was reported for wheat (Wakabayashi et al., 1997). Such a modification in the composition of the cell wall may be involved in turgor maintenance through changes in wall elasticity. The situation in this respect, however, is not clear. In species that show OA and accumulate significantly high solute concentrations, a rigid cell wall may be necessary to maintain cell/tissues integrity on rehydration following a period of stress, and rigid cell walls may facilitate the maintenance of lower water potential at any given volume better than elastic walls (Clifford et al., 1998). In contrast, woody species with elastic cell walls were reported to have a high inherent drought tolerance in the absence of OA because in this situation, turgor potential is maintained over a wide range of RWC (Fan et al., 1994).

It has been shown that, for a given plant material and a given water stress intensity, the extent of OA varies depending on external parameters such as light (Delpérée et al., 2003) or N nutritional status (Ashraf et al., 2001). Thus, at this stage, the absence of a close relationship between OA and water stress resistance estimated in terms of growth in *Atriplex halimus* is valid under our experimental conditions only.

In the present work, the experimental evidence indicates that proline is not involved in OA of water stressed *A. halimus* plants since its content in leaves was relatively low and was not modified in response to the water deficit, although leaf RWC and Ψs were significantly decreased. In salt stress conditions, Bajji et al. (1998) observed increased proline levels in leaves of *A. halimus*. This finding could suggest that alterations due to water deficit are less important than damages caused by salt or perhaps that Na^+ and Cl^- ions may have additional toxic effects directly triggering proline overproduction independent of the osmotic component of salt stress. An impact of specific ions on proline-synthesizing enzymes was indeed reported in some experimental systems (Rout and Shaw, 1998; Girija et al., 2002). It is interesting to note that seeds of Tensift were collected on a salt-affected site while those of Kairouan originated from a non-saline dry area. Seedlings from those populations did not differ for their abilities to accumulate proline under drought conditions in the absence of NaCl.

In contrast, glycinebetaine concentration increased in leaves of *A. halimus* in response to the water stress treatment. The accumulation of this solute has been frequently reported under saline and water stress conditions and has usually been considered to have important physiological roles in the osmoregulation of the cytoplasmic cell compartment, in protein protection, and in membrane stabilization (Sakamoto and Murata, 2000 and references therein). From our results, we estimated that the quantitative contribution of glycinebetaine to the OA^{acc} process in response to water stress at the whole leaf level was limited to less than 1%, regardless of the population. Under salinity conditions, total quaternary ammonium compound levels were found to increase in *A. halimus* leaves (Bajji et al., 1998), but once again, their estimated contribution to OA was very low (no more than 0.3%). However, because glycinebetaine, the major quaternary compound in chenopods, is predominantly localised in chloroplasts (Hanson et al., 1985; Rhodes and Hanson, 1993), the concentration inferred here greatly underestimates the levels in intact organelles. In photosynthetic systems, for instance, glycinebetaine efficiently protects various components of the photosynthetic machinery, including 1,5 bisphosphate carboxylase/oxigenase (Rubisco) and oxygen evolving photosystem II (PSII) complex, from salt-induced inactivation and dissociation into subunits (Papageorgiou and Murata, 1995; Allakhverdiev et al., 2003). Interestingly, the increase in glycinebetaine content was larger in Tensift, where photosynthesis activity was less affected by the water stress, than in Kairouan as was recently shown by Martìnez et al. (2003).

The accumulation of sugars in plants in response to water stress is also quite well documented and is considered to play an important role in OA (Kameli and Lösel, 1995; Hare et al., 1998; Bajji et al., 2001). In the case of *Atriplex halimus*, the leaf-soluble sugar concentrations increased in both populations at a rate closely corresponding to the decrease in leaf RWC (Figs. 3 and 4). From a quantitative point of view, the contribution of soluble sugars to OA would be significant and represent, if all soluble sugars were hexoses, 44% and 68% to OA^{acc} in Kairouan and Tensift, respectively, if expressed on a water content basis. However, the actual contribution of sugars to Ψs should be lower, since disaccharides should also be present. Decrease of starch levels when water availability is limited has been observed in leaves of many different plant species (Geigenberger et al., 1997; Geiger et al., 2000). The decrease in starch content could result from a decrease in synthesis, since CO_2 assimilation was reduced in response to water stress as shown by Martìnez et al. (2003). However, this reduction could also be caused by a stimulation of starch degradation, regulated by hydrolytic and/or phosphorolytic pathways (Geiger et al., 2000). For example, Todaka et al. (2000) observed that beta-amylase activity in cucumber cotyledons increased in response to PEG water stress.

In conclusion, this study demonstrates that OA is not an absolute pre-request for water stress resistance in *Atriplex halimus*. It also provides some evidence supporting the hypothesis that accumulation of sugars plays a preponderant role in the contribution to OA in this species while glycinebetaine plays a minor role in OA, but is probably associated with the protection of enzymes and cellular structures.

