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Full Length Research Paper

Physiological behavior of wheat genotypes from Algerian semi-arid regions grown under salt stress

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The performances of the Algerian local genotypes Mohamed Ben Bachir and Oued Zenati tested under NaCl stress, show an ability to withstand moderate salt concentrations. It appears that salinity affected normal physiological functions of wheat genotypes. This was expressed by the imbalance in water relation, mineral balance and proline accumulation in the two genotypes. It was noted that these genotypes showed a low leaf water potential (Ψw) which is associated with suitable relative water content (RWC), which maintains the tissues hydration. It seems that the decline in water and osmotic potential is not due to water loss but to a significant accumulation of Na^+ and proline in which tissues can feed satisfactorily with water and this is possible through osmorégulation mechanism sealed by the fundamental role of membrane integrity to regulate cellular permeability. Physiologically, it is a quantitative rather than a qualitative difference between the two genotypes tested in this study. The better physiological mechanisms associated with less affected water relation and Na^+ efflux probably contributed to the higher salt tolerance in M.B. Bachir than in O. Zenati genotype. Therefore, these genotypes could be considered as salt tolerant and they are suitable in improving durum wheat for salt tolerance.

Key words: NaCl, wheat genotypes, physiological responses, membrane integrity, proline, K^+/Na^+ selectivity.

INTRODUCTION

Soil salinity is one of the main environmental problems affecting plant growth and crops productivity (Parida et al., 2004), especially in arid and semi-arid regions of the world both in irrigated and dryland agriculture (Degl'Innocenti et al., 2009). Salinity induces water deficit even in well watered soils by decreasing the osmotic potential of soil solutes thus making it difficult for roots to extract water from their medium (Sairam et al., 2002).

Although, high ionic concentrations compete with the uptake of other nutrients (Munns, 2002). Increased treatment of NaCl raise Na^+ and Cl^- and reduce Ca^{2+}, Mg^{2+} and particularly K^+ levels in plant (Rontein et al., 2002). Salinity stress, changes water permeability of the cell membrane (Mansour et al., 2005). Excess of Na^+ may produce detrimental effects on the membrane integrity and water availability in a root medium (Zang and Komatsu, 2007). Water stress induce decrease in water level of tissues (Zhu et al., 2006). Two approaches could be heeded in order to escape the salinity problems; leaching salts from the soil profile by irrigation (Zhao-Zhong et al., 2005) and/or selecting more salt-tolerant genotypes (El Hendawy et al., 2005). However, water scarcity in semiarid conditions makes the first approach impractical. Therefore, the selection and breeding of salt-tolerant genotypes would be more successful, in achieving maximum attainable tolerance, if it were based directly on the relevant agronomic and physiological mechanisms for increasing wheat productivity under saline conditions (Abdelghani, 2009). Improving salinity tolerance of wheat is a key target for many wheat breeding

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programs worldwide (Dreccer et al., 2004). Salt stress physiology and plant responses to high salinity have been discussed over the last decades (Zhu, 2002; Sairam and Tyga, 2004).

However plant species differ in their sensitivity or tolerance to salts (Walia et al., 2009). The varietal differences in salinity tolerance and sensitivity existed among species can be used through screening programs for selection and plant breeding (Ashkani et al., 2007). Wheat is commonly classified as a moderately salt tolerant crop; the threshold value for wheat is around 4.48 mg/l (Mass and Hoffman, 1977). Genotype variation for agronomic and physiological traits has been reported for drought tolerance in wheat (Fischer and Maurer, 1978; Tavakol and Pakniyat, 2007). However, a difference in the salt tolerance among wheat genotypes may also occur at different growth stages (Kingsbury and Epstein 1984; El-Hendawy et al., 2005). Therefore, the salt tolerance of different wheat genotypes must be evaluated. It has been reported that the salt tolerant barley genotypes maintained lower Na^+ than non-tolerant ones (Pakniyat et al., 1997; Schachtman and Lio, 1999; Rivelli et al., 2002). Salt tolerance in wheat is mostly related to its enhanced ability to discriminate between K^+ and Na^+ during transport of these ions to the shoot (Gorham, 1990). Many other traits could be used for the assessment of salt tolerance (Flowers and Yeo, 1995).

The use of physiological markers such as, plant water relations, mineral balance and proline accumulation could be useful (Ashkani et al., 2007). The use of plant ionic status along with agronomic traits has been shown to be applicable and their relationship with salt tolerance indices are considered strong enough to be exploited, as a selection tool in the breeding of salt tolerant genotypes (Allakhverdiev et al., 2000). Little information is available on the response of local durum wheat genotypes adapted to arid and semiarid Algerian regions to salinity. Therefore, the objectives of this study were to assess the potential of two Algerian wheat genotypes in tolerating salt stress and to set advices on the probable introduction of this genetic material for future salt tolerance improvement.

