African Journal of Biotechnology Vol. 9(37), pp. 6061-6069, 13 September, 2010
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB09.1783
ISSN 1684–5315 © 2010 Academic Journals

Full Length Research Paper

Long-term mannitol-induced osmotic stress leads to stomatal closure, carbohydrate accumulation and changes in leaf elasticity in *Phaselous vulgaris* leaves

Sameh Sassi[1], Samir Aydi[1]*, Kamel Hessini[1], Esther M. Gonzalez[2], Cesar Arrese-Igor[2] and Chedly Abdelly[1]

[1]Laboratoire des Plantes Extrémophiles, Centre de Biotechnologie de Borj Cedria, BP 901, 2050 Hammam Lif, Tunisia.
[2]Dpto. CC. Medio Natural, Universidad Pública de Navarra, Campus Arrosadía, E-31006 Pamplona, Spain.

Accepted 17 June, 2010

The effect of long-term osmotic stress was investigated in leaves of two common bean lines, with contrasting tolerance: Flamingo (tolerant) and coco blanc (sensitive). Water relations, organic solute, ion accumulation and amino acids content as well as osmotic adjustment (OA) were studied during an extended exposure to osmotic stress. Osmotic stress was applied by means of 50 mM mannitol for 15 days. At the end of the stress period, both osmotic potential at full turgor (Ψ^{100}) and at turgor loss point (Ψ^{0}) decreased significantly in stressed plants compared with the control. The decrease being greater in the sensitive line, showed a greater OA compared with flamingo. Sugars contents increased in stressed plants and seem to be the major components of osmotic adjustment in stressed common bean leaves. The increase was more marked in coco blanc. Osmotic stress tolerance could thus not be associated with higher OA. The possible role of decreased leaf cell elasticity (ε_{max}) is discussed in relation to osmotic stress tolerance in this species.

Key words: Common bean, carbohydrate accumulation, growth, osmotic stress, osmotic adjustment, P-V curve, water relations.

INTRODUCTION

Plants may be subjected to environmental stresses that adversely affect growth, metabolism and yield (Lawlor, 2002). The degree of tolerance of plants to environmental stress varies greatly not only between species but in different varieties of the same species. 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 differences in stress tolerance could be used to select or create new varieties of crops that have increased productivity under such conditions. A large number of environments stress, including drought, salinity and low temperature, limit crop growth and productivity by imposing osmotic stress on plants. Water stress decreases plant growth and productivity, by slowing the rate of cell division and expansion mainly due to loss of turgor, which results in a decline of the water status components of the plant cells (Kiani et al., 2007). Furthermore, water stress result in stomatal closure, which limits CO_2 entry required for photosynthesis (Zhu, 2001; Tonon et al., 2004).

To cope with the problem, plant cells must readjust their osmotic potential to prevent water losses. That can be achieved by either uptake of inorganic ions from the external solution, or *de novo* synthesis of a number of metabolites, which are termed compatible solutes because they do not interfere with biochemical reactions (Bohnert et al., 1995). These metabolites include carbohydrates, such as mannitol, sucrose and oligosaccharides, and nitrogen-containing compounds, such as

*Corresponding author. E-mail: aydisamir@yahoo.fr. Tel: 00 216 97 431 733.

Abbreviations: AWC, Apoplastic water content; **DAS,** days after seedlings emergence; **DW,** dry weight; **LWP,** leaf water potential; **OA,** osmotic adjustment; **PAR,** photosynthetic active radiation; **P-V curve,** pressure-volume curve; **RWC,** relative water content; **SEM,** scanning electron microscopy; **$\Delta\Psi$I,** degree of osmotic adjustment; **ε,** tissue elastic modulus; **ε_{max},** cell elasticity; **Ψ,** osmotic potential; **Ψ^{0},** osmotic potential at turgor loss point; **Ψ^{100},** osmotic potential at full turgor.

amino acids. The function of compatible solute accumulation is often associated with osmotic adjustment, by lowering the osmotic potential (Ψ) to improve the uptake of water against the external gradient (Bohnert and Shen, 1999). The decrease in osmotic potential (Ψ) 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. Turgor is known to play an important role in the physiology of leaves (Passioura and Fry, 1992, Passioura, 1994). A positive turgor is necessary to sustain leaf growth (McDonald and Davies, 1996; Passioura, 1992). Turgor maintenance is affected by changes in both osmotic and elastic properties of leaf tissue (Dale, 1988). Osmotic potential at full turgor (Ψ^{100}) as well as at turgor loss point (Ψ^{0}), derived from pressure-volume curves, have proved to be reliable indices of the osmotic adjustment ability of 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 solute content of the symplast. Thus, it is difficult to be represented by a single mathematical formula (Wu and Spence, 1985). A graphical evaluation of the contribution of elastic and osmotic changes to turgor maintenance, based on the comparison of different areas under a pressure-volume curve, was proposed by Kikuta and Richter (1986). A number of researches have studied the water relations and osmotic and elastic adjustment of different crops under saline stress (Rodriguez et al., 2005; Navarro et al., 2006) and under water stress (Stoyanov, 2005). Nevertheless, the effects of these stresses on tissue elasticity are not clear, with increases reported in some species (Joly and Zaerr, 1987; Blake et al., 1991) and decreases observed in others (Nabil and Coudret, 1995). Some other authors even suggested that elastic adjustment does not play a role in adaptation mechanism (Mustard and Renault, 2004). Recently, we compared the behaviour of four common bean lines under osmotic stress conditions (Sassi et al., 2008). Results therein showed the relative tolerance of the line flamingo to osmotic stress under controlled conditions as compared with the line coco blanc. The present study focuses on revealing and quantitatively evaluating the participation of various osmotica in the osmotic adjustment of common bean leaves grown under long-term osmotic stress using the pressure volume technique.