Acknowledgements

This work was supported by the EU (convention ERB IC18-CT98-0390). The authors wish to thank Prof. S. Bouzid (University of Tunis) and Prof. A. Benchaâbane (University of Marrakech) for kindly providing the seeds and Prof. P. Dutuit (University Paris-Sud) for stimulating discussions. Juan-Pablo Martìnez is also very grateful to the Université catholique de Louvain (Secrétariat à la Coopération) for the valuable financial support.

References

Allakhverdiev SI, Hayashi H, Nishiyama Y, Ivanov AG, Aliev JA, Klimov VV, Murata N, Carpentier R. Glycinebetaine protects the D1/D2/Cyt *b559* complex of photosystem II against photo-induced and heat induced inactivation. J Plant Physiol 2003;160:41–9.

Ashraf M, Shabaz M, Mahmood S, Rasul E. Relationships between growth and photosynthetic characteristics in pearl millet (*Pennisetum glaucum*) under limited water deficit conditions with enhanced nitrogen supplies. Belg J Bot 2001;134:131–44.

Bajji M, Kinet JM, Lutts S. Salt stress effects on roots and leaves of *Atriplex halimus* L. and their corresponding callus cultures. Plant Sci 1998;137:131–42.

Bajji M, Lutts S, Kinet JM. 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 2001;160:669–81.

Bates LS, Waldren RP, Teare ID. Rapid determination of free proline for water stress studies. Plant Soil 1973;39:205–7.

Bianchi G, Gamba A, Limiroli R, Pozzi N, Elster N, Salamini F, Bartels D. The unusual sugar composition in leaves of the resurrection plant *Myrothamnus flabellifolia*. Physiol Plant 1993;87:223–6.

Blum A. Osmotic adjustment and growth of barley genotypes under drought stress. Crop Sci 1989; 29:230–3.

Blum A, Munns R, Passioura JB, Turner NC, Sharp RE, Boyer JS, Nguyen HT, Hsiao TC. Genetically engineered plants resistant to soil drying and salt stress: how to interpret osmotic relations? Plant Physiol 1996;110:1051–3.

Bohnert HJ, Jensen RG. Strategies for engineering water-stress tolerance in plants. Trends Biotech 1996;14: 89–97.

Chimenti CA, Pearson J, Hall AJ. Osmotic adjustment and yield maintenance under drought in sunflower. Field Crop Res 2002;10:235–46.

Clifford SC, Arndt SK, Corlett JE, Joshi S, Sankhla N, Popp M, Jones HG. The role of solute accumulation, osmotic adjustment and changes in cell wall elasticity in drought tolerance in *Ziziphus mauritiana* (Lamk.). J Exp Bot 1998;49:967–77.

Colmer TD, Epstein E, Dvorak J. Differential solute regulation in leaf blades of various ages in salt-sensitive wheat and a salt-tolerant wheat x *Lophopyrum elongatum* (Host) A Löve amphiploid. Plant Physiol 1995;108:1715–24.

Crowe JH, Hoekstra FA, Crowe LM. Anhydrobiosis. Annu Rev Physiol 1992;54:579–99.

Delpérée C, Kinet JM, Lutts S. Low irradiance modifies the effect of water stress on survival and growth-related parameters during the early developmental stages of buckwheat (*Fagopyrum esculentum*). Physiol Plant 2003;119:1–10.

Fan S, Blake TJ, Blumwald E. The relative contribution of elastic and osmotic adjustments to turgor maintenance in woody species. Physiol Plant 1994;90:408–13.

Geigenberger P, Reimholz R, Geiger M, Merlo L, Canale V, Still M. Regulation of sucrose and starch metabolism in potato tubers in response to short-term water deficit. Planta 1997;201:501–18.

Geiger DR, Servaites JC, Fuchs MA. Role of starch in carbon translocation and partitioning at the plant level. Aust J Plant Physiol 2000;27:571–82.

Gibon Y, Bessieres MA, Larher F. Is glycinebetaine a non-compatible solute in higher plants that do not accumulate it? Plant Cell Environ 1997;20:329–40.

Girija C, Smith BN, Swamy PM. Interactive effects of sodium chloride and calcium chloride on the accumulation of praline and glycinebetaine in peanut (*Arachis hypogaea* L.). Environ Exp Bot 2002;47:1–10.

Girma FS, Krieg DR. Osmotic adjustment in sorghum. I. Mechanisms of diurnal osmotic potential changes. Plant Physiol 1992;99:577–82.

Guo Y, Zhang L, Xiao G, Chen SY. Expression of the BADH gene and salinity tolerance in rice transgenic plants. Sci China (Ser C) 1997;27:151–5.