MATERIALS AND METHODS

The experiment was conducted under greenhouse controlled conditions with day and night temperatures of 25±2°C and 18±2°C respectively. Photoperiod was adjusted to 14 h with light intensity of 10 000 lux. Relative humidity was maintained at 60%. Local Algerian genotypes of wheat (Triticum durum Desf.), Oued Zenati (O.Z) and Mohamed Ben Bachir (M.B.B), were tested in this study under salinity. Wheat seeds were surface sterilized by dipping the seeds in 1% mercuric chloride solution for 2 min and rinsed thoroughly with sterilized distilled water. The seeds were germinated in Petri dishes at 10 seeds per box.

Then, seedlings were transplanted into pots filled with soil and compost (2v:1v) and sufficient water, equivalent to 3/4 of the pot capacity, was added each three days. Three levels of NaCl salinity, namely 2, 4, 6 g/l and tap water as the control were applied, till the fourth leaf emergence. Alternatively and at an interval of two salt supplies, plants were irrigated with tap water to avoid salt precipitation around roots. The plants were harvested fifteen days after salt treatment. The plants were rinsed with de-ionized water and separated into root and shoot portions.

Measurement of plant water status

Water potential (Ψw) is measured, the early morning on the last sheet, using a pressure chamber or chamber of Scholander on leaf blades (Scholander Pressure Bomb, Arimad 2, Germany). Five fresh leaves of same size and same age of five plants from each treatment were collected and weighted (Fw). Leaf segments were kept immersed in distilled water for 24 h at room temperature in the dark. The turgid weight (Tw) of leaves were measured and then oven-dried at 80°C for 72 h until constant weight and reweighting (Dw). The fresh weights, turgidity and dry weights of the leaf segments were used to determine the hydratation and relative water content following Sangakkara et al. (1996). Hydration was determined as H(%) = 100 - 100 (Dw / Fw). The relative water content (RWC) was determined as RWC(%) = [(Fw-Dw) / (Tw-Dw)] x 100.

Measurement of the membrane integrity percentage

The membrane integrity was evaluated by conductivity method following Blum and Evercon (1981). It is a measure of the electrolytes release subsequent to the partial destruction of cell membranes. The percentage of membrane integrity is given as MIP (%) = (1-FC/TC) x 100 where FC = free conductivity and, TC = total conductivity.

Proline determination

Proline accumulation is one of the most remarkable characteristic in stressful conditions. Proline was determined according the method described by Bathes et al. (1973). Approximately 0.5 g of fresh leaf material was homogenized in 10 ml of 3% aqueous sulfosalicylic acid and then this aqueous solution was filtered through Whatman's No. 2 filter paper and finally 2 ml of filtrated solution was mixed with 2 ml acid-ninhydrin and 2 ml of glacial acetic acid in a test tube. The mixture was placed in a water bath for 1 h at 100°C. The reaction mixture was extracted with 4 ml toluene and the chromophore containing toluene was aspirated, cooled to room temperature and the absorbance was measured at 520 nm with a spectrophotometer; appropriate proline standards were included for calculation of proline in the sample.

Determination of K^+ and Na^+

Collected samples were washed in distilled water to remove any external salt and dried at 80°C oven for 48 h. The dried samples were ground into a fine powder using a mortar and pestle. Samples (1 g) were ashed by putting them into crucibles and placed in 600°C electric furnace, for 4 h, 5 ml of 2 N HCl was added to cooled ash samples, dissolved in boiling deionized water, filtered and made to a final volume to 50 ml. Na^+ and K^+ were measured using standard flame photometer procedure (Vogel, 1955) and reported as $mM.g^{-1}$ dry weight.

Statistical analysis

The variance of homogeneity of the data was assessed and

Table 1. Comparison between wheat genotypes (O. Zenati and M. B. Bachir) for water status under salinity.

NaCl levels (g/l)	Water potential (MPa)		Hydration (%)		Relative water content (%)	
	O.Z	M.B.B	O.Z	M.B.B	O.Z	M.B.B
Control	-1.98	-1.67	88.05	89.44	94.09	95.69
2	-2.20	-1.91	79.10	80.12	80.32	90.10
4	-2.60	-2.05	72.64	73.22	74.10	80.35
6	-3.10	-2.82	70.10	72.92	70.30	78.92

Data are the mean ± SE (n=5). Different letters a column indicate significant difference (P<0.05, Student-Newman-Keuls test).

conformed to the model which would permit analysis of variance (ANOVA) on the data set. Results were analyzed using the General Linear Model (GLM) procedure implemented in the statistical software SPSS 16.0 (SPSS Inc, Chicago, USA) by ANOVA analysis. The term significant indicates differences for which $P < 0.05$ under the confidence level $\alpha = 95\%$.