MATERIALS AND METHODS

Plant material and growth conditions

Seeds of two contrasting varieties of common bean (*Phaseolus vulgaris* L.), coco blanc (sensitive) and flamingo (tolerant), were selected. Coco blanc, originated from local population and flamingo from Colombia. Seeds were surface sterilized in 80% ethanol for 30 s, 5% sodium hypochlorite for 2 min and washed ten times in sterilized distilled water. Sterilised seeds were pre-germinated in agar 0.9%. They were transferred in 1 L glass bottle wrapped with aluminium foil to maintain darkness in the rooting environment. The roots of selected uniform seedlings were gently passed through the hole of a rubber stopper on the bottle neck, and a cotton wool was fitted at the hypocotyl level to maintain the root system suspended in the nutrient solution. The later contained 0.25 mM KH_2PO_4, 0.7 mM K_2SO_4, 1 mM $MgSO_4 \cdot 7H_2O$, 1.65 mM $CaCl_2$, 22.5 µM Fe for macronutrients, 6.6 µM Mn, 4 µM Bo, 1.5 µM Cu, 1.5 µM Zn and 0.1 µM for micronutrients. The nutrient solution was renewed every 2 weeks. It was initially added with 1 ml of *Rhizobium tropici* CIAT 899 containing approximately 10^8 cells ml^{-1}. Medium pH was maintained to 7.0 by adding 0.2 g L^{-1} $CaCO_3$. It was aerated with a flow of 400 ml min^{-1} of filtered air via a compressor and "spaghetti tube" distribution system. Plants were grown in a temperature-controlled glasshouse with night/day temperatures of circa 20/28°C and a 16 h photoperiod with additional lights of 400 µmol PAR. m^{-2} s^{-1}.

Dry weight determinations

After harvest, different plant parts were separated. Leaves, stems and roots were then weighed. Leaf areas were determined with a portable area metre (Model LI-3000A, LI COR). Dry weights (DW) of different plant parts were determined after drying for 3 days at 70°C.

Osmotic treatment

Osmotic stress was applied by means of 50 mM mannitol, an osmotic component used generally to generate water deficit stress when added to nutrient solution. In order to avoid any osmotic shock, mannitol was progressively added as 25 and 50 mM at 15 and 18 days after seedlings emergence (DAS), respectively. Osmotic stress started 15 DAS. At the onset of flowering, 45 DAS, plants were harvested for water relations and growth parameters determination and compared with non-stressed plants (controls).

Scanning electron microscopy (SEM)

Only leaves (second or third pair from top) of plants subjected to 0 and 50 mM mannitol were observed and photographed with scanning electron microscopy (FEI quanta 200). Stomatal counting was assessed directly on three micrographs of different fresh leaves. Micrographs were randomly taken from a middle portion of a different upper second leaf. Each micrograph has known dimensions, and also a defined surface which correspond to a determined leaf surface.

Measurements of leaf water potential

Leaf water potential (LWP) was measured on fully expanded mature leaves (n = 3 per treatment) at the end of the osmotic stress period, using the pressure chamber (Soil Moisture Equipments Corp., Santa Barbara, CA, USA) according to Scholander and Hammel, (1965).

Stomatal conductance assay

Leaf conductance were measured in the second fully-expanded leaf with a portable IRGA (LI-6200, Li-Cor, Lincoln, NE, USA).

Pressure-volume (P-V) curves

The various components of water potential, osmotic adjustment and the elastic modulus of tissues were calculated using P-V curves analysis on 3 completely expanded leaves per treatment. Leaves were collected predawn at 05.00 h (am), wrapped in damp paper and enclosed in a plastic bag. The tissues were rehydrated by immersing in distilled water in a beaker sealed with para-film. Beakers were then stored in complete darkness at 2 - 4°C. Full rehydration was, on average, achieved in 24 - 48 h. Water potential at full turgor (Ψ_{100}) of leaves were close to zero (-0.01 MPa). P-V curves were determined using the Scholander pressure-chamber technique (Scholander and Hammel, 1965). The flow of nitrogen was set to give a pressure rise of approximately 0.005 MPa $^{-1}$.

