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Adjustments in leaf water relations of wild wheat relative *Aegilops geniculata* Roth. and wheat (*Triticum durum* Desf.) plants grown in a salinity gradient

Khaled Mguis[1, 2, 3 *]**, Ali Albouchi**[2]**, Ayda khadhri**[1]**, Mejda Abassi**[2]**, Mbarka Yakoubi-Tej**[4]**,**
Asma Mahjoub[3]**, Zeineb Ouerghi**[1] **and Nadia Ben Brahim**[3]

[1]Unit of Physiology and Biochemistry of Salt tolerance of Plants, Department of Biology, Faculty of Sciences of Tunis, University Tunis El Manar, 2092 Tunis, Tunisia
[2]Unit of Agrosylvopastrolism, INERGREF, University of Carthage, Hedi karray Street, 2080 Ariana, Tunisia
[3]Botanic laboratory, INRAT, Hedi karray Street, 2080 Ariana, Tunisia
[4]Department of Biology, Faculty of Sciences of Tunis, University Tunis El Manar, 2092 Tunis, Tunisia

***Corresponding author: k_guigis@yahoo.fr**

Abstract

In order to study salt effect on wild and cultivated wheat, three accessions of *Aegilops genicula* Roth. (wild wheat) from Ain Zana, Zaghouan, and Sbitla and a variety of *Triticum durum* L. (cultivated wheat) were grown in greenhouse and treated with different salinity levels. Leaf growth, water relations, and proline and sugar contents were measured for each plant at the reproductive stage and analyzed using SAS software. *T. durum* and the three accessions of *A. geniculata* responded to saline stress by developing avoidance and tolerance mechanisms. The avoidance mechanisms took place at the morphological and physiological levels. Morphologically, a reduction of leaf number and length were observed, which are considered as mechanisms that regulate water loss *via* transpiration. Tolerance mechanisms included osmotic adjustment, a decrease of leaf cell elasticity, and an increase of apoplast water content. This study showed a high variability in these characteristics, which is mainly related to seed geographical origins. It was also observed that Sbitla accession was the least affected by the imposed salt stress (57% at 200 mM) while Ain zana was the most affected (84% at 200 mM).

Keywords: *Aegilops geniculata*, osmotic adjustment, P-V curve, salinity, water relations, wheat.

Abbreviations: LL, Per-flag leaf length; LN, Leaf number; DW, leaf dry weight; g_s, Stomatal conductance; Ψ_{pd}, Pre-dawn potential; Ψ_{md}, Midday water potential; AWC, Apoplastic water content ; OA, Osmotic adjustment; ε_{max}, Modulus of elasticity; Ψ_{π}^{100}, Osmotic potential at full turgor; Ψ_{π}^{0}, Osmotic potential at turgor loss point; RWC_0, Relative water content at loss point.

Introduction

Salinity is a complex environmental constraint that presents two main components: an osmotic component due to the decrease in the external osmotic potential of the soil solution and an ionic component linked to the accumulation of ions that are toxic at high concentrations (mainly Na^+ and Cl^-). The accumulation of these elements leads to a decrease in essential nutrient (e.g, potassium and calcium) uptake (El-Bassiouny and Bekheta, 2001). The most crop species in the world are glycophytes; hence, they do not grow under high soil salinity (Kao et al., 2005). However, with an increasing food demand from the growing human population, the need to develop salt-tolerant crop varieties is necessary. Therefore, it is compulsory to identify the degree of salinity tolerance within crops and their wild-type relatives. Durum wheat represents only 8% of total wheat production but 80% is growing under Mediterranean climates (Monneveux et al., 2000). In these regions, salinity considerably limits its yield since its effect is always reinforced by those of drought and heat. Consequently, special efforts should be made to increase abiotic stress tolerance in this species (Monneveux et al., 2000). The genus *Aegilops* is closely related to *Triticum* (Van Slageren, 1994). A growing interest has been developed in the recent years to exploit *Aegilops* spp as important genetic resources for wheat improvement (Farooq et al., 1996; Zaharieva et al., 2001). *Aegilops geniculata* Roth. (= *A Aegilops ovate* L.) is an annual, selfing allo-tetraploid species (2n = 4x = 28) with MU genome (Van Slageren, 1994). This species grows in Mediterranean regions characterized by a dry summer season with high temperature and high irradiance. As many other wild species, they can acclimate to these constraints by escaping, avoiding, or tolerating it (Colmer et al., 2006). Plants may be subjected to environmental stresses that adversely affect growth, metabolism, and yield (Lawlor, 2002). Their degrees of tolerance vary greatly not only between species but also between varieties. Genotypic differences in drought tolerance could be attributed to the ability of plants to grow (Turtola et al., 2006). A thorough understanding of the physiological basis of such difference in stress tolerance could be used to select or create new crop varieties able to increase productivity under such conditions. A large number of environmental stresses, such as drought, salinity, and low temperature may limit crop growth and productivity by imposing osmotic stress on plants. Salt stress decreases plant growth and productivity by slowing the rate of cell division and expansion mainly through a loss of turgor, which results in a decline of the water status components of the plant cells (Navarro et al., 2007; Mguis, 2010). To cope with this problem, plant cells must readjust their osmotic potential to prevent water losses either by an uptake of inorganic ions from the external solution or by *de novo* synthesis of a number of metabolites termed compatible solutes because they do not interfere with biochemical reactions (Bohnert et al., 1995). These metabolites include carbohydrates such as mannitol, sucrose, oligosac-charides, nitrogen-containing compounds, and amino acids. The function of compatible solute accumulation is often associated with osmotic adjustment by lowering the osmotic potential (Ψ_π) to improve water uptake against the external gradient (Bohnert et al., 1999). The decrease in osmotic potential (Ψ_π) can result either from simple passive solute concentration resulting from dehydration or from net solute accumulation. It has been pointed out that only the latter can be regarded as active osmotic adjustment (Morgan et al., 1991). In many plants, net accumulation of osmotically active solutes allows turgor-dependent processes to continue to some extent under water stress conditions. Furthermore, tolerance to water deficits can be improved by increasing intracellular solute concentration by ion uptake (Scholander and Hammel, 1965). The resulting decrease in osmotic potential allows mangroves to substantially lower their xylem water potential below that of seawater and maintain positive water uptake by roots (Scholander and Hammel, 1965). Turgor is known to play an important role in leaf physiology (Passioura, 1994). A positive turgor is necessary to sustain leaf growth (McDonald and Davies, 1996). Turgor maintenance is affected by changes in both osmotic and elastic properties of leaf tissue (Dale, 1988). Osmotic potential at full turgor (Ψ_π^{100}) as well as at turgor loss point (Ψ_π^0) derived from pressure-volume curves have proved to be reliable indices of the osmotic adjustment ability in plants (Abrams and Kubiske, 1994, Livingston and Von, 1992; Tan and Hogan, 1995). However, the changes in tissue elasticity are related to cell turgor, cell dimensions, and symplast solute content. A number of researches explored water relations and osmotic and elastic adjustment of different crops under saline stress (Rodriguez et al. 2005; Navarro et al., 2007). The specific objectives were: (1) to evaluate leaf growth in three accessions of *A. geniculata* (Ain zana, Zaghouan, and Sbitla) and a variety of *T. durum* (Chili) under experimental salinity concentrations from 0 to 200 mM NaCl; (2) to analyse leaf water relations; (3) to determine osmolyte (proline and total sugars) contents in response to increasing external salinity; and (4) to select the most tolerant accession in order to improve wheat varieties. This study derived forms a part of a large wheat improvement program in Tunisia that focuses on the use of *A. geniculata* to expand genetic variability and develop alternate plant types through an understanding of physiological processes that are responsible for its tolerance to salt, drought, and high temperature.