Hanson AD, May AM, Grumet R, Bode J, Jamieson GC, Rhodes D. Betaine synthesis in chenopods: localization in chloroplasts. Proc Natl Acad Sci USA 1985;82:3678–82.

Hare PD, Cress WA, Van Staden J. Dissecting the roles of osmolyte accumulation during stress. Plant Cell Environ 1998;21:535–53.

Ingram J, Bartels D. The molecular basis of dehydration tolerance in plants. Annu Rev Plant Physiol Plant Mol Biol 1996;47:377–403.

Jia GX, Zhu ZQ, Chang FQ, Li YX. Transformation of tomato with the BADH gene from *Atriplex* improves salt tolerance. Plant Cell Rep 2002;21:141–6.

Jones GP, Naidu BP, Starr RK, Paleg LG. Estimates of solutes accumulating in plants by ^{1}H nuclear resonance spectroscopy. Austral J Plant Physiol 1986; 13:649–58.

Kameli A, Lösel DM. Contribution carbohydrates and other solutes to osmotic adjustment in wheat leaves under water-stress. J Plant Physiol 1995;145:363–6.

Kramer PJ, Boyer JS. Roots and root systems. In: Kramer PJ, editor. Water relations of plants and soils. London: Academic Press; 1995. p. 115–65.

Martìnez JP. Mechanisms of resistance to water stress at plant, cellular levels in *Atriplex halimus* L. PhD thesis, Université catholique de Louvain, Louvain-la-Neuve, Belgium, 2001. 224pp.

Martìnez JP, Ledent JF, Bajji M, Kinet JM, Lutts S. Effect of water stress on growth, Na^+ and K^+ accumulation and water use efficiency in relation to osmotic adjustment in two populations of *Atriplex halimus*. Plant Growth Regul 2003;41:63–73.

McCready R, Guggolz J, Silviera V, Owens HS. Determination of starch and amylose in vegetables. Anal Chem 1950;22:1156–8.

McCue KF, Hanson AD. Drought and salt tolerance: towards understanding and application. Trends Biotech 1990;8:358–62.

Mullet JE, Whitsitt MS. Plant cellular responses to water deficit. Plant Growth Regul 1996;20:119–24.

Nonami H, Wu Y, Boyer JS. Decreased growth-induced water potential: a primary cause of growth inhibition at low water potentials. Plant Physiol 1997;114:501–9.

Nonami H. Plant water relations and control of cell elongation at low water potentials. J Plant Res 1998;111:373–82.

Papageorgiou GC, Murata N. The unusually strong stabilizing effects of glycinebetaine on the structure and function of the oxygen-evolving photosystem-II complex. Photosynthet Res 1995;44:243–52.

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

Rathinasabapathi B. Propionate, a source of β-alanine, is an inhibitor of β-alanine methylation in *Limonium latifolium*, Plumbaginaceae. J Plant Physiol 2002; 159:674.

Rhodes D, Hanson AD. Quaternary ammonium and tertiary sulfonium compounds in higher-plants. Annu Rev Plant Physiol Plant Mol Biol 1993;44:357–84.

Rout NP, Shaw BP. Salinity tolerance in aquatic macrophytes: probable role of proline, the enzymes involved in its synthesis and C_4 type of metabolism. Plant Sci 1998;136:121–30.

Sakamoto A, Murata N. Genetic engineering of glycinebetaine synthesis in plants: current status and implication for enhancement of stress tolerance. J Exp Bot 2000;51:81–8.

Shen YG, Du BX, Zhang WK, Zhang JS, Chen SY. *AhCMO*, regulated by stresses in *Atriplex hortensis*, can improve drought tolerance in transgenic tobacco. Theor Appl Genet 2002;105:815–21.

Todaka D, Matsushima H, Morohashi Y. Water stress enhances beta-amylase activity in cucumber cotyledons. J Exp Bot 2000;51:739–45.

Trossat C, Rathinasabapathi B, Weretilnyk EA, Shen TL, Huang ZH, Gage DA, Hanson AD. Salinity promotes accumulation of 3-dimethylsuflfoniopropionate and its precursor S-methylmethionine in chloroplasts. Plant Physiol 1998;116:165–71.

Wakabayashi K, Hoson T, Kamisaka S. Changes in amounts and molecular mass distribution of cell-wall polysaccharides of wheat (*Triticum aestivum* L) coleoptiles under water stress. J Plant Physiol 1997; 151:33–40.

Wang ZL, Huang BR, Xu QZ. Effects of abscissic acid on drought responses of Kentucky bluegrass. J Am Soc Hort Sci 2003;128:36–41.

Yemm EW, Willis J. The estimation of carbohydrates in plant extracts by anthrone. Biochem J 1954;57: 508–14.

Zhang J, Nguyen HT, Blum A. Genetic analysis of osmotic adjustment in crop plants. J Exp Bot 1999;50:291–302.