RESULTS

Plant water status

Water potential

This parameter is of great importance in assessing the degree of water stress, which applies to the plant. The results (Table 1) show that both wheat genotypes recorded leaf water potentials which decreased significantly with increasing salt concentration in the medium ($P < 0.01$**). Water potential reduction was higher in O.Z than in M.B.B genotype; indeed, it decreased from -1.98 MPa in control to -3.1 MPa in the treatment with 6 g/l NaCl against -1.67 MPa and -2.82 MPa respectively, in the same conditions. It should be noted that up to 4 g/l decline is not significant but it becomes more pronounced at 6 g/l NaCl in both genotypes.

The relative water content

Results analysis show that, the levels of applied salt stress induced a decrease in the relative water content, more pronounced in O.Z genotype ($P<0.01$**). The decrease of RWC in plant tissues is correlated with the decline of water potential (Ψw) and osmotic potential (Ψs). The lowest values of RWC were 70.3% and 78.9% respectively, in O.Z and M.B.B genotypes under stress induced by 6 g/l NaCl (Table 1).

Hydration

The results of hydration show that, the local genotypes are able to maintain proper hydration in its tissues under salt concentrations up to 4 g/l, and despite the presence of stress, water deficit is not very pronounced and substantial moisture is up 6 g/l. Tissue hydration ranged between 88% and 70% in O.Z genotype whilst between 89% and 73% in M.B.B genotype (Table 1).

The membrane integrity percentage (IP)

The percentage of cellular integrity is a measure of the release of electrolytes after partial destruction of cell membranes. We can see from the results (Table 2), the variation of the integrity of membrane structures under the effect of gradual salt concentrations. The IP is high in genotypes tested and leaves retain a significant structural integrity despite the presence of salt which causes physiological drought to plants.

This difference is slightly significant in M.B.B genotype which divulge a small variation ($P < 0.05$*) and highly significant in O.Z genotype which disclose a weakness to preserve its membrane integrity compared to the other genotype ($P < 0.01$**). The ability of O.Z to maintain the integrity of its membranes appears to be associated with avoidance mechanisms of salt stress, although at 6 g/l NaCl, the percentage of integrity decreased due to the disruption of walls ultra-structure caused by stress (Blum and Ebercon, 1981). These alterations may result from mechanical destruction by plasmolysis (Mansor et al., 2005).

Proline content

The applied salt concentrations had significant effects causing an increase in leaves proline levels of two wheat genotypes ($P < 0.05$*, Table 2), this increase in proline concentration was observed in many plants subjected to water deficit such as wheat (Bathes et al., 1973). Comparing between genotypes, it was found that O.Z leaves accumulated higher proline content compared with M.B.B leaves. Proline accumulation could be a discriminatory factor for varietal resistance to various stresses.

The almost linear increase in proline content in this genotype of wheat has also been observed in tea (Chakraborty et al., 2002) and tomato (Claussen, 2005). This increased accumulation of proline up to 6 g/l reached 360 µg/g FM in O.Z leaves against 320 µg/g FM

Table 2. Comparison between wheat genotypes (O.Zenati and M.B.Bachir) for percentage membrane integrity, proline content and mineral balance under salinity.

NaCl levels (g/l)	Membrane integrity (%)		Proline (µg.g-1 FM)		K^+ (mM.g-1 DM)				Na^+ (mM.g-1 DM)			
					Shoot		Root		Shoot		Root	
	O.Z	M.B.B	O.Z	M.B.B	O.Z	M.B.B	O.Z	M.BB	O.Z	M.B.B	O.Z	M.B.B
Control	88.73	89.06	15.45	14.40	1.55	1.56	1.17	1.15	0.04	0.03	0.06	0.05
2	80.10	80.59	65.12	62.30	1.15	1.17	0.75	0.70	0.75	0.81	0.51	0.60
4	76.42	79.25	180.15	160.12	0.85	0.85	0.50	0.55	1.50	1.32	1.01	0.92
6	72.60	77.50	360.80	320.75	0.75	0.80	0.51	0.50	1.96	1.96	1.51	1.22

Data are the mean ± SE (n=5). Different letters a column indicate significant difference (P<0.05, Student-Newman-Keuls test).

in M.B.B leaves. The ability of leaves to accumulate proline in plants subjected to salt stress could be a factor of resistance; and could lead to the osmoregulation which is evidenced by a decline of water potential (Ψw) and osmotic potential (Ψs) from hand, and an increase in relative water content (RWC) and hydration (H) from the other hand.