Results

Variance analysis revealed a highly significant species; population and treatment effects (Table 2). Population effect was significant in all traits (leaves growth, stomatal conductance, water relations and osmolytes contents). Indeed the fourteen traits showed significant difference ($P < 0.05$) between *Aegilops* accessions and wheat variety indicating a high level of genetic variability (Table 2). This included three leaf growth (LL, LN and DW), stomatal conductance (g_s), eight water relations traits (Ψ_{pd}, Ψ_{md}, Ψ_π^0, Ψ_π^{100}, OA, RWC_0, ε_{max} and AWC) and solute concentrations (proline and total soluble carbohydrates). The fourteen parameters were significantly affected by the salt treatments ($P < 0.05$). Population X treatment interactions were also detected for all traits except Apoplastic water content (AWC) indicating variable performance of populations in different salt levels

Leaf growth and biomass

For all saline levels, the length of pre-flag leaf showed clear difference between the three *Ae.geniculata* accessions and durum wheat variety (Table 3). Indeed, wheat plants present the most elevated leaf length compared to the *Ae.geniculata* plants. *Ae. geniculata* Sbitla accession presented the most elevated leaf length and Ain zana population displayed each value. The leaf length decreases progressively with increasing stress (Table 3). However, at 200 mM NaCl, pre-flag leaf length was reduced to 67, 58, 50 and 55% for Ain zana, Zaghouan, Sbitla and wheat variety (Chili) respectively compared with the plants control. Increasing NaCl reduced significantly leaf number. However, the reduction depended on the NaCl level. In fact, at 200 mM, leaf number was reduced to 78, 67, 56 and 68 % for Ain zana, Zaghouan, Sbitla and wheat variety (Chili) respectively, compared with the plants control. In three *Ae. geniculata* accessions and wheat variety salinity induced a decrease in the total leaf biomass (Table 3). However, clear differences were noted between wheat and *Ae geniculata*, and between *Aegilops* accessions. At the reproductive stage, total leaf biomass was reduced at 200 mM NaCl by 80, 72, 66 and 77 % for Ain zana, Zaghouan, Sbitla and wheat variety (Chili) respectively, compared with the control.

Stomatal conductance

Midday stomatal conductance (g_s) in pre-flag leaf was similar among the three *Aegilops* accessions in the control, but g_s in this treatment was higher in the Wheat variety `Chili`. g_s decreased progressively with increasing salt concentration (Fig. 1). Indeed, at 200 mM these decreases of g_s were 89; 82; 77 and 85% for Ain zana, Zaghouan, Sbitla and wheat variety `Chili` respectively.

Water relations

Pre-dawn leaf water potential (Ψ_{pd}) and Middy leaf water potential (Ψ_{md}) of the pre-flag leaf of all *Ae. geniculata* accessions and wheat variety were reduced to a similar extent by salinity. Ψ_{pd} and Ψ_{md} in control were lower in the wheat variety than in *Aegilops* accessions (Fig. 2). However, the effect of salinity on Ψ_{pd} and Ψ_{md} was similar with the two species. An increasing NaCl treatment led to a decrease Ψ_{pd} and Ψ_{md}. Under salt stress Ain zana accession had the highest leaf water potential, compared Zaghouan and Sbitla. However, wheat had the least values of Ψ_{pd} and Ψ_{md}. At 200 mM, Ψ_{pd} reached -0,87 , -0,95, -1,09 and -1.23 MPa and Ψ_{md} was -2.76, -2.87, -2.98 and -3.36 MPa respectively for Ain zana, Zaghouan, Sbitla and wheat variety (Chili). Osmotique potential at the loss point (Ψ_π^0) and at the full trugor (Ψ_π^{100}) of the pre-flag leaf in the three accessions of *Ae. geniculata* and cultivar of wheat decreased significantly with increasing of concentrations of NaCl (Figs. 3a, b) . In fact, Ψ_π^0 and Ψ_π^{100} showed similar values in the plants controls of *Ae. geniculata* accessions and wheat variety. However, from 50 Mm NaCl, the height increment in Sbitla accession was lower than Ain zana. *Ae. geniculata* accessions and wheat can be arranged in

Table1. Accessions of *Aegilops geniculata* Roth. and characteristics of sites of origin.

Code des accessions	Site	Province	Altitude (m)	Rainfall (mm)	Bioclimatic area
AZ	Ain Zana	Kroumirie	641	700	Sub-humid
Z	Zaghouan	Dorsale	400	496	Uper semi arid
S	Sbitla	Centre	670	320	Uper arid

Table 2. Summary of analysis of variance for 10 growth, water statut, and gas exchange traits of three *Ae. geniculata* Roth accessions `Ain zana, Zaghouan and Sbitla` and durum Wheat variety `Chili` grown under different salinity levels (0, 50, 100, 150, 200 mM) at Ariana, Tunisia.

Parameters	Abbr	Sources of variation		
		Population (Pop)	Treatment (Tr)	Pop & Tr
Growth parameters				
Per-flag leaf length	LL	704.93**	210.7**	738.99**
Leaves number	LN	27.34**	120.94**	1493.77**
Total leaves dry weight	DW	4.31**	31.45**	0.45**
Gas exchange parameters				
Stomatal conductance	g_s	0.0063**	0.327**	0.0012**
Water relations				
Pre-dawn potential	Ψ_{pd}	235.741**	127.037**	0.598*
Midday water potential	Ψ_{md}	1.60**	68.58**	1.95**
Apoplastic water content	AWC	161.36**	78.208**	9.55^{ns}
Osmotic adjustment	OA	0.028**	0.771**	0.003*
Modulus of elasticity	ε_{max}	1.119**	5.657**	0.159***
Osmotic potential at full turgor	Ψ_π^{100}	0.43**	0.33**	0.38**
Osmotic potential at turgor loss point	Ψ_π^{0}	0.892**	0.775**	0.0031**
Relative water content at loss point	RWC_0	61.14**	815.73**	10.987**
Solute accumulations				
Soluble carbohydrate		73.76**	1827.14**	7.37**
Proline		81.04**	6841.35**	9.07**

F-probabilities are indicated by symbols , • ns = non significant differences at 0.05. • * = significant difference at $p < 0.05$. • ** = significant difference at $p < 0.01$.