To obtain the first points on the P-V curve, the leaves were subjected to stepwise increases of 0.2 MPa. At pressures above 2.5 MPa, stepwise increases of 0.5 MPa were applied to obtain the remaining points on the curve up to pressures of 5.0 MPa. At each step, the quantity of xylem sap expressed from the cut surface was determined. The dry weight of the leaves was measured afterwards. The P-V curves of each leaf were obtained by expressing the relationship between the relative water content (RWC) values and the reciprocals of the water potentials measured (-1/Ψ). Osmotic potential at full turgor (Ψ^{100}) (which equals the cell turgor at full hydration) was estimated via linear regression of data in the straight-line region of the P-V curves. Osmotic potential at zero turgor (Ψ^{0}) was derived from the X and Ψ coordinates, respectively, of the first point in the straight-line region of the P-V curves (Patakas and Notsakis, 1999). The degree of osmotic adjustment ($\Delta\Psi$) was defined as the difference in Ψ^{100} and Ψ^{0} between the control and the stressed plants, respectively. The bulk tissue elastic modulus (ε) was calculated according to Patakas and Notakis (1999). All leaf water relation parameters were measured at the middle of the photoperiod on three upper most fully expanded leaves from three randomly selected plants for each treatment.

Carbohydrate extraction and determinations

Fresh material was exhaustively extracted in boiling 80% (v/v) ethanol. Ethanol-soluble extracts were dried in a Turbovap LV evaporator (Zymark Corp, Hopkinton, MA, USA) and soluble compounds were re-dissolved with 4 ml of distilled water, mixed and centrifuged at 20000 *g* for 10 min. The ethanol insoluble residue, remaining after the extraction of soluble compounds, was extracted for starch as in MacRae (1971) and the glucose produced was analyzed, as well as the sucrose and mannitol of the ethanol soluble fraction, by high-performance capillary electrophoresis (CE) in a Beckman Coulter PACE system 5500 (Beckman Instruments, Fullerton, CA, USA) as described by Marino et al. (2006).

Determination of organic acids and inorganic ions

Organic acids (malate, α-ketoglutarate, oxalate and citrate) and inorganic ions content in nodules was determined by ion chromatography in a DX-500 system (Dionex, Salt Lake City, UT, USA) by gradient separation with a Dionex IonPac AS11 column as described by Gálvez et al. (2005).

Amino acid analysis

The samples were extracted in 80% ethanol and the extracts partitioned by ion exchange chromatography into neutral, basic, and acidic fractions as previously described (Madore, 1990). The basic fractions were taken to dryness, re-suspended in 100 µl of a drying reagent consisting of triethylamine: absolute ethanol: water (1:1:1, by vol.) and dried again. The amino acids were converted to their PITC derivatives, and analysed as previously described (Mitchell and Madore, 1992).

Osmotic contribution of solutes

Concentration of soluble sugars, inorganic ions, and free amino acids were calculated for symplastic water volume at full turgor, according to the different fractions in control (0 mM mannitol) and stressed leaves (50 mM mannitol), estimated by the pressure-volume technique (Patakas et al., 2002). These concentrations were used to estimate the contribution of each solute to osmotic adjustment, assuming that 40 µmol. g^{-1} of symplastic water corresponds to 0.1 MPa (Patakas et al., 2002).

Statistical analysis

To calculate the number of leaves and dry weight, 8 replicates were used per treatment. Data were subjected to analysis of variance and comparison of means by ANOVA test. The analysis of variance and the lowest standard deviation (LSD) of the means were used to determine the significance ($P < 0.05$) between lines.

RESULTS

Morphological responses to osmotic stress

As shown in Figure 1, osmotic stress induced a signify-cant decrease in growth, leaf area and leaf number either in flamingo or in coco blanc, as compared with control. This decrease being higher in the susceptible variety, coco blanc. By this time, osmotic stress resulted in signifi-cant reduction in leaf water status (Table 1). Indeed, water potential was reduced by 25 and 80% in flamingo and coco blanc leaves, respectively, as compared with control values.

Stomatal frequency

The data in Figure 2 indicated differences in the stomatal frequency (number cm^{-2} leaf surface) in flamingo and coco blanc. Under control and stressed conditions, there was a high frequency of stomata on the abaxial surface and a low frequency on the adaxial surface in both flamingo and coco blanc (Figure 2). Observations also showed that the frequency of stomata on both the abaxial and adaxial surfaces was slightly higher in flamingo than in coco (Figure 2) mainly in controls. Furthermore stomata seemed to be closed in treated coco leaves while they looked closed in treated leaves of flamingo plants mainly in the abaxial surface.

Stomatal conductance

Stomatal conductance may have been influenced by stomatal frequency. In controls, both cultivars showed