Mineral balance

Results in Table 2 show that, the uptake and the accumulation of Na^+ increases with the raise of salt concentration in the medium, both in leaves and roots of the two genotypes ($P < 0.01^{**}$), whereas the K^+ content decrease in the same organs ($P < 0.01^{**}$). M.B.B genotype showed higher K^+ and the lowest Na^+ concentrations in leaves compared to O.Z genotype, resulting in the higher ratio K^+/Na^+, in this genotype under increased salt levels. The reverse result was observed in roots. The decrease of K^+ content is more pronounced in roots than in leaves; similarly, in the two genotypes which could be explained thus, that roots seem to drain their K^+ in favor of leaves. The preferential accumulation of Na^+ in leaves than in roots was observed with respect to all treatments and this corroborates the results of Zid et al. (1991) and Cramer et al. (1991).

DISCUSSION AND CONCLUSION

The performances of the local genotypes were tested under NaCl stress, in order to obtain results that can characterize the effect of salt stress on its physiological responses from a hand, and the varietal differences in salinity tolerance could be used through screening programs for selection and plant breeding on the other hand (Ashkani et al., 2007). It was noted that, wheat genotypes subjected to salt stress showed a low leaf water potential (Ψw) which is associated with relative water content (RWC) quite high, which maintains the tissues hydration better pronounced in M.B.B genotype. It seems that, the fall in water potential is not due to water loss but to an accumulation of solutes confirmed by low osmotic potentials recorded. The decline of (Ψw) was accompanied with significant accumulation of Na^+ and proline in the leaves which can feed tissues satisfactorily with water and this is possible through osmoregulation mechanism. This ability to maintain a moisture level that allows the leaves to remain in a state of turgor is considered as criteria of drought adaptation and hence salinity (Maggio et al., 2005).

Regarding the preservation of membrane integrity, both genotypes and especially M.B.B genotype are able to maintain resistance despite the accumulation of solutes which lead to preservation of metabolic activities and membrane structure. It is well documented that a greater degree of salt tolerance in plants is associated with a more efficient system for selective uptake of K^+ over Na^+ (Noble and Rogers, 1992). Salt tolerance in the Triticeae is associated with better ability to discriminate between Na^+ and K^+ at the uptake sites of plasmalemma and to preferentially accumulate K^+ and exclude Na^+ (Omielan and Epstein, 1991; Ali et al., 2004). Gorham (1990), Rashid et al. (1999) and Sarwar et al. (2003) reported that in wheat, genetic variation in salt tolerance is associated with low rates of Na^+ transport to shoot and high selectivity for K^+ over Na^+.

As for the nutritional aspect, there is a high accumulation of Na^+ correlated with a lower K^+ content especially in the roots. The possible cause of varietal difference most likely involves membrane ion transport properties and cellular compartmentation (Munns, 2002). Schachtmann and Munns (1992) reported that sodium exclusion was a general characteristic of salt tolerance in wheat genotypes; whereas, salt tolerant display much higher shoot sodium level than sensitive genotypes and M.B.B appear more tolerant to NaCl than O.Z genotype. Wheat genotypes could adjust to high salt concentrations by lowering tissue osmotic potential with the accumulation of inorganic ions, such as Na^+ and K^+, as well as organic solutes such as proline (Fricke 2004; Munns et al., 2006) with respect to cell structural changes and regulation of membrane permeability (Cooke and Burden, 1990; Mansour et al., 2004). As the plasma membrane is one of the cell parts that salt reaches first,

membrane integrity plays a fundamental role in regulating water and salt permeability and triggering primary responses to salinity (Zang and Komatsu, 2007).

In this study, pronounced increase of proline content was observed in the presence of increasing NaCl concentration in the medium. The negative correlation between proline amounts and leaf water potential (Ψw) suggests that proline plays an essential role in osmotic adjustment under salt stress (Shao et al., 2006). In wheat, proline acts as an endogenous osmotic regulator and the levels of proline in plants tissue correlates with the ability of the plants to tolerate or to adapt to saline conditions (Fricke 2004; Munns et al., 2006). The stimulation of proline accumulation under salinity was reported before in other crop species, such as barley (Pesci and Beffagna, 1986), rice (Dubey and Rani, 1989) and *Brassica juncea* (Jain et al., 1991). It appears that salinity affected normal physiological functions of wheat genotypes. This was expressed by the imbalance in water relation, mineral ions and proline accumulation in the two genotypes. The better physiological mechanisms associated with less affected water relation and Na^+ efflux probably contributed to the higher salt tolerance in M.B. Bachir than in O. Zenati genotype.

In conclusion, physiologically, it is a quantitative rather than qualitative difference between the two genotypes tested in this study. We noted the superiority of the genotype M.B.B, in order to maintain its physiological functions under salinity. The different parameters studied in the present study, may prove very useful for selecting wheat genotypes against salt stress. Therefore, these genotypes could be considered as salt tolerant and they are suitable in improving durum wheat for salt tolerance. Moreover, further research is required to confirm these results under field conditions.

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