the following order on the base of osmotic potential: `Ain Zana`> `Zaghouan` > wheat > `Sbitla`. The lower values of Ψ_π^{100} (Fig. 3b) showed an osmotic adjustment (OA) in stressed rather than in control plants. In the three accessions of *Ae. geniculata* and wheat cultivar, OA increase with increasing of NaCl concentrations (Fig. 3c). However, clear differences between *Ae. geniculata* and wheat were noted. For all salt level, the higher values of OA was noticed in Sbitla and the lower in Ain Zana. Wheat cultivar and Zaghoaun accession showed intermediary behaviours. In three accessions of *Ae. geniculata* and wheat, the relative water content at the turgor loss point (RWC_0), the volumetric modulus elasticity (ε_{max}) and the apoplastic water content (AWC) increased significantly with an increase of salinity (Figs. 3d, e, f) . Nonetheless clear differences were observed between accessions of *Ae. geniculata* and cultivar of wheat. In fact, for all NaCl concentrations, the higher values of RWC_0 and ε_{max} obtained in Ain Zana accession and the lower in Sbitla (Figs. 3d, e). In contrast, Sbitla showed the greatest increment of AWC and Ain Zana the least. Wheat cultivar and Zaghoaun accession showed intermediary behaviours (Fig. 3f).

Osmolytes accumulation

Proline and total soluble carbohydrates concentrations in control weren't different between *Ae. geniculata* and wheat. The salt stress provoked a markedly accumulation of proline and total soluble carbohydrates. However, from 100 mM NaCl, strongly differed between population (Tables 2 and 4). In fact, the highest contents were recorded in Sbitla followed by Zaghouan, wheat and finally Ain Zana.

Discussion

Substantial variation was observed in our study for traits related to the adaptation of *Ae. geniculata* Roth and wheat to salt stress. However, each population behaved differently under the influence of different salt treatments. The variation among species and accessions for growth leaves, water relation and organic solutes was mainly explained by geographical origin, suggesting that those traits are mainly constitutive and result from natural pressure selection exerted by the climatic constraints (Table 2). Although data were obtained from plants grown in pots, the result can be related to *in situ* performance. Sbitla accession, which is better adapted to a naturally occurring salt stress, was more tolerant to experimentally an imposed salt experiment stress than Ain Zana and Zaghoaun accessions. Ain Zana was the most sensitive to the salt stress. Growth of both species (wheat variety and the three *Aegilops geniculata* Roth accessions) decreased with an increasing salt stress as indicated by before-flag leaf length, leaf number and total leaves dry weight (Table 3). This was observed in other species, such as wheat (Mahar et al., 2003; Munns et al., 2003; El-Bassiony et al., 2005) and Olive (Gucci et al., 1996). The results reported by Munns and Rawsan (1999) in wheat showed that saline stress generally appears by a weak growth, a reduction of area and number of leaves and an acceleration of senescence of the mature leaves. Besides, Cramer and Quarrie (2002) in maize noted that salt stress reduced the development of the

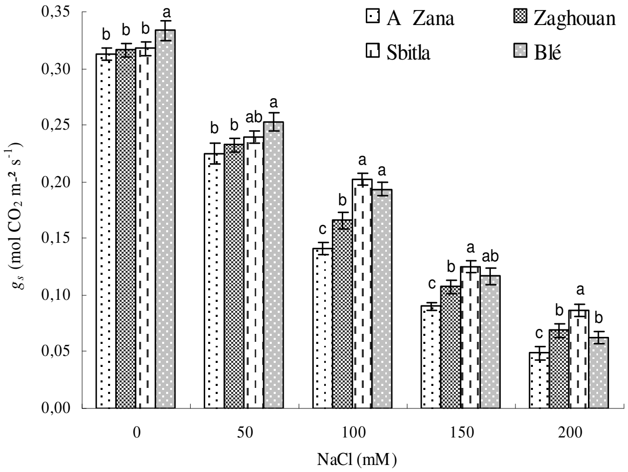

Fig 1. Stomatal conductance (*gs*) of three *Ae. geniculata* accessions `Ain zana., Zaghouan and Sbitla` and durum Wheat variety `Chili` grown under different salinity levels (0, 50, 100, 150, 200 mM NaCl) the data are mean values of four replications with three measurements per replicate and vertical bars are $LSD_{0.05}$.

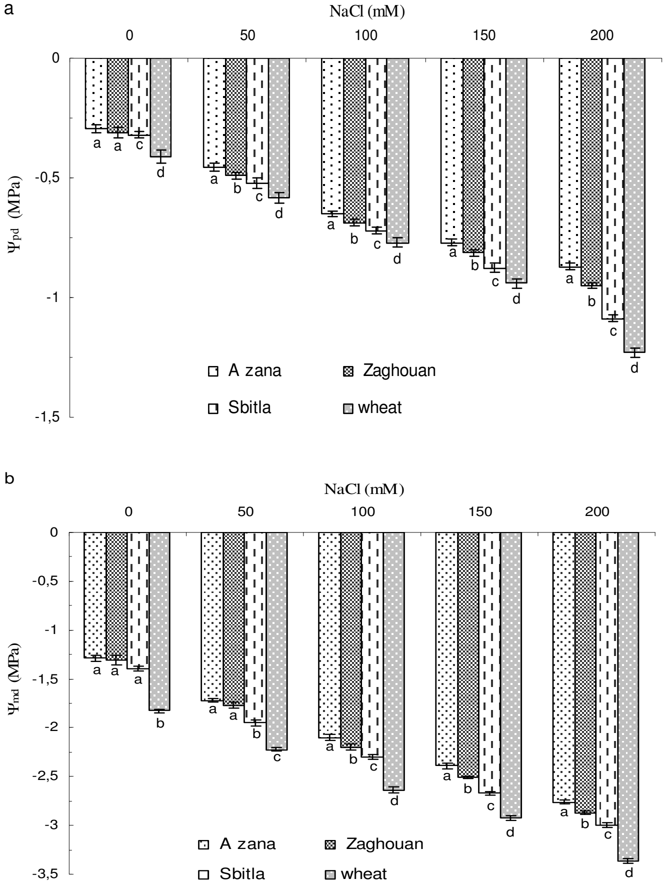

Fig 2. (a) Pre-dawn (Ψ_{pd}) and (b) midday (Ψ_{md}) leaf water potential for three *Ae. geniculata* accessions `Ain zana., Zaghouan and Sbitla` and durum Wheat variety `Chili` grown under different salinity levels (0, 50, 100, 150, 200 mM NaCl) the data are mean values of four replications with three measurements per replicate and vertical bars are $LSD_{0.05}$.