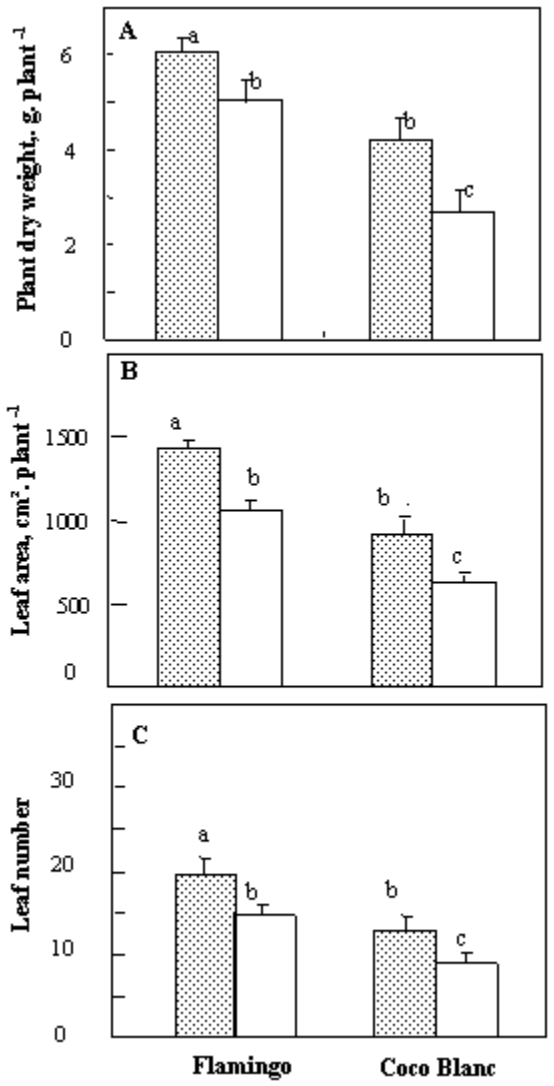

Figure 1. Effects of osmotic stress on plant biomass, leaf area and leaf number of Flamingo and Coco blanc plants. A: plant dry weight; B: Leaf area; C: Leaf number. Values represent mean ± SE (n = 8). Numbers followed by a different letter within a column are significantly different at $P \leq 0.05$ according to LSD analysis.

similar values reaching almost 0, 8 mol H_2O m^{-1} S^{-1} (Figure 3). Mannitol treatment induced significant reductions in this parameter in both lines. However, flamingo leaves exhibited the lowest stomatal conductance under stressed conditions, reaching ~50% of reduction as compared to its respective control (Figure 3).

Pressure-volume analysis

The water relations parameters obtained from pressure-volume curves indicated that, in both lines, stressed leaves exhibited lower osmotic potential at full turgor (Ψ^{100}) and this at turgor loss point (Ψ^{0}), and also a higher symplastic portion of the total water content than the stressed leaves (Table 1). The bulk modulus of elasticity was higher in stressed plants than in mannitol-stressed plant. The degree of osmotic adjustment ($\Delta\Psi$) was higher in coco blanc leaves, either Ψ^{100} or Ψ^{0} were considered (Table 1).

Leaf carbohydrate-amino acid content

When compared to control leaves, total soluble carbohydrate concentration, expressed on the basis of symplastic water content at full turgor, appeared to be significantly higher in stressed leaves. Greater carbohydrate content was measured in coco blanc leaves in stressed plants (Table 2). Furthermore, sugars content was much higher in stressed leaves in both lines. By the time, mannitol concentration which was not detected in controls, increased significantly in stressed leaves of both lines. Higher mannitol concentration was measured in coco blanc under osmotic stress. Starch levels increased significantly in stressed leaves, representing about three to ten fold than those of controls, in Flamingo leaves and coco blanc ones, respectively. The differences between lines and osmotic treatments were statically significant. Free amino acid, contents exhibited also significant differences between osmotic treatments (Table 2). Decreased levels were measured in Flamingo after mannitol supply, while in coco blanc leaves, it was essentially constant

Leaf organic acids and inorganic ions

Under control conditions, the accumulation of malate, citrate and oxalate was similar in leaves of both lines. However, ketoglutarate and succinate were more accumulated in coco leaves. In mannitol-treated leaves, flamingo showed a significant decrease in oxalate content. Nevertheless, coco blanc leaves showed a considerable decrease in malate and oxalate, while increased contents of ketoglutarate and succinate were observed. The exception to this was citrate content which remained equal to control values. It should be noted that malate was the major organic acid in both varieties either in control conditions or after mannitol supply. Decreased contents of inorganic ions were measured in both lines under osmotic stressed conditions (Table 2).

Contribution of solute to osmotic adjustment

The contribution of sugars to the osmotic potential at full turgor (Ψ^{100}) was about 20% in flamingo and about 40% in coco blanc leaves, mannitol was the main sugar in osmotic adjustment, while the contribution of amino acids was lower in both lines reaching only 5% in flamingo and

Table 1. Water potential and water relation parameters as derived from P-V curve analysis.

Line	Treatment	Ψ^{100} (MPa)	Ψ^{0} (MPa)	AWC (%)	RWC_{0} (%)	ε	Ψ_{W} (MPa)
Flamingo	Control	-1 ± 0.1 a	-1.58 ± 0.1 a	9 ± 0.1 c	79.66 ± 0.1 a	0.06 ± 0.1 c	-0.3 ± 0.1 b
	Treated	-1.25 ± 0.1 b	-2.5 ± 0.1 b	62 ± 0.1 a	74.66 ± 0.1 a	1.04 ± 0.1 a	-0.4 ± 0.1c
Coco blanc	Control	-1.25 ± 0.1 b	-1.87 ± 0.1 a	16 ± 0.1 d	76.00 ± 0.1 a	0.12 ±0.1b	-0.1 ± 0.1a
	Treated	-1.66 ± 0.1 c	-3.33 ± 0.1 c	46 ± 0.1 b	69.33 ± 0.1 b	1.10 ± 0.1a	-0.5 ± 0.1d

Values represent the mean ± SE (n = 6). Numbers followed by a different letter within a row are significantly different at P ≤ 0.05 according to LSD analysis.

