

Physiological Behavior of Two Algerian Wheat Genotypes Grown Under Saline Conditions

دراسة السلوك الفسيولوجي لبعض أصناف القمح الصلب من المناطق الشبه الجافة الجزائرية تحت تأثير ضغط الملح

Taibi Khaled^{1,2} and Chorfi Abdelmalek¹

طبيبي خالد وشرفي عبد المالك

¹Department of Biology, Faculty of Sciences, Hadj Lakhdar University of Batna, Algeria.

²Department of Biology, Faculty of sciences, Laboratory of Plant Physiology,

Es-senia University of Oran, Algeria.

E-Mail: khaledtaibi@hotmail.com

Abstract: The performances of two Algerian local genotypes: Mohamed Ben Bachir and Oued Zenati, tested under NaCl stress showed an ability to withstand moderate salt concentrations. It appeared that salinity affected normal physiological functions of these wheat genotypes, expressed by the imbalance in water relations, mineral balance and proline accumulation in the two genotypes. It was noted that these genotypes showed a low leaf water potential (Ψ_w) associated with suitable relative water content (RWC), which maintained tissue hydration. It appears the decline in water potential is not due to water loss but to significant accumulation of Na^+ and proline which can satisfactorily supply tissues with water. This is possible through osmoregulation mechanism sealed by the fundamental role of membrane integrity to regulate cellular permeability. Physiologically, this is a quantitative rather than a qualitative difference of physiological behavior between these genotypes. The physiological mechanisms associated with less affect on water relation and Na^+ afflux probably contributed for the higher salt tolerance in M.B. Bachir than in the O. Zenati genotype. Therefore, these genotypes could be considered salt tolerant and are suitable in improving durum wheat's salt tolerance. **Keywords:** NaCl, wheat genotypes, physiological responses, membrane integrity, proline, K^+/Na^+ selectivity.

المستخلص: لقد تم اختبار قدرات الأنماط الوراثية المحلية الجزائرية للقمح الصلب 'محمد بن بشير' و 'واد زناتي' تحت ضغط كلوريد الصوديوم، أظهرت هذه الأنماط الوراثية قدرتها على الصمود أمام تركيز معتدل للملح فيما يبدو أن الملوحة تضر بالوظائف الفسيولوجية الطبيعية للأنماط الوراثية للقمح وقد تجلى ذلك باختلال التوازن في العلاقات المائية و التوازن المعدني و تراكم البرولين في هذه الأنماط الجينية. ولوحظ أن هذه الأنماط الجينية أظهرت انخفاض محتمل لتركيز كمون المياه (Ψ_w) و الذي يرتبط مع محتوى الماء النسبي المناسب (RWC) مما يحافظ على رطوبة الأنسجة. ويبدو أن الانخفاض في تركيز كمون المياه ليس بسبب فقدان المياه ولكن نظرا لتراكم كبير من الصوديوم والبرولين الذين يمكن أن يضمنا تغذية ملائمة للأنسجة بالماء وذلك من خلال آلية التنظيم الاسموزي المرتبطة أساسا بسلامة الغشاء الخلوي المنظم للنفاذية. فمن الناحية الفسيولوجية فهناك فارق كمي بدلا من وجود فارق نوعي بين سلوك المورثتين المختبرتين في هذه الدراسة. وأفضل الآليات الفسيولوجية مرتبطة بأقل نسبة تضرر للعلاقات المائية و أقل تدفق للصوديوم ساهمت على الأرجح في مقاومة أكبر للنمط الوراثي 'محمد بن بشير' مقارنة بالنمط الوراثي 'واد زناتي'. ويمكن اعتبار هذه الأنماط الوراثية متحملة للملح و مناسبة لبرامج تحسين القمح الصلب لتحمل ملوحة التربة.

كلمات مدخلية: كلوريد الصوديوم، أنماط القمح الوراثية، الآليات الفسيولوجية، الغشاء الخلوي، البرولين، نفاذية تفاضلية صوديوم/بوتاسيوم.