aerials parts by inhibition of the apparition of new leaves. The observed reduction in plant biomass might be due to a combination of slow growth and development as a result of osmotic stress (Shani and Ben-Gal, 2005). Nonetheless, the reduction in leaf area can be considered as avoidance mechanisms minimising water losses when stomata are closed and which happens to many species under osmotic stress (Blum 1986; Savé al., 1994; Ruiz-sháchez et al., 2000). Under saline condition it is known that the reduction in total leaf area can be explained by a decrease in leaf turgor, changes in cell wall proprieties or a decreased photosynthesis rate (Franco et al., 1997). In our conditions, it could be due to cell wall changes and a decrease in the photosynthesis rate, because Ψ_π values are reduced (Figs. 3a, b) and the net assimilation rate decreased (Mguis, 2010). Indeed, at the reproductive stage, salinity stress markedly inhibited the leaf water potentials (Ψ_{pd} and Ψ_{md}), the stomatal conductance (g_s) and the osmotic potential (Ψ_π^0 and Ψ_π^{100}). Similar results were found on wheat (Rivelli, 2002a), *Plantago cronopus* (Koyro, 2006), Arbutus unedo (Navarro et al., 2007). The highest Ψ_{pd} and Ψ_{md} was found in Ain zana accession as well as in the tow author accessions of Zaghouan and Sbitla. Low values were found in wheat (Fig. 2). For instance, the existence of difference between Ψ_{pd} and Ψ_{md} values suggested that some recovery in leaf water potential took place during the night. In three accessions of *Ae. geniculata* and wheat, stomatal conductance decreased (Fig. 1) proportionally Ψ_{md} (Fig. 2b) with an increase of NaCl, which an indicates that saline stressed plants have a capacity to favour water absorption and to conserve. For instance the strong correlation ($r^2 = 0.98$, $p < 0.001$) which was observed between *g*s and Ψmd (Fig. 4) suggested that leaf water potential was the main factors inducing stomatal closure. However, stomata close under salinity in response to soil-toleaf chemical signals (James et al., 2002, LeNoble et al. 2004), leaf water potential (Navarro et al., 2007) or leaf specific hydraulic conductivity (Schultz, 2003). On the other hand, the gradual closure of the stomata in salt stress over a wide range of Ψmd may be important to maintain some level of photosynthesis (Savé al., 1994). The decrease $\Psi\pi^0$ Contributes to maintain a positive cell turgor at lower water potentials. Nonetheless, the reduced $\Psi\pi^0$ could be attributed to osmotic adjustment following a departure from pure water to the dehydration of tissues and / or an active accumulation of solutes. The first hypothesis can be accepted as lower $\Psi\pi^0$ (Fig. 3a) is accompanied by an increase RWC_0 (Fig. 3d) and the second hypothesis will be discussed later. The observed sharp decrease in leaf osmotic potential at full turgor ($\Psi\pi^{100}$) in treated plants of three *Ae. geniculata* and wheat indicated the ability of those plants to develop osmotic adjustment under saline conditions (Fig. 3d). However, the reaction of those plants was different. Under all NaCl concentrations, Sbitla accession showed a high accumulation of osmotic compounds and also with the mechanisms of physiological adaptation to drought followed by Zaghouan accession and wheat which showed intermediate behavior. The highest $\Psi\pi^{100}$ were obtained from Ain Zana accession (Fig. 3c).The osmotic adjustment which occurs through salt accumulation in plant under saline stress is less energetic and demanding than the adjustment of inorganic ions and organic solutes may perform osmotic adjustment (Netondo et al., 2004a; Munns et al., 2006), proline and glycine betaine (Yang et al., 1990). In the three *Ae. geniculata* accessions and wheat the effect of saltiness occurs by a high accumulation of soluble carbohydrates and proline in leaves (Table 4). Thus, under different salt treatments, the highest accumulations of soluble sugars and proline were showed in Sbitla accession and the

Table 3. Pre-flag leaf length (LL) in "cm" of the tagged tillers, leaves number (LN, number of leaves emerged in principal tiller) ant total leaves dry weight (DW) (mean ± SE., n = 8) of three *Ae geniculata* accessions `Ain zana, Zaghouan, Sbitla` and one durum Wheat variety `Chili` grown under salt stress (0, 50, 100, 150, 200 mM) conditions.

Salinity levels	*Ae* Ain Zana	*Ae* Zaghouan	*Ae* Sbitla	Wheat
		LL		
0 mM	9.21 ± 0.93	9.57 ± 0.84	9.54 ± 0.94	31.45 ± 3.84
50 mM	6.50 ± 0.73	7.59 ± 0.79	8.51 ± 0.84	27.64 ± 1.40
100 mM	4.92 ± 0.42	6.39 ± 0.60	7.04 ± 0.44	22.01 ± 2.09
150 mM	3.72 ± 0.63	5.04 ± 0.61	5.84 ± 0.58	19.39 ± 1.92
200 mM	2.99 ± 0.39	3.96 ± 0.57	4.58 ± 0.79	14.28 ± 2.17
		LN		
0 mM	9 ± 1	9 ± 1	9 ± 1	12 ± 2
50 mM	6 ± 1	8 ± 1	8 ± 1	9 ±1
100 mM	4 ± 1	5 ± 1	6 ± 1	7 ± 1
150 mM	3 ± 1	4 ± 1	5 ± 1	5 ± 1
200 mM	2 ± 1	3 ± 1	4 ± 1	4 ± 1
		Leaf DW		
0 mM	3.86 ± 0.155	3.82 ± 0.133	3.82 ± 0.119	3.79 ± 0.093
50 mM	2.73 ± 0.087	2.92 ± 0.102	3.26 ± 0.087	2.18 ± 0.052
100 mM	1.67 ± 0.052	2.07 ± 0.062	2.36 ± 0.092	1.48 ± 0.048
150 mM	1.18 ± 0.049	1.47 ± 0.070	1.47 ± 0.057	1.27 ± 0.031
200 mM	0.78 ± 0.042	1.06 ± 0.048	1.29 ± 0.052	0.89 ± 0.047

Table 4. Solute concentrations in leaves (mean ± SE., n = 4) of three *Ae geniculata* accessions `Ain zana, Zaghouan, Sbitla` and one durum Wheat variety `Chili` grown under salt stress (0, 50, 100, 150, 200 mM) conditions.

Salinity levels	*Ae* Ain Zana	*Ae* Zaghouan	*Ae* Sbitla	Wheat
		Proline content (μmol. g^{-1} MS)		
0 mM	10.05 ± 1.03	10.35 ± 1.14	10.15 ± 2.14	11.09 ± 1.34
50 mM	17.16 ± 1.78	20.88 ± 1.66	24.17 ± 2.17	19.45 ± 1.47
100 mM	35.37 ± 2.03	42.91 ± 2.07	51.74 ± 2.08	41.18 ± 2.22
150 mM	58.37 ± 2.38	69.10 ± 2.41	73.85 ± 2.75	65.45 ± 1.77
200 mM	71.78 ± 1.89	79.82 ± 2.61	88.73 ± 2.12	77.82 ± 2.45
		Soluble carbohydrates (μmol. g^{-1} MS)		
0 mM	581.75 ± 15.46	568.75 ± 12.19	571.79 ± 20	591.79 ± 10.24
50 mM	635.71 ± 11.55	656.64 ± 11.61	686.14 ± 18.57	663.23 ± 13.58
100 mM	694.85 ± 20.31	723.32 ± 12.62	759.59 ± 16.95	730.68 ± 12.83
150 mM	714.66 ± 12.56	761.44 ± 8.18	797.51 ± 14.42	755.49 ± 16.97
200 mM	782.34 ± 19.32	823.51 ± 11.01	866.81 ± 12.54	825.15 ± 15.23