2.5% in coco. Furthermore, low mass antioxidant molecules represent about 1.5 in flamingo and about 2% in coco. Both organic and inorganic ion concentrations exhibited negative values indicating their non implication in osmotic adjustment in common bean leaves under osmotic stressed conditions. Thus, it seems that osmotic adjustment in stressed common bean leaves was mainly due to the accumulation of carbohydrates.

DISCUSSION

Morphological responses of common bean lines grown under osmotic stress

Water stress tolerance is seen in almost all plant species but its extent varies from species to species (Sinclair and Serraj, 1996; Chaitanya et al., 2003). Such diversity can also be intra specific (Ramos et al., 2003, Wentworth et al., 2006). Flamingo and coco blanc are two contrasting lines of common bean which have different sensitivities to osmotic stress under controlled conditions (Sassi et al., 2008). In the present study, the morphological differences in *P. vulgaris* due to exposure osmotic stress conditions were in terms of reduction in total biomass, leaf area and leaf number (Figure 1). Reduction in the biomass was indicative of severe growth limitations (Bhatt and Srinivasa, 2005).

Water relations of common bean lines under osmotic stress

The analysis of P-V curve data suggested a development of an active osmotic adjustment in both cultivars, in response to osmotic-water stress. The decrease of osmotic potential at full hydration in water deficient leaves corresponded to the degree of osmotic adjustment. The relation between the decrease of Ψ and osmotic adjustment has already been documented in several studies (Martinez et al., 2004; Stoyanov, 2005). In the present study the degrees of osmotic adjustment was intra species dependent. Values of osmotic adjustments (0.25 - 0.41) reported in this work (Table 1), are within those reported for others studies on young *P, vulgaris* plants submitted to water stress (Stoyanov, 2005). On the other hand, water stress also induced a significant decrease in leaf cell elasticity (that is, the bulk modulus of elasticity, ε_{max}, increased), which caused the turgor loss point to move to a higher lower RWC (Table 1). 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., 2006). An increase in ε_{max} (stiffness) is expected when the cell walls become more rigid or thicker. This increase is observed to be higher in the tolerant line (flamingo) as shown in Table 1. The greater stiffness of the cell wall, given an equal decrease in cell volume, will be responsible for the drop in water potential. These drought related changes in tissue elasticity of both lines lead to an alteration of the relationship between turgor pressure and cell volume which might contribute to drought tolerance. Indeed in species which achieves osmotic adjustment through the accumulation of large amounts of solutes, a rigid cell may be more effective for maintaining cell/tissue integrity on re-hydration after a period of stress (Patakas et al., 2002). The overall effect of these changes was the maintenance of a positive cell turgor at lower RWC. Moreover, a positive turgor is required for cell expansion and growth under water-stress conditions (Martínez et al., 2004; Patakas et al., 2002) and is crucial for the continuation of many biochemical and physiological processes of plants (Martínez et al., 2004). Indeed, the maintenance of turgor during changes in plant water status associated with osmotic adjustment preserve the metabolic processes of the plant and contributes to limiting stress effects on plant growth (Martínez et al., 2004). In addition to elastic adjustments, the observed increase in water partitioning between symplastic and apoplastic water fractions had also a significant role in turgor maintenance (Maury et al., 2000).

Solute accumulation in common bean lines under osmotic stress

In this study, leaf sugars concentrations increased in both lines (Table 2). The contribution of soluble sugars to osmotic adjustment reached about 10 and 20% in flamingo and coco blanc, respectively (Table 3). Sugars accumulation in plants in response to water stress is quite well documented and is considered to play an