INTRODUCTION

Soil salinity is one of the main environmental problems affecting plant growth and crop 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). High ionic concentrations compete with the uptake of other nutrients (Munns, 2002). Increased concentration of NaCl raise Na^+ and Cl^- and reduces Ca^{2+} , Mg^{2+} , and particularly K^+ levels in plants (Rontein, *et al.* 2002). Salinity stress changes the water permeability of the cell membrane (Mansour, *et al.* 2005). Excess Na^+ may produce detrimental effects on membrane integrity and water availability in a root medium (Zang and Komatsu, 2007).

Induced water stress decreases water levels within tissues (Zhu, *et al.* 2006). Two approaches could be taken to escape 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 semi-arid conditions makes the first approach impractical. Therefore, selection and breeding of salt-tolerant genotypes would be more successful in achieving maximum attainable tolerance, if they were based directly on 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 programs worldwide (Dreccer, *et al.* 2004). Physiological salt stress and plant response to high salinity have been discussed over the last decade (Sairam and Tyga, 2004). However, plant species differ in their sensitivity to salts (Walia, *et al.* 2009).

Varietal differences in salinity tolerance and sensitivity that exist among species can be used in 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). Genotypic

variations in agronomic and physiological traits have been reported for drought tolerance in wheat (Tavakol and Pakniyat, 2007). However, differences in salt tolerance among wheat genotypes may also occur at different growth stages (El-Hendawy, *et al.* 2005). Therefore, the salt-tolerance of different wheat genotypes must be evaluated. It has been reported that salt tolerant barley genotypes maintained lower Na^+ than non-tolerant ones (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 as well (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 with agronomic traits has been shown to be applicable and their relationship to salt tolerance indices were considered strong enough to be exploited as a selection tool in breeding 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.

MATERIAL AND METHODS

The experiment was conducted under greenhouse-controlled conditions with day and night temperatures of $25 \pm 2^\circ\text{C}$ and $18 \pm 2^\circ\text{C}$ respectively. Photoperiod was adjusted to 14h with a 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 minutes 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 every three days. Three levels of NaCl salinity, viz. 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.

Plant Water Status Measurement

Water potential (Ψ_w) was measured 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 the same size and age by five plants from each treatment were collected and weighed (Fw). Leaf segments were kept immersed in distilled water for 24 hours at room temperature in the dark. The turgid weight (Tw) of the leaves was measured, then leaves were oven-dried at 80°C for 72 hours until constant weight (Dw). Fresh weights, turgidity and dry weights of leaf segments were used to determine hydration and relative water content following Sangakkara, *et al.* (1996). Hydration was determined as $H (\%) = 100 - 100 (Dw / Fw)$. Relative water content (RWC) was determined as $RWC (\%) = [(Fw - Dw) / (Tw - Dw)] \times 100$.

Membrane Integrity Percentage Measurement

Membrane integrity was evaluated using conductivity method following Blum and Evercon (1981), a measure of electrolyte release following partial destruction of cell membranes. The percentage of membrane integrity is given as $MIP (\%) = (1 - FC/TC) \times 100$ where FC = free conductivity and TC = total conductivity.

Proline Determination

Proline accumulation is one of the most remarkable characteristics under stress conditions. Proline was determined according to 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, then this aqueous solution was filtered through Whatman's

No. 2 filter paper. 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 chromophore containing toluene which was aspirated, cooled to room temperature after which its absorbance was measured at 520 nm with a spectrophotometer. The appropriate proline standards were followed for calculation of proline in the sample.

Determination of K⁺ and Na⁺

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

Statistical Analysis

The variance of homogeneity of the data was assessed and 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). Means were separated at the 5% level.

RESULTS

Plant Water Status

Water potential

Results (Table 1) show 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 decreases from -1.98 MPa in the control to -3.1 MPa in treatment with 6 g/l NaCl compared with -1.67 MPa and -2.82 MPa respectively, for MBB in the same conditions. It should be noted that up to 4 g/l decline is not

significant, but becomes more pronounced at 6 g/l NaCl, in both genotypes.

Relative water content

Results showed the levels of applied salt stress induced a decrease in relative water content more pronounced in O.Z genotype ($P < 0.01^{**}$) (Table 1). The decrease of RWC in plant tissues was correlated with a decline in water potential (Ψ_w) and Osmotic Potential (Ψ_s). 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.

