PHOTOSYNTHETICA 40 (4): 615-620, 2002

Gas exchange characteristics and water relations in some elite okra cultivars under water deficit

M. ASHRAF*,***, M. ARFAN*, M. SHAHBAZ*, ASHFAQ AHMAD*, and A. JAMIL**

Department of Botany and Department of Biochemistry**, University of Agriculture, Faisalabad, Pakistan*

Abstract

Thirty-days-old plants of two cultivars of okra (*Hibiscus esculentus* L.), Sabzpari and Chinese-red, were subjected for 30 d to two water regimes (100 and 60 % field capacity). Leaf water potential and osmotic potential of both lines decreased significantly with the imposition of drought. Both the leaf pressure potential and osmotic adjustment were much lower in Chinese-red than those in Sabzpari. Chlorophyll (Chl) *b* content increased, whereas Chl *a* content remained unchanged and thus Chl *a/b* ratios were reduced in both lines. Drought stress also caused a significant reduction in net photosynthetic rate (P_N), transpiration rate (E), stomatal conductance (g_s), and water use efficiency (WUE) especially in cv. Sabzpari. The lines did not differ in intrinsic WUE (P_N/g_s) or intercellular/ambient CO_2 ratio. Overall, the growth of two okra cultivars was positively correlated with P_N, but not with g_s or P_N/E, and negatively correlated with osmotic adjustment.

Additional key words: chlorophyll; drought stress; *Hibiscus esculentus*; osmotic adjustment; photosynthetic rate; stomatal conductance; transpiration rate; water relations.

Introduction

Drought stress is one of major abiotic stresses limiting agricultural productivity throughout the world. Plants under drought stress exhibit a number of morphological, physiological, and biochemical responses. Photosynthetic rate is among the most common physiological responses, and this is changed due to closure of stomata and/or inhibition in the activity of enzymes involved in photosynthesis (*e.g.* Tabaeizadeh 1998). Determination of water relation components at the whole plant or cellular level is important for determination of resistance of species or cultivars to drought stress (Turner 1986). Osmotic adjustment is one of the most effective physiological phenomena involved in plant resistance to drought stress (Morgan 1984, Ashraf *et al.* 1992, Fukai and Cooper 1995, Morgan and Tan 1996, Bray 1997, Zhu *et al.* 1997, Ranjbarfordoei *et al.* 2000, Srinivasa Rao *et al.* 2000).

Okra (*Hibiscus esculentus* L.) is one of the major vegetable crops in many countries (Ashraf *et al.* 2003). Since it is a summer crop, occasional drought significantly reduces crop growth and pod yield. Little work has been done with this species with reference to its response to water limitation. Thus the major objective of the present study was to uncover the crop response to water deficit by examining its growth, water relations, and gas exchange.

Materials and methods

Seeds of two cultivars (Sabzpari and Chinese-red) of okra (*Hibiscus esculentus* L.) were obtained from the Director Vegetables, Ayub Agricultural Research Institute, Faisalabad, Pakistan. Sabzpari has been developed through selection and breeding at Ayub Agricultural Research Institute, Faisalabad, whereas the origin of Chinese-red is China. All seed samples were surface sterilised in 5 % sodium hypochlorite solution for 10 min before sowing. The experiment was carried out in a naturally-lit glasshouse in the Botanical Garden of the University of Agriculture, Faisalabad, Pakistan (latitude 31°30'N, longitude 73°10'E, altitude 213 m), where the average irradiance (PAR) measured at noon ranged from 929 to 1 760 μmol m^{-2} s^{-1}, day/night relative humidity was 28/54 %, and temperature 44/31 °C. In April 2000, 15 seeds of each cultivar were sown randomly about

---

Received 30 September 2002, *accepted* 25 November 2002.

[***] *Address for correspondence*: 51-C Sheikh Colony, ABC Road, Faisalabad, Pakistan; e-mail: ashrafm@fsd.paknet.com.pk

Abbreviations: Chl, chlorophyll; E, transpiration rate; g_s, stomatal conductance; P_N, net photosynthetic rate; WUE, water use efficiency.

5 mm deep in earthen pots (25 cm diameter × 25.5 cm length) which contained 10.0 kg sandy loam soil (pH = 7.76, electrical conductivity of the saturation paste = 1.40 dS m^{-1}). After the emergence of seedlings the plants were thinned to six in each pot. All the pots were irrigated for 30 d with normal irrigation water after which time drought was imposed by maintaining the soil moisture equivalent to 60 % field capacity, whereas the well-watered pots were maintained at full field capacity. The soil moisture was monitored daily by weighing the pots. The experiment was arranged in a completely randomised design with 5 replicates and two water regimes. Plants were harvested 30 d after the start of water deficit treatments.

For measuring leaf water potential