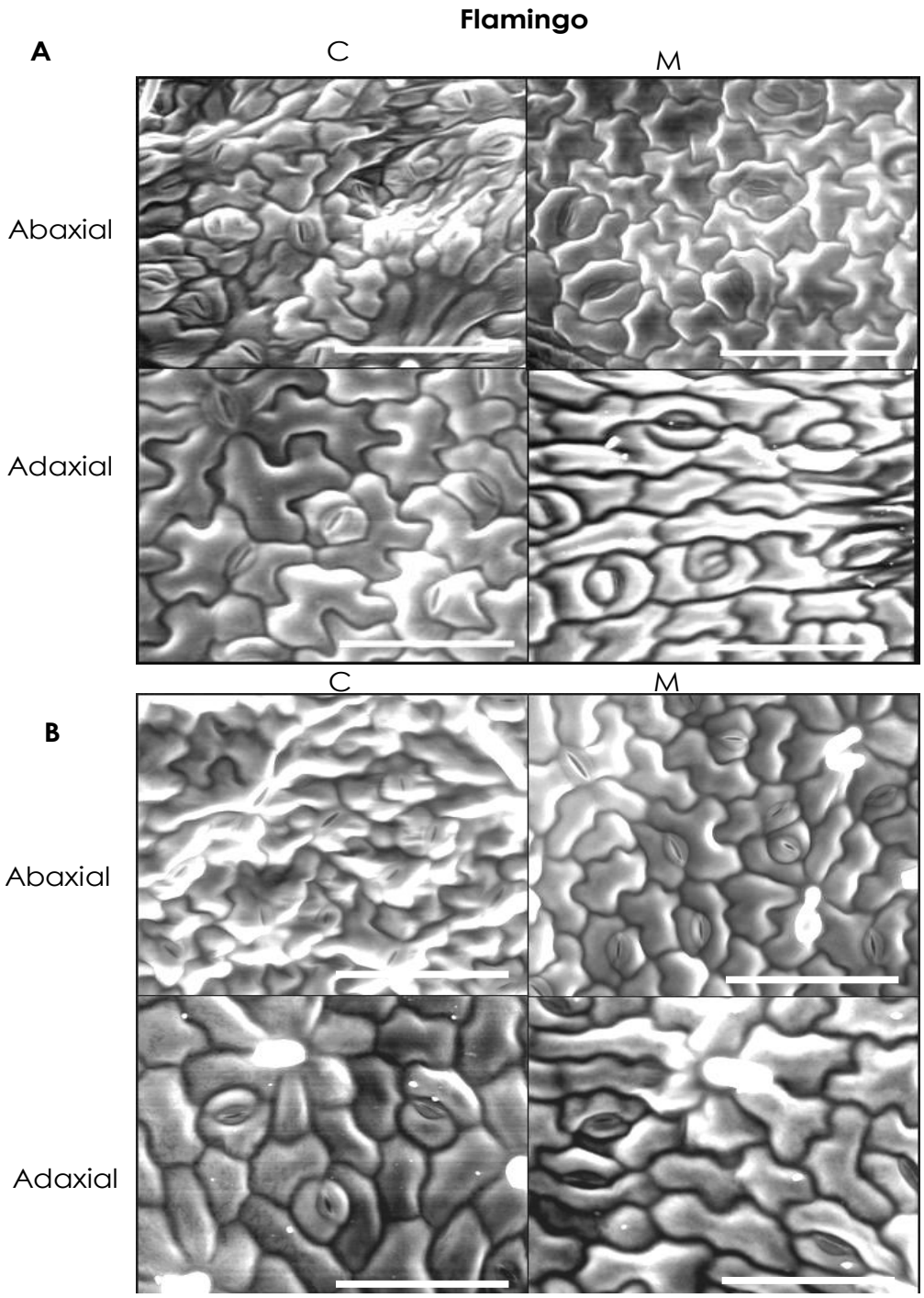

Figure 2. SEM micrographs of stomates observed on abaxial and adaxial leaf surface from control (C) and mannitol treated (M) common bean plants. Flamingo (A) and Coco blanc (B). (**A**) Control flamingo abaxial surface, mannitol treated Flamingo abaxial surface, control flamingo adaxial surface, mannitol treated flamingo adaxial surface. (**B**) Control coco abaxial surface, mannitol treated coco abaxial surface, control coco adaxial surface, mannitol treated coco adaxial surface. White bars show scale = 100 µm.

important role in osmotic adjustment (Bajji et al., 2001), which leads to lowering leaf osmotic potential, thus maintaining the driving force for extracting soil water under water deficit conditions (Morgan, 1984; Sharp et al., 1990). The sensitive line accumulated more soluble sugars than the osmotic-stress tolerant line (Table 2).

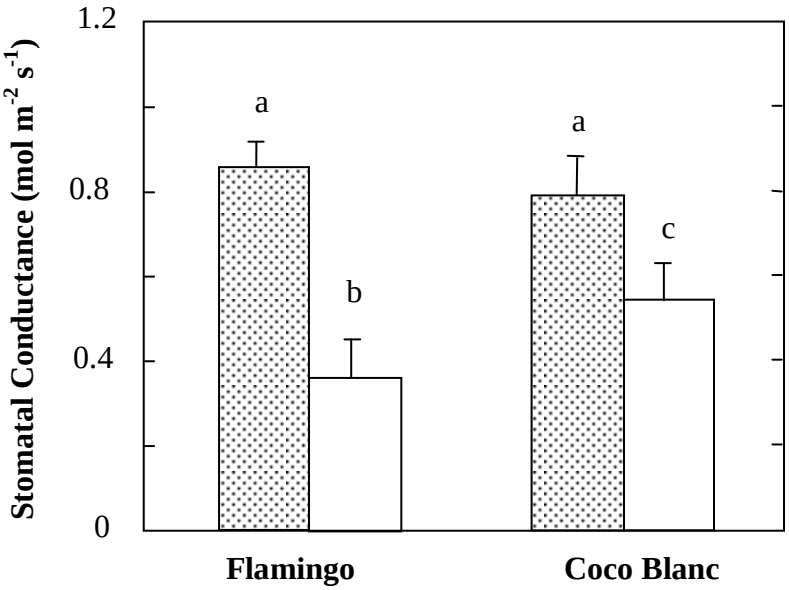

Figure 3. Effect of osmotic stress on stomatal conductance (mol m^{-2} s^{-1}) of control and osmotic-stressed bean leaves.