Hydration

The results of hydration showed the local genotypes were able to maintain proper hydration in tissues under salt concentrations up to 4 g/l. despite the stress, water deficit was not very pronounced and there was substantial moisture up to 6 g/l. Tissue hydration ranged between 88% and 70% in the O.Z genotype and between 89% and 73% in the M.B.B genotype.

Membrane Integrity Percentage (IP)

The percentage of cellular integrity is a measure of the release of electrolytes after partial destruction of cell membranes. IP is high in the genotypes tested. The leaves retained a significant structural integrity despite the presence of salt which causes physiological drought to plants (Table 2). This difference was slightly significant in the M.B.B genotype which provided a small variation ($P < 0.05^*$) and was highly significant in the O.Z genotype which disclosed 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 the membrane appears to be associated with an avoidance mechanism to salt stress, although at 6 g/l NaCl, the percentage of integrity decreased due to disruption of walls' ultra-structure caused by stress (Blum and Ebercon, 1981). These alterations may have resulted from mechanical destruction by plasmolysis (Mansor, *et al.* 2005).

Proline Content

The applied salt concentrations had significant effects, causing an increase in leaf proline levels in the 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 genotypes, it was found that O.Z leaves accumulated a higher proline content compared with M.B.B leaves. Proline accumulation could be a discriminating factor for varietal resistance to various stresses such as wheat. The almost linear increase in proline content in the O.Z. genotype has also been observed in tea (Chakraborty, *et al.* 2002) and tomatoes (Claussen, 2005). This increased accumulation of proline, up to 6 g/l, reaching 360 $\mu\text{g/g}$ FM in O.Z leaves against 320 $\mu\text{g/g}$ FM in M.B.B leaves. The ability of leaves to accumulate proline in plants subjected to salt stress could be an element of resistance, and could lead to the osmoregulation evidenced by a decline in water potential (Ψ_w) and osmotic potential (Ψ_s). On the one hand an increase in relative water content (RWC) and hydration (H) was observed

Table 1. Water status under salinity conditions in two wheat genotypes (O. Zenati and M.B. Bachir).

NaCl level (g/l)	Water potential (MPa)		Relative water content (%)		Hydration (%)	
	O.Z	M.B.B	O.Z	M.B.B	O.Z	M.B.B
Control	-1.98±0.26a	-1.67±0.19a	94.09±2.21a	95.69±1.77a	88.05±2.33a	89.44±1.44a
2	-2.20±0.13b	-1.91±0.22b	80.32±3.27b	90.10±2.52b	79.10±2.13b	80.12±1.55b
4	-2.60±0.16c	-2.05±0.11c	74.10±3.31c	80.35±2.46c	72.64±1.58c	73.22±1.88c
6	-3.10±0.08d	-2.82±0.02d	70.30±1.24c	78.92±1.11c	70.10±1.66d	72.92±1.79c

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

Table 2. Percentage membrane integrity, proline content and mineral balance under salinity in two wheat genotypes (O. Zenati and M.B. Bachir).

NaCl level (g/l)	Membrane integrity (%)		Proline ($\mu\text{g}\cdot\text{g}^{-1}$ 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.B.B	O.Z	M.B.B	O.Z	M.B.B
Control	88.73±1.36a	89.06±1.13a	15.45±1.22a	14.40±0.88a	1.55±0.17a	1.56±0.21a	1.17±0.13a	1.15±0.16a	0.04±0.01a	0.03±0.01a	0.06±0.00a	0.05±0.00a
2	80.10±2.12b	80.59±1.79b	65.12±4.17b	62.30±5.15b	1.15±0.11b	1.17±0.15b	0.75±0.10b	0.70±0.17b	0.75±0.12b	0.81±0.13b	0.51±0.16b	0.60±0.09b
4	76.42±2.33c	79.25±2.66b	180.15±18.16c	160.12±11.33c	0.85±0.09c	0.85±0.13c	0.50±0.07c	0.55±0.14c	1.50±0.34c	1.32±0.29c	1.01±0.46c	0.92±0.17c
6	72.60±1.67d	77.50±2.05c	360.80±31.23d	320.75±21.11d	0.75±0.07d	0.80±0.07d	0.51±0.09c	0.50±0.10d	1.96±0.47d	1.96±0.52d	1.51±0.44d	1.22±0.23d