lowest accumulations were recorded in Ain Zana, while Zaghouan and wheat showed intermediate accumulations. Nevertheless, the Strong relationships observed between accumulation of proline and the decrease of Ψ_{π}^{100} ($r^2 = 0.92$, $p < 0.001$) and between increases of total carbohydrates and decreases of Ψ_{π}^{100} ($r^2 = 0.96$, $p < 0.001$) suggested the involvement of those elements in maintaining the osmotic balance (Figs. 4 a, b). Similar effects have been reported in many species such as, wheat (Rivelli et al., 2002a; Tambussi et al., 2005), sorghum (Netondo et al., 2004a), *Arbutus unedo* (Navarro et al., 2007). The salt stress induced a significant decrease in leaf cell elasticity (that is, the bulk modulus of elasticity, ε_{max}, increased) in the three *Ae. geniculata* and wheat (Fig. 3e). An increase in ε_{max}, tended to enhance relative water content at zero turgor (RWC0). However, osmotic adjustment compensated for this effect. Increased ε_{max} concomitant with osmotic adjustment is an effective means of counteracting the negative effects of osmotic stress on water balance (Navarro et al., 2007). An increase in ε_{max} (stiffness) is expected when the cell walls become more rigid or thicker (Stuiver et al., 1978; Kuiper, 1984). This increase is observed to be higher in the tolerant accession (Sbitla) followed by Zaghouan, wheat, and Ain Zana. The greater stiffness of the cell wall, given an equal decrease in cell volume, will be responsible for the drop in water potential. These saltiness related changes in tissue elasticity of three Ae. *geniculata* and wheat accession lead to an alteration in the relationship between turgor pressure and cell volume which might contribute to salinity tolerance. Similar results were showed in wheat (Rivelli et al., 2002a), in *Cornus stolinifera* (Jennifer and Sylvia, 2004), *Arbutus unedo* (Navarro et al., 2007). On the other hand in fourth populations (three *Ae. geniculata* accessions and wheat cultivar), the salt stress also provoked a significant increase in apoplast water content (AWC) compared with controls varies with the cultivar or accession and the intensity of stress (Fig. 3f). Increasing water reserve of apoplast compartment could be attributable to thicker cell walls or reduces cell volume (Cutler and Rains, 1978) by accumulation of materials such as hemicellulose (Rascio et al., 1992) and salts (Cachorro et al., 1993). For instance, Water apoplast protects tissues against water loss from symplast (Tyree and Jarvis, 1982) and this seems to prove that in wheat and *Ae. geniculata*, the increase of apoplast water played a role in maintaining or increasing the turgidity. This behavior would have a buffer role during the installation of water deficit to preserver water symplast. In conclusion, the *Ae. geniculata* accessions provides germaplasm that has the potential of crop improvement; it belongs to the wild species. Wild related species have been considered until now much more as sources of resistance to pests and diseases than as sources of diversity permitting deep modification of architecture and physiology of the

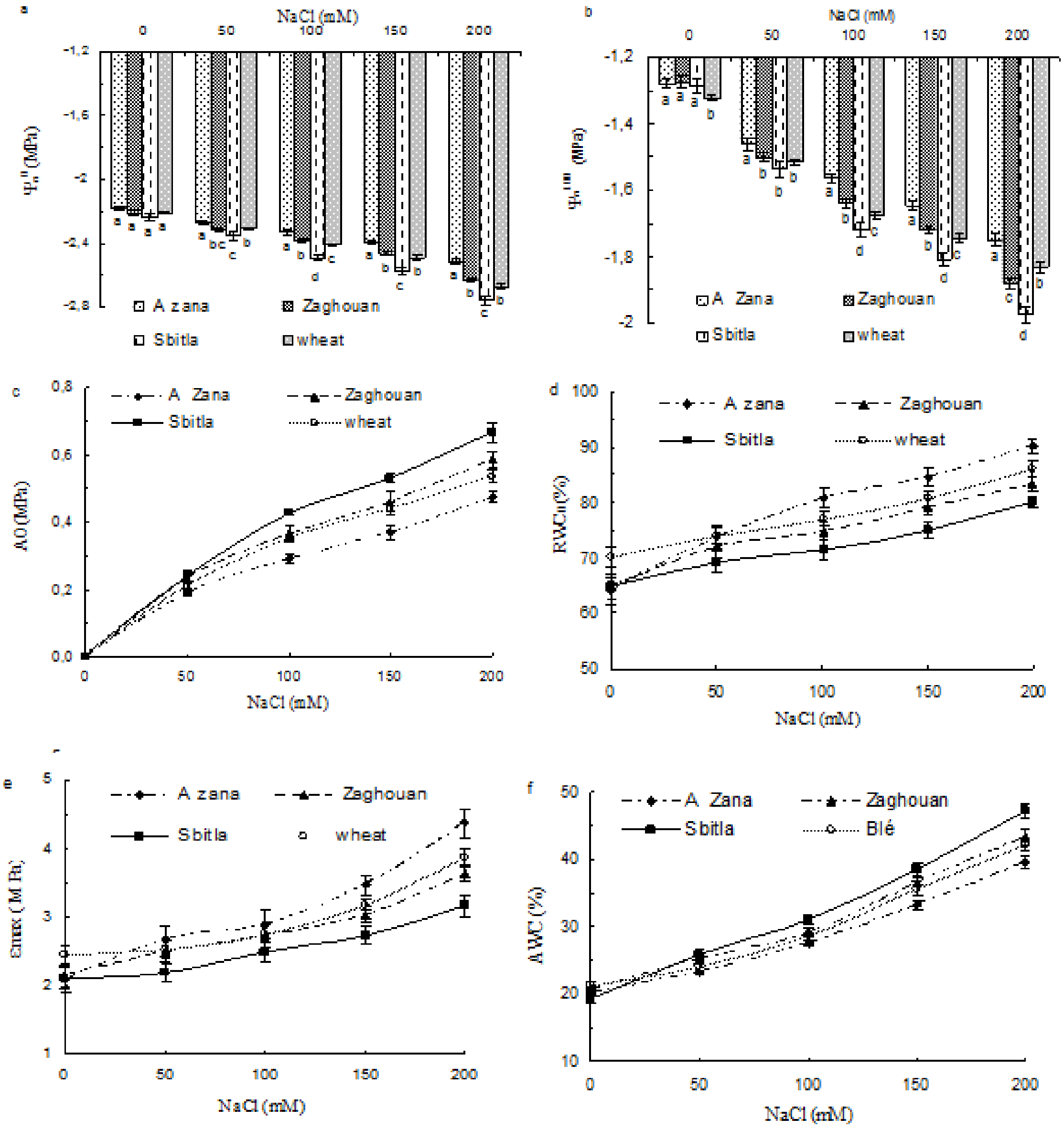

Fig 3. Water relation parameters derived from P-V curve analysis: (a) the osmotic potential at zero trugor (Ψ_π^0), (b) the osmotic potential at full trugor (Ψ_π^{100}), (c) the osmotic adjustment (OA), (d) the relative water content at the turgor loss point (RWC_0), (e) the volumetric modulus elasticity (ε_{max}) and (f) the apoplastic water content (AWC)of three *Ae. geniculata* accessions `Ain zana., Zaghouan and Sbitla` and durum Wheat variety `Chili` grown under different salinity levels (0, 50, 100, 150, 200 mM NaCl) the data are mean values of four replications with three measurements per replicate and vertical bars are $LSD_{0.05}$.

