water content (RWC)

The relative water content was calculated from the equation:

$$RWC = \frac{FW - DW}{TW - DW} \times 100$$

where FW is the fresh weight of the shoots, TW is the weight at full turgid, measured after floating the shoots for 24 h in distilled water in the light at room temperature and DW is the weight estimated after drying the shoots at 80°C or until a constant weight is achieved.

The hydration (H)

The hydration was evaluated from the equation:

H = 100 - 100 (DW/FW)

Chlorophyll content

The extraction of the chlorophyll pigments is carried out by crushing 2 g of the third leaf-blade in 80% (v/v) acetone/water. The extracts are proportioned by optical density at 663 and 645 nm using a spectrophotometer.

Chlorophyll fluorescence

The initial fluorescence (Fo) and maximal fluorescence (Fm), variable fluorescence (Fv) and maximal photochemical efficiency of PSII (Fv/Fm) were measured immediately after dark-adapted the leaves for 30 min. Measurement was performed using a portable Fluorescence Induction Monitor (FIM 1500, Analytical Development Company Limited, ADC) from three leaves stage.

Proline content

The extraction of the proline is carried out by crushing of the third leaf in ethanol solution at 40%, after heating with 85°C of the precipitate witch added 1ml of acetic acid, 25 mg ninhydrin and 1 ml of a solution containing 120 ml distilled water, 300 ml glacial acetic acid and 80 ml phosphoric acid. After cooling of the tubes in ice, the products were extracted with 5 ml of toluene by vortex mixing and the upper (toluene) phase decanted into a glass cuvette and absorbance read at 528 nm. Proline concentrations were calculated using proline standards (0 to 50 mg/ml) in identical manner.

Statistic analysis

The experimental design was arranged in a completely randomized design with 3 replications. The data were analyzed using ANOVA and subsequent comparison of means was performed using the Duncan's Test at 5% probability. Statistic analysis was carried out using computer software SPSS 10.

RESULTS AND DISCUSSION

Relative water content (RWC)

The mean values of the RWC across genotypes under normal conditions (0 mM/L) and salt stress treatments (100 mM/L) were 86.8 and 84.7%, respectively (Table 1). The genotypes Swebâa Eljia (81.92 mM) and Jenah
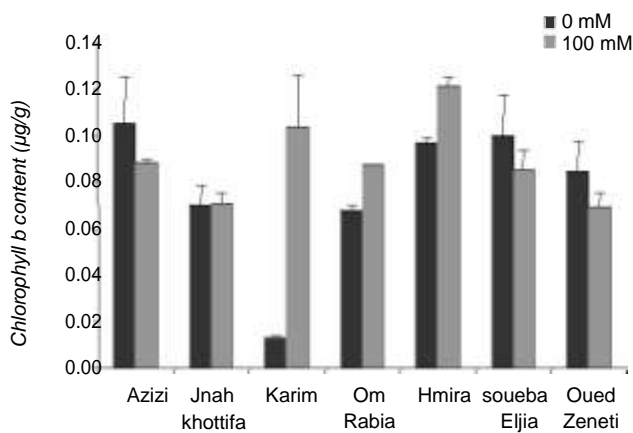
Table 1. Variance analysis for seven durum wheat genotypes for the relative water content (%) and hydration (%).

Variation source	Degree of freedom	RWC (%)	H (%)
Genotype	6	20.18^{ns}	27.36^{ns}
Treatment	1	13.6^{ns}	41.41^{ns}
Genotype* treatment	6	34.42^{ns}	21.23^{ns}

^{ns}: Not significant at the 0.05 probability level.
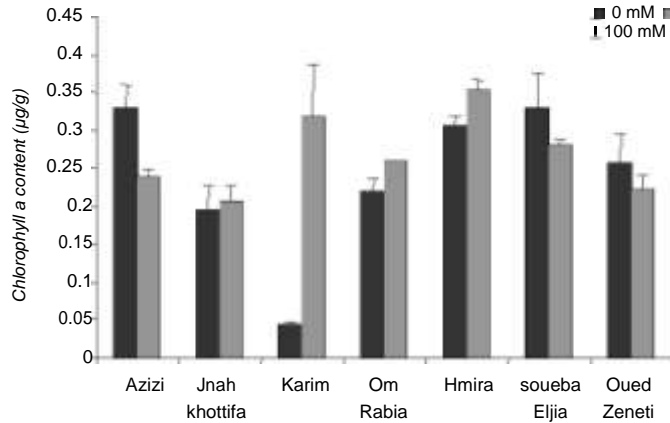
(A)

(B)

Figure 1. Chlorophyll (a) and (b) content at third leaves stage of seven durum wheat genotypes cultivated in hydroponie. (A) Chlorophyll a content under normal (0 mM) and salt stress conditions (100 mM); (B) Chlorophyll b content under normal (0 mM) and salt stress conditions (100 mM).