Table 2. Solute concentrations in leaves of control and stressed plants expressed on the basis of symplastic water content at full turgor.

Solute concentration (µmol. g^{-1} symplastic water)	Flamingo		Coco blanc	
	Control	Treated	Control	Treated
Glucose	0.21± 0.01 c	0.91± 0.O2 b	0.05±0.01d	2.89± 0.11 a
Fructose	0.14± 0.01 c	1.18± 0.03 a	0.09±0.01c	0.87± 0.01b
Sucrose	0.91± 0.13 c	2.26± 0.14 b	0.36±0.12d	3.32± 0.11 a
Mannitol	ND	6.24± 0.21 b	ND	25.38± 3.8 a
Total soluble carbohydrate	1.26± 0.16 c	10.59±3.21 b	0.50±0.21d	32.46± 9.1 a
Starch	21.3± 3.6c	65.9± 4.3 a	3.9±0.1d	40.2± 2.5 b
Total amino acids	7.35± 1.2 a	0.95± 0.13 b	1.2±0.6b	1.61± 0.2b
Total inorganic ions	0.7± 0.1 a	0.4± 0.07 b	0.9±0.1a	0.4± 0.08 b
Total organic acids	0.54± 0.3 a	0.47± 0.2 a	0.55± 0.3 a	0.29± 0.2b

ND denotes not detected compound. Values represent the mean ± SE (n = 6). Numbers followed by a different letter within a row are significantly different at P ≤ 0.05 according to LSD analysis.

Table 3. Contribution of starch, total soluble carbohydrate, total inorganic ions, total organic acids and total amino acids, at full turgor Ψ^{100}, to osmotic adjustment in stressed plants.

Parameters	Contribution to osmotic adjustment (%)	
	Flamingo	Coco blanc
Total soluble carbohydrate	22.33	39.5
Mannitol	18.24	25.47
Total amino acids	5.23	2.5
Total inorganic ions	-0.3	-0.3
Total organic acids	-0.06	-0.16

Nevertheless, the production of sufficient organic osmotica is metabolically expensive and potentially limits plant growth in this line by consuming significant quantities of carbon that could otherwise be used for growth (Greenway and Munns, 1980). Decreased starch levels when water availability is limited have been observed in leaves of different plant species. Nevertheless, in this study, the increase of starch content, in stressed common bean leaves (Table 2) strengthen the hypothesis of the unloading of sucrose and its conversion to starch witch has to be coordinated with the synthesis or the uptake of other cell components for the maintenance of a suitable turgor (Patrick, 1990), a mannitol scenario may be considered, as this polyol was easily available for cellular uptake in this experiment. Osmotic adjustment by the culture medium mannitol was also reported by Shabala et al. (2000).

The better osmotic stress tolerance in flamingo line seems to be associated with 1) Saving energy by accumulating less organic ions to adjust its osmotic potential which in turn enables it to use more carbon for maintaining adequate growth, 2) limiting mannitol uptake avoiding its toxicity at higher levels and 3) maintaining a small cell volume and large apoplastic water content (AWC) associated with decreased cell tissue elasticity thus providing an effective means of counteracting the negative effects of osmotic stress on water balance.

ACKNOWLEDGEMENT

This work was supported by the AQUARHIZ Project: “Modulation of plant-bacteria interactions to enhance tolerance to water deficit for grain legumes in the Mediterranean dry lands” PT6 Project INCO-CT-2004-509115, and by the Tunisian Ministry of Higher Education and Scientific Research (LR10CBBC02). Authors thank Gustavo Garijo, Arantzazu Ederra and Joseba Aldasoro from Dpto. CC. Medio Natural, Navarra for technical assistance.

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.

Bajji M, Lutts S, Kinet JM (2001). Water deficit effects on solute contribution to osmotic adjustment as a function of leaf ageing in three durum wheat (*Triticum durum* Desf.) cultivars performing differently in arid conditions. Plant Sci. 160: 669-681.

Bhatt RM, Srinivasarao NK (2005). Influence of pod load on response of okra towater stress. Indian J. Plant Physiol. 10: 54-59.

Blake TJ, Bevilacqua E, Zwiazek JJ (1991). Effects of repeated stress on turgor pressure and cell elasticity chages in black spruce seedlings. Can. J. For. Res. 21: 1329-1333.

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 (eds). Molecular Responses to Cold, Drought, Heat, and Salt Stress in Higher Plants. Landes RG Company, Austin, TX. pp. 29-62.

Chaitanya KV, Sundar D, Jutur PP, Ramachandrareddy A (2003). Water stress effects on photosynthesis in different mulberry cultivars. Plant Growth Regul. 40: 75-80.

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

Gálvez L, Gonzalez EM, Arrese-Igor C (2005). Evidence for carbon flux shortage and strong carbon/nitrogen interactions in pea nodules at early stages of water stress. J. Exp. Bot. 56: 2551-2561.

Greenway H, Munns R (1980). Mechanisms of salt tolerance in nonhalophytes. Ann. Rev. Plant Physiol. 31: 49-190.