cultivated species. There are indications that *Ae. geniculata* Roth can contribute useful genes for salt tolerance (Monneveux et al., 2000). Systematic approaches to increase the level of a biotic stress tolerance require the evaluation of genetic variability in *Ae. geniculata* Roth gene pool, within and among accessions. In this study, a large variation for salt-stress tolerance was found within accessions. Single population such as Sbitla or Ain zana or Zaghouan that exhibit rich growth and water relations diversity may provide valuable resources for traits of agronomic importance. The salt-stress tolerant identified in the *Ae. geniculata* accessions and wheat variety `Chili` may be combined with breeding lines exhibiting high yield potential. Genotypic analysis in the combination with physiological studies is required to establish whether the salt-stress-tolerant lines are similar, i.e. the same mechanisms. If different mechanisms are identified there will be potential for recombining these for further improvement.

Materials and methods

Plant Materials

The three *A. geniculata* accessions originated from Ain Zana, Zaghouan, and Sbitla (Table 1) were collected on a large range of climatic conditions and at different altitudes. The

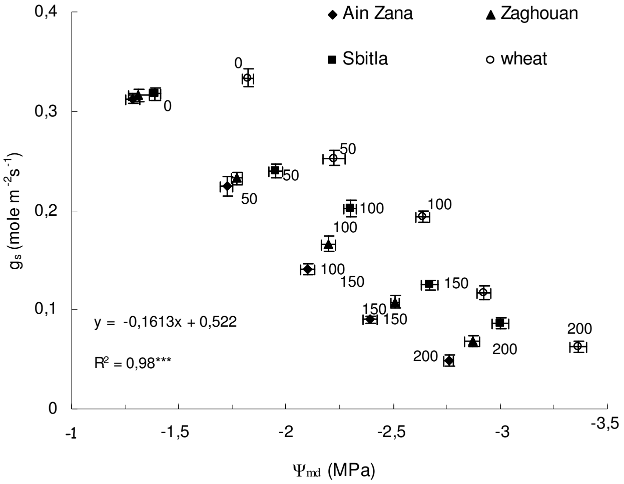

Fig. 4. Relationship between leaf conductance (*gs*) and midday leaf water potential (Ψ_{md}) of three *Ae. geniculata* accessions `Ain zana., Zaghouan and Sbitla` and durum Wheat variety `Chili` grown under different salinity levels (0, 50, 100, 150, 200 mM NaCl) the data are mean values of four replications with three measurements per replicate and vertical bars are $LSD_{0.05}$.

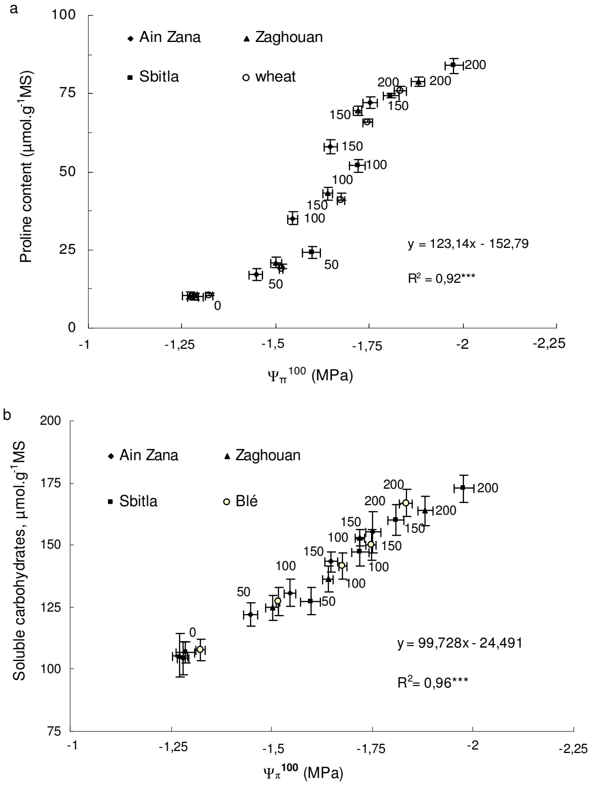

Fig 5. Relationships between accumulation of proline and osmotic potential at full trugor (Ψ_{π}^{100}) and between total soluble carbohydrates and osmotic potential at full trugor (Ψ_{π}^{100}) of three *Ae. geniculata* accessions `Ain zana., Zaghouan and Sbitla` and durum Wheat variety `Chili` grown under different salinity levels (0, 50, 100, 150, 200 mM NaCl) the data are mean values of four replications with three measurements per replicate and vertical bars are $LSD_{0.05}$.

durum wheat variety (Chili) was provided by the Cereal Laboratory of the National Institute of Agronomic Research of Tunis (INRAT). It is an old and precocious variety that has a high length average (120 cm). It slows late (20 days after modern one) and low yielding. Furthermore, it is susceptible to lodging days to heading and it is one of the most ancient salt-tolerant varieties (Maamouri et al., 1998).

Growing conditions

Experiments were carried out during (2009-2010) in a greenhouse at the INRAT in Ariana (Northeast Tunisia at 10 m above sea level). Seed sowing was performed in pots of 28 cm of diameter and 25 cm of height. The pots were filled with a mixture of 50% loam and 50% sand. After replenishment of the pots, 10 seeds per pot were sowed in a homogeneous way to a depth of 2-3 cm, and after emergence (7 days), seedling number per pot was reduced to 6. During the first seven days, pots were watered daily with tap water while adding the necessary volume to bring soil to its field capacity. Thereafter, pots were watered twice a week. Salt treatment started when the seedling had approximately 3 to 4 leaves (4 weeks after sowing). Sodium chloride was added in four concentrations: 50, 100, 150, and 200 mM. Prior to salt treatment, nutrient solution was supplied instead of water. The nutrient solution was prepared according to Maas et al. (1986). Pots were irrigated at each stage of plant development with nutrient solution. The experiment was set up as completely randomized design of five salt levels, four populations (three *Aegilops* accessions and one durum wheat variety) and four replicates. Each pot (six seedlings) was considered as one replicate. Hence, each treatment contained 24 seedlings per accession or variety.

Measurements

Measurements were made at intervals of 6 or 7 days between 6:00 and 8:00 h, beginning at the heading stage (16 weeks after emergence) and continuing during the flowering one.