Khotifa (92.12) showed respectively the minimum and the maximum values RWC in normal conditions but Azizi (80.20 mM) and Karim (90.26) showed respectively the minimum and the maximum RWC in stress conditions. Variance analysis showed no significant difference among genotypes in maintaining their RWC under both normal and stressed conditions (Table 1). According to the results, it can be expressed that studied genotypes

had the ability to avoid the water stress induced by salinity because changes in the RWC of leaves are considered as a sensitive indicator of salt stress. Our results are in agreement with those of Sairam et al. (2002) who report a greater reduction in the RWC of salt sensitive wheat cultivar as compared with tolerant one under salt stress. These results confirm also those of Bounaqba (1998) which showed that salt stress (100 mM of NaCl) not affected the water relative contents of roots and leaves of the plants of wheat, triticale and barley in seedling stage. However, Jones and Turner (1978) found that salt stress decreased RWC and reduce the leaf turgor potential which accompanies the loss of water from leaf tissue.

According to Çiçek and Çakirlar (2002), RWC decreased the slow development of water deficits resulted not only in osmotic adjustment, but also a decrease in leaf tissue elasticity. Results based on statistical analysis further showed significant variation of hydration (%) among genotypes in the control and in the treatment (Table 1). In the control, Jenah Khotifa had the highest hydration and Swebâa Eljia had the lowest hydration. Under salt stress, genotypes exhibited various behaviours and were classified in two groups significantly different at p<0.05. Azizi, Om Rabia, Karim, Hmira and Swebâa Eljia formed the first group witch presenting an increase in the level of hydration. The second group included Oued Zeneti and Jenah Khotifa showed weak reduction rates of hydration (%) of 1.36 and 3.26%, respectively (Table 1).

Variation in chlorophyll content

Results pertaining to chlorophyll a and b showed a significant variation among the seven durum wheat genotypes studied (Figure 1). The chlorophyll (a) and (b) content were significantly increased for Jenah Khotifa, Karim, Om Rabia and Hmira under salt stress. The genotypes Azizi, Oued Zeneti and Swebâa Eljia showed a reduction of 27.63, 13.98 and 15.08% for chlorophyll *a* content and reduction of 16.32, 17.63 and 14.63% for chlorophyll (b) content (Table 2). An increase in chlorophyll content has been thought to be due to the accumulation of NaCl in the chloroplast (Kirst, 1989) or the increase in the number of chloroplasts in stressed leaves (Misra et al., 1997).

Table 2. Variation of chlorophyll *a* / b ratio at third leaves stage of seven durum wheat genotypes cultivated in hydroponie under normal (0 mM) and salt stress conditions (100 mM).

Parameter	Control (0 mM)	Treated (100 mM)
Azizi	3.16^{ab}	2.70^{d}
Jenah Khotifa	2.76^{b}	2.89^{c}
Karim	3.19^{ab}	3.05^{bc}
Om Rabia	3.23^{a}	2.95^{c}
Hmira	3.07^{ab}	2.89^{c}
Swebâa Eljia	3.30^{a}	3.28^{a}
Oued Zeneti	3.05^{ab}	3.18^{ab}

Means in the same column followed by different letters are significantly different at 0.05 level.

Table 3. Variance analysis for seven durum wheat genotype for F_0, F_m and F_v parameters.

Variation source	F_0	F_m	F_v
Genotype	143900.1^{**}	9478.4^{ns}	104479.6^{ns}
Treatment	226.6^{ns}	31889.7^{ns}	313228.5^{ns}
Genotype*treatment	1879.4^{ns}	139636^{ns}	125537.6^{ns}

^{ns}: Not significant at the 0.05 probability level; **: Significant at the 0.01 probability level.

Table 4. Variation (F_v /F_m) and (F_v/F_0) parameters on the seven durum wheat under normal (0 mM) and salt stress conditions (100 mM) at three leaves stages.

Parameter	F_v / F_m		F_v / F_0	
	0 mM	100 mM	0 mM	100 mM
Azizi	0.817^{ab}	0.818^{c}	4.49^{bc}	4.53^{c}
Jenah Khotifa	0.835^{a}	0.832^{a}	5.09^{ab}	5.00^{a}
Karim	0.820^{ab}	0.834^{a}	4.61^{bc}	5.03^{a}
Om rabia	0.845^{a}	0.832^{ab}	5.49^{a}	4.96^{a}
Hmira	0.828^{a}	0.823^{bc}	4.88^{ab}	4.67^{bc}
Swebâa Eljia	0.789^{c}	0.827^{ab}	3.85^{c}	4.83^{ab}
Oued Zeneti	0.817^{ab}	0.815^{c}	4.46^{bc}	4.41^{c}

Means in the same column followed by different letters are significantly different at 0.05 level.