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

Mineral Balance

Table (2) shows that uptake and the accumulation of Na⁺ increases with the increase in salt concentration in the medium in both leaves and roots of the two genotypes ($P < 0.01^{**}$), whereas the K⁺ content decreased in the same organs ($P < 0.01^{**}$). The M.B.B genotype showed higher K⁺ and lowest Na⁺ concentrations in leaves, compared to the O.Z genotype, resulting in higher K⁺/Na⁺ in this genotype under increased salt levels. The reverse was observed in the roots. The decrease in K⁺ content is more pronounced in roots than in leaves of the two genotypes which could be explained by roots seeming to drain their K⁺ in favor of leaves. The preferential accumulation of Na⁺ in leaves, rather than in roots, was observed in all treatments which corroborates the results of Zid, *et al.* (1991) and Cramer, *et al.* (1991). Table (2) Comparison between wheat genotypes O.Zenati and M.B.Bachir for percentages of membrane integrity, proline content and mineral balance under salinity.

DISCUSSION AND CONCLUSION

The performances of the local genotypes were tested under NaCl stress in order to characterize the effect of salt stress on physiological responses and varietal differences in salinity tolerance which could be used in screening programs for plant selection and breeding (Ashkani, *et al.* 2007). It was noted that these wheat genotypes, when subjected to salt stress, showed a low leaf water potential (Ψ_w) which was associated with relative water content (RWC) which was quite high. This maintained tissue hydration which was better

pronounced in the M.B.B genotype. It seems the drop in water potential was not due to water loss, but to accumulation of solutes confirmed by the low osmotic potentials recorded.

The decline in Ψ_w was accompanied by a significant accumulation of Na⁺ and proline in leaves which satisfactorily supply tissues with water; is possible through an osmoregulation mechanism. This ability to maintain a moisture level that allows leaves to maintain turgor is considered a criteria of drought adaptation, and hence salinity (Maggio, *et al.* 2005). Regarding the preservation of membrane integrity, both genotypes, especially the M.B.B genotype, were able to maintain resistance despite accumulation of solutes which preserve 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 its 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), Sarwar, *et al.* (2003) reported that in genetic wheat variation in salt tolerance is associated with low rates of Na⁺ transport to shoot and high selectivity for K⁺ over Na⁺. As regards the nutritional aspect, there was 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).

Schachtman and Munns (1992) reported

that sodium exclusion was a general characteristic of salt tolerance in wheat genotypes, whereas salt tolerances display much higher shoot sodium levels than sensitive genotypes. Consequently, M.B.B appears more tolerant to NaCl than the O.Z genotype. Wheat genotypes could adjust to higher salt concentrations by lowering their tissue osmotic potential upon 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 structural cellular changes and regulation of membrane permeability (Mansour, *et al.* 2004). As the plasma membrane is one cell part 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, a pronounced increase of proline content was observed upon increasing NaCl concentration in the medium. The negative correlation between proline amounts and leaf water potential (Ψ_w) 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 levels of proline in plants tissue correlated with the ability of plants to tolerate or adapt to saline conditions (Fricke, 2004; Munns, *et al.* 2006).

Stimulation of proline accumulation under salinity conditions was reported for 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 the normal physiological functions of wheat genotypes, expressed by the imbalance in water relation, mineral ions and proline accumulation in the two genotypes. Physiological mechanisms associated with less effect on water relation and Na⁺ afflux probably contributed to higher salt tolerance of M.B.Bachir than the O.Zenati genotype. In conclusion, physiologically, it is a quantitative rather than a qualitative difference between the two genotypes tested. Therefore, these genotypes could be considered salt-tolerant and are suitable to improve durum wheat for salt tolerance. Moreover, further research would be required to confirm these results under field conditions.

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