Joly RJ, Zaerr JB (1987). Alteration of cell wall water content and elasticity in Douglas fir during periods of water deficit. Plant physiol. 83: 418-422.

Kiani PS, Talia P, Maury P, Grieu P, Heinz R, Perrault A (2007). Genetic analysis of plant water status and osmotic adjustment in recombinant inbred lines of sunflower under two water treatments. Plant Sci. 172: 773-787.

Kikuta SB, Richter H (1986). Graphical evaluation and partitioning of turgor responses to drought in leaves of durum wheat. Planta, 168: 36-42.

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

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

MacRae JC (1971). Quantitative measurement of starch in very small amounts of leaf tissue. Planta, 96: 101-108.

Madore M (1990). Carbohydrate metabolism in photosynthetic and non-photosynthetic tissues of variegated leaves of Coleus blumei Benth. Plant Physiol. 93: 617-22.

Marino D, González EM, Arrese-Igor C (2006). Drought effects on carbon and nitrogen metabolism of pea nodules can be mimicked by paraquat: evidence for the occurrence of two regulation pathways under oxidative stresses. J. Exp. Bot. 57: 665-673.

Martinez JP, Lutts S, Schanck A, Bajji M, Kinet JM (2004). Is osmotic adjustment required for water stress resistance in the Mediterranean shrub Atriplex halimus L? J. Plant physiol. 161: 1041-1051.

Maury P, Berger M, Mojayad F, Planchon C (2000). Leaf water characteristics and drought acclimation in sunflower genotypes. Plant Soil, 223: 153-160.

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.

Mitchell D, Madore M (1992). Patterns of assimilate production and translocation in muskmelon (*Cucumis melo* L.) II. Low temperature effects. Plant Physiol. 99: 966-971.

Morgan JM (1984). Osmoregulation and water stress in higher plants. Ann. Rev. Plant Physiol. 35: 299-319.

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.

Mustard J, Renault S (2004). Effects of NaCl on water relations and cell wall elasticity and composition of red-osier dogwood (*Cornus stolonifera*) seedlings. Physiol. Plant. 121: 265-271.

Nabil M, Coudret A (1995). Effects of sodium chloride on growth, tissue elasticity and solute adjustment in two *Acacia nilotica* subspecies. Physiol. Plant, 93: 217-224

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

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

Passioura JB, Fry SC (1992). Turgor and cell expansion: beyond the Lockhart equation. Aust. J. Plant Physiol. 19: 565-579.

Patakas A, Noitsakis B (1999). Osmotic Adjustment and Partitioning of Turgor Responses to Drought in Grapevines Leaves. Am. J. Enol. Vitic. 50: 76-80.

Patakas A, Nikolaou N, Zioziou E, Radoglou K, Noitsakis B (2002). The role of organic solute and ion accumulation in osmotic adjustment in drought-stressed grapevines. Plant Sci. 163: 361-367.

Patrick JW (1990). Sieve element unloading: cellular pathway, mechanism and control. Physiol. Plant, 78: 298-308.
Ramos MLG, Parsons R, Sprent JI, James EK (2003). Effect of water stress on nitrogen fixation and nodule structure of common bean. Pesq. Agro. Bras. 38: 339-347.
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.
Sassi S, Aydi S, Gonzalez EM, Abdelly C (2008). Osmotic stress affects water relations, growth, and nitrogen fixation in Phaseolus vulgaris plants. Acta physiol. Plant 30:441-449.
Scholander PF, Hammel HT (1965). Sap pressure in vascular plants. Science, 148: 339-346.
Shabala S, Babourina O, Newman I (2000). Ion-specific mechanisms of osmoregulation in bean mesophyll cells. J. Exp. Bot. 51: 1243-1253.
Sharp RE, Hsiao TC, Silk WK (1990). Growth of the maize primary root at low water potentials. II. Role of growth and deposition of hexose and potassium in osmotic adjustment. Plant Physiol. 93: 1337-1346.
Sinclair TR, Serraj R (1996). Processes contributing to N2 -fixation insensitivity to drought in the soybean cultivar Jackson. Crop Sci. 36: 961-968.
Stoyanov Z (2005). Effects of water stress on leaf water relations of young bean plants. J. Cent. Eur. Agric. 6: 5-14.
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.
Tonon G, Kevers C, Faivre RO, Graziani M, Gaspar T (2004). Effect of NaCl and mannitol iso-osmotic stresses on proline and free polyamine levels in embryogénic F*raxinus angustifolia* callus. J. Plant Physiol. 161: 701-708.
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.
Wu HRD, Spence IA (1985). Cell wall elasticity: Critique of the bulk elastic modulus approach and an analysis using polymer elastic principles. Plant Cell Environ. 8: 563-570.
Yadav SK, Jyothi LN, Maheswari M, Vanaja M, Venkateswarlu B (2005). Influence of water deficit at vegetative, Anthesis and grain filling stages on water relation and grain yield in sorghum. Indian J. Plant Physiol. 10: 20-24.
Zhu JK (2001). Plant salt tolerance. Trends Plant Sci. 6: 66-71.