Chlorophyll a / b ratio

Chlorophyll *a* / b ratio is significantly affected by salt stress. The addition of NaCl (100 mM) in the nutrient solutions caused the reduction of chlorophyll *a* / b ratio for all genotypes except for Oued Zeneti and Jenah Khotifa which showed both an increase of about 13% (Table 2).

Chlorophyll fluorescence

The value of the minimal fluorescence yield (Fo) was increased under salt stress as compared to the control. On the other hand, the value of the variable fluorescence (Fv), and the maximal fluorescence yield (F_m) were not affected under salt stress (Table 3). Same results were also recorded by Ayari (2000). Similarly, the Fv/Fm ratio, which characterizes the maximum yield of the primary photochemical reaction in dark-adapted leaves and frequently used as a measure of the maximal photochemical efficiency of PSII (Krause and Weis, 1991), was not affected by salt stress. An increase in F_O under stress (100 mM) showed for all genotypes studied is a characteristic of PSII inactivation (Havaux, 1993; Bounaqba, 1998). Table 4 showed that the optimum quantum yield fluorescence (Fv/Fm) was decreased under salt stress. Dark-adapted values of Fv/Fm reflect the potential quantum efficiency of PSII and are used as a sensitive indicator of photosynthetic performance, with optimal values of around 0.832 measured from most plant species (Johnson et al., 1993). Values lower than
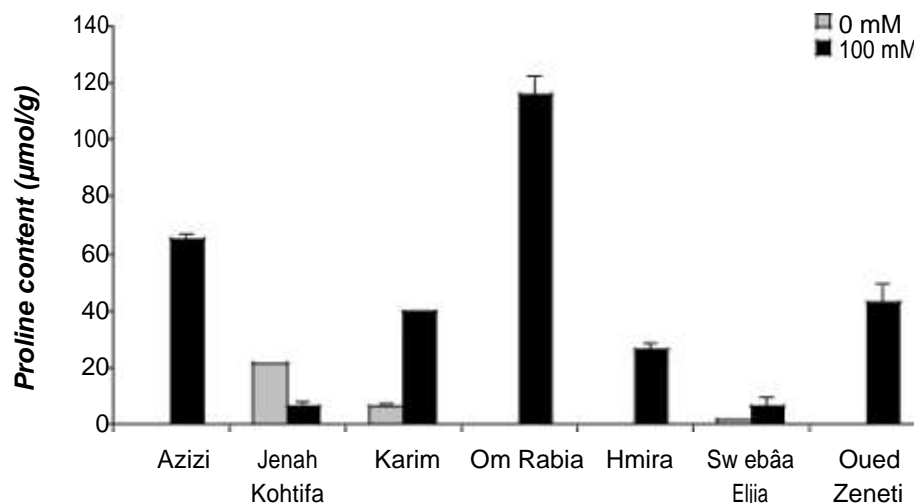
Figure 2. Proline accumulation of seven genotypes of durum wheat under normal and salt stressed conditions.

this are measured when the plant is exposed to stress, indicating a particular phenomenon of photo-damage to PSII reaction centers, and the development of slowly relaxing quenching process (Maxwell and Johnson, 2000; Baker and Rosenqvist, 2004) which reduce the maximum efficiency of PSII photochemistry.

On the basis of (Fv /Fm) and (FV/F0) parameters, Jenah Khotifa and Karim accessions which showed the best tolerance to salt stress, but Hmira, Swebâa Eljia, Azizi and Oued Zeneti accessions considered as the more sensitive toward NaCl stress.

Proline content

The study of proline content showed variability among genotypes in the control and in the salt stressed treatment (Figure 2). In the control, Jenah Khotifa had the highest proline content, while Om Rabia, Hmira, Azizi and Oued Zeneti had the smallest ones. Under salt stress, proline content was significantly increased in shoot in all the stressed genotype over non-saline control expect the genotype Jenah Khotifa which showed the highest proline content in normal conditions (21 and 13 µmol/g). Results further showed differences in proline content in leaf tissue between genotypes under salt stress (Figure 2). In all genotypes, proline increased significantly at salt stress except of the genotype Jenah Khotifa. The genotype Om Rabia and Azizi showed the higher proline content. According to Palfi and Juhasz (1971) measurement of proline accumulation is an important criterion for determination of plant tolerance to salt stress; in fact, many plants accumulate proline as a non-toxic and protective osmolyte under saline or water stress conditions. The accumulation of compatible solutes may help to maintain the relatively high water content

necessary for growth and cellular function (Yoshiba et al., 1997). We found that salt stress at different osmotic potential increased the amounts of proline of leaf tissues in all genotypes. Proline accumulation in response to environmental stresses has been considered by a number of authors as an adaptive trait concerned with stress tolerance, and it is generally assumed that proline was acting as a compatible solute in osmotic adjustment (Larher et al., 1993; Aziz and Larher, 1995; Aziz et al., 1999; Mansour, 2000). Its accumulation is caused by both the activation of its biosynthesis and inactivation of its degradation (Mattioni et al., 1997).

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