Leaf growth parameters

The pre-flag leaf length (LL) in "cm" of the tagged tillers and leaf number (LN, number of leaves emerging from the principal tiller) of *Aegilops* accessions and the durum wheat variety were measured. After harvest, leaves were separated and dried for 3 days at 70°C for dry-weight (DW). Measurements were taken from eight plants (2 plants per replicate) per population and per treatment.

Stomatal conductance

Stomatal conductance (g_s, mol m^{-2} s^{-1}) was measured at midday for a similar number and type of leaves as for leaf water potential using a steady-state promoter (LI 1600, LI-COR Inc.).

Leaf water relations

- *Water potential*

Leaf water potential was measured pre-dawn (6:00 am) (Ψ_{pd}) and at midday (Ψ_{md}) for one mature leaf of the tagged tillers (pre-flag leaves) per plant and per replicate (n = 4 measurements per treatment) using a pressure chamber (Soil Moisture Equipments Corp., Santa Barbara, CA, USA) following the recommendations of Turner (1988).

- *Pressure-volume (P-V) curves*

P-V curves were determined using the Scholander pressure-chamber technique (Scholander and Hammel 1965). The P-V curves of each leaf were obtained by expressing the relationship between relative water content (RWC) values and the reciprocals of the measured water potentials ($-1/\Psi w$). Osmotic potential at full turgor (Ψ_{π}^{100}) was estimated *via* linear regression of data in the straight-line region of the P-V curve. Osmotic potential at the turgor loss point (Ψ_{π}^{0}) was derived from the RWC and $-1/\Psi w$ coordinates respectively of the first point in the straight-line region of the P-V curves (Patakas and Notsakis 1999). The osmotic adjustment (OA) was defined as the difference in Ψ_{π}^{100} between stressed and control plants.

$OA = (\Psi_{\pi}^{100})_{stressed} - (\Psi_{\pi}^{100})_{control}$

The apoplastic water content (AWC) or intercellular water is estimated from the extension of the linear part to the axis (OX) (Girma and Krieg, 1992). It tells about the no displaced water (Ritchie and Roden, 1985). The relative water content at the turgor loss point (RWC_0) was obtained from the extension of the intersection of curve and linear portions (Wilson et al., 1979). The volumetric modulus elasticity (ε) was calculated as the slope of the relationship between turgor pressure and RWC (Patakas and Notakis, 1999).

$\varepsilon_{max} = (\Psi_t^{100} - \Psi_t^{0}) \text{ x } (1- AWC) / (1- RWC_0)$

Relative water content (RWC) was determined on three to four fully expanded leaves. Leaves were excised before dawn, weighed freshly (FW) and placed in vials to rehydrate in the dark for 20 h. Preliminary experiments had indicated that full rehydration was complete after 18 h. The next morning, leaf turgid weight (TW) was measured and then leaves were dried at 80 °C for 48 h and dry weight (DW) was determined. The RWC was calculated from the equation:

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

Osmolytes contents

Proline was determined following the ninhydrin method described by Bates et al. (1973), using L-proline as a standard. Proline in the test samples was calculated from a standard curve prepared against L-Proline (5-30 µg, from MERCK KGaA): ($y = 0.059x - 0.014$, $R^2 = 0.99$). The content of total soluble carbohydrates in the studied samples was determined according to Mc Cready et al. (1950) and Staub (1963), using glucose as a standard. From the standard curve, the concentrations of soluble carbohydrates in the test samples were calculated ($y = 0.0095x - 0.0299$, $R^2 = 0.979$).

Statistical Analysis

The experiment was a complete randomized design consisting of five salinity treatments, four populations (three *Aegilops* accessions and one durum wheat variety) and four replicates. The data were analyzed using appropriate procedures of the SAS software 6.12 (Khawarizmi Center, Tunis version 1998). Analysis of variance (ANOVA) was performed with the statistical program Minitab (Minitab Inc.; College Park), involving two levels of classification (salinity and population) with interactions. A Duncan's multiple range test was carried out to determine if significant ($P < 0.05$) difference occurred between accessions and treatments.

References

Abrams MD, Kubiske MD (1994) Synchronous changes in tissue water parameters of mature foliage from well-watered and periodically droughted tree seedlings. J Exp Bot 45: 171-177

Bates LS, Waldren RP, Teare ID (1973) Rapid determination of free proline for water stress studies. Plant and Soil 39: 205-207

Blum A (1986) Salinity resistance In: CRC (ed): Plant Breeding for Stress Environments. Press, Boca Raton

Bohnert HJ, Nelson DE, Jensen RG (1995) Adaptation to environmental stresses. Plant Cell 7: 1099-1111

Bohnert HJ, Su H, Shen B (1999) Molecular mechanisms of salinity tolerance. In: Shinozaki K, Yamaguchi-Shinozaki K (edts) Molecular Responses to Cold, Drought, Heat and Salt Stress in Higher Plants. Landes RG Company. Aust. TX

Cachorro P, Ortiz A, Cerda A (1993) Growth, water relations and solute composition of *Phaseolus vulgaris* L. under saline conditions. Plant Sci 95: 23-29

Colmer TD, Flowers TJ, Munns R (2006) Use of wild relatives to improve salt tolerance in wheat. J Exp Bot 57: 1059-1078

Cramer GR, Quarrie SA (2002) Abscisic acid is correlated with the leaf growth inhibition of four genotypes of maize differing in their response to salinity. Funct Plant Biol 29: 111-115.

Cutler JM, Rains DW (1978) Effects of water stress and hardening on the internal relations and osmotic constituents of cotton leaves. Physiol Plant 42: 261-268

Dale JB (1988) The control of leaf expansion. Ann Rev Plant Physiol 39: 267-295

El-Bassiouny HMS, Bekheta MA (2001) Role of putrescine on growth, regulation of stomatal aperture, ionic contents and yield by two wheat cultivars under salinity stress. E J Physiol Sci 2-3: 239-258

Farooq S, Shah TM and Asghar M (1996) Intergenic hybridization for wheat improvement: V. Production of and metaphase 1 chromosome analysis in F1 hybrids of wheat (*Triticum aestivum*) with *Aegilops ovata* L. Cereal Res Comm 24: 155-161

Gucci R, Lombardini L, Tattini M (1996) Analysis of leaf water relations in leaves of two olive (*Olea europaea*) cultivars differing in tolerance to salinity. Tree Physiol 17: 13-21

James RA, Rivelli AR, Munns R, Von Caemmerer S (2002) Factors affecting CO_2 assimilation, leaf injury and growth in salt stressed durum wheat. Funct Plant Biol 29: 1393-1403

Jennifer M, Sylvia R (2004) Effects of NaCl on water relations and composition of red rosier dogwood (*Cornus Stolonifera*) seedlings. Physiol Plant. 121: 265-271

Kao WY, Tsai TT, Tsai HC, Shih CN (2005) Response of three *Glycine* spieces to salt stress. Environ Exp Bot 56:120- 125

Koyro HW (2006) Effect of salinity on growth, photosynthesis, water relations and solute composition of the potential cash crop halophyte *Plantago coronopus* (L.) Environ Exp Bot. 56: 136–146

Kuiper PJC (1984) Functionning of plant cell membranes under saline conditions; Membrane lipid compostion and ATPase. In: Staples RC, Toenniessen DH (edts) Salinity Tolerance in Plants. Wiley, New York

Lawlor DW (2002) Limitation to photosynthesis in water stressed leaves: Stomata *Vs* metabolism and role of ATP. Ann Bot 89: 871- 885

LeNoble ME, Spollen WG, Sharp RE (2004) Maintenance of shoot growth by endogenous ABA: genetic assessment of the involvement of ethylene suppression. J Exp Bot 55: 237-245

Livingston NIP, Von A (1992) Water relations parameters of embryonic cultures and seedlings of larch. Plant Physiol 100: 1304-1309

Maamouri A, Deghais M, El Felah M, Halila H (1988) Les variétés de céréales recommandées en Tunisie. Document technique

Maas EV, Poss JA, Hoffman GJ (1986) Salinity sensitivity on sorghum at three growth stages. Irrig Sci **7**: 1-11

Mahar AR, Hollington PA, Virk DS, Witcombe JR (2003) Selection for early heading and salt-tolerance in bread wheat. Cereal Res Commun 31: 81-88

Mc Cready RM, Guggolz J, Silviera V, Owes HS (1950) Determination of starch and amylase in vegetables, application to peas. Anal Chemistry 22:1156-1158

McDonald JS, Davies WJ (1996) Keeping in touch: responses of the whole plant to deficits in water and nitrogen supply. Adv Bot Res 22: 229-300

Mguis K (2010) Comportement morphologique, ecophysiologique et nutritionnel de trois accessions *d'Aegilops geniculata* (Roth.) et d'un cultivar du blé dur soumis à la contrainte saline. Thèse de doctorat Tunisie

Monneveux P, Zaharieva M, Rekika D (2000) The utilization of *Triticum* and *Aegilops* species for the improvement of durum wheat. Génétique et Amélioration des Plantes INRA 71-81

Morgan JM, Rodriguez-Maribona B, Knights EJ (1991) Adaptation to water-deficits in chickpea breeding lines by osmoregulation: relationship to grain-yield in the field. Field Crops Res 27: 61-70

Munns R, James RA (2003) Screening methods for salt tolerance: a case study with tetraploid wheat. Plant and Soil, 253: 201-218

Munns R, Rawson HM (1999) Effect of salinity on salt accumulation and reproductive development in the apical meristem of wheat and barley. Aust J Plant Physiol 26: 459-464

Munns R, James RA, Läuchli A (2006) Approaches to increasing the salt tolerance of wheat and other cereals. J Exp Bot 57: 1025-1043

Navarro A, Bañon S, Olmos E and Sahchez-Blanco MJ (2007) Effects of sodium chloride on water potential components, hydraulic conductivity, gas exchange and leaf ultrastructure of *Arbutus unedo* plants. Plant Sci 172: 473-480

Netondo GW, Onyango JC, Beck E (2004a) Sorghum and salinity: Response of growth, Water Relations, and Ion Accumulation to NaCl salinity. Crop Sci 44: 797- 805

Passioura JB (1994) The physical chemistry of the primary cell wall: implications for the control of expansion rate. J Exp Bot 45, 1675-1682

Patakas A, Noitsakis B (1999) Mechanisms involved in diurnal changes of osmotic potential in grapevines under drought conditions. J Plant Physiol 154:767–74

Rascio A, Platini C, Di Fonzo N, Wittmer G (1992) Bound water in durum wheat under drought stress. Plant Physiol 98 908-912

Richardson SG, McCree KJ (1985) Carbon balance and water relations of Sorghum exposed to salt and water stress. Plant Physiol 79: 1015-1020

Rivelli AR, Lovelli S, Perniola M (2002) Effects of salinity on gas exchange, water relations and growth of sunflower (*Helianthus annuus*). Funct Plant Biol 29: 1405-1415

Rodriguez P, Torrecillas A, Morales MA, Ortuño MF, Blanco MJS (2005). Effects of NaCl salinity and water stress on growth and leaf water relations of *Asteriscus maritimus* plants. Environ Exp Bot 53: 113-123

Ruiz-sháchez MC, Domingo R, Torricillas A, Alarcon JJ (1998) Diurnal and seasonal osmotic potential changes in *Lotus crestus* plants grown under saline stress. Plant Science 136: 1-10

Savé R, Olivella C, Biel C, Adillòn J, Rabella R (1994) Sesonal patterns of water relationships photosynthetic pigments and morphology of *Actinidia deliciosa* plants of the *Haywards* and *Tomouri* cultivars. Agronomie 2: 121-126

Scholander PF, Hammel HT (1965) Sap pressure in vascular plants. Science 148: 339-346.

Schultz HR (2003) Differences in hydraulic architecture account for near isohydric and anisohydric behaviour of two field-grown *Vitis vinifera* L. cultivars under drought. Plant Cell Environ 26: 1393-1405

Shani U, Ben-Gal A (2005) Long-term response of grapevines to salinity: osmotic effects and ion toxicity. Am J Enol Vitic 56: 148-154

Staub AM (1963) Extraction, identification et dosages des glucides dans les extraits d'organes et les corps bactériens. In: Masson and Compagnie (edts) Techniques de laboratoire (Tome 1 et 2) Paris

Stuiver CEE, Kuiper PJC, Marshner H (1978) Lipids from bean, barely and suguar beet in relation to salt resistance. Physiol Plant 42: 124-128

Tambussi EA, Nogués S, Araus JL (2005) Ear of durum wheat under water stress: water relations and photosynthetic metabolism. Planta 221: 446-458

Tan W, Hogan GD (1995) Effects of nitrogen limitations on water relations of jack pine *(Pinus banksiana* Lamb.) seedlings. Plant Cell Environ 18: 757-764

Turtola S, Rousi M, Pusenius J, Yamaji K, Heiska S, Tirkkonen V, Meier B, Julkunen-Tiitto R (2006) Genotypic variation in drought response of willows grown under ambient and enhanced UV-B radiation. Environ Exp Bot 56: 80-86

Tyree MT, Jarvis PG (1982) Water in tissues and cells. In: Lange OL, Nobel PS, Osmand CB, Ziegler H (edts) Springer-Verlag Encyclopedia of plant physiology, New series, Berlin

Van Slageren MW (1994) Wild wheats: A monograph of *Aegilops* L. and *Amblyopyrum* (Jaub. et Spach) Eig (*Poaceae*). Agricultural University, Wageningen – International Center for Agricultural. Research in Dry Areas, Aleppo, Syria

Yang YW, Newton RJ, Miller FR (1990) Salinity tolerance in sorghum.I. Whole plant reponse to sodium chloride in *S. Bicolour* and *S. halpense*. Crop Sci 30 : 775-781.

Zaharieva M, Gaulin E, Havaux M, Acevedo E, Monneveux P (2001) Drought and heat response in the wild wheat relative *Aegilops geniculata* Roth: Potential interest for wheat improvement. Crop Sci 41: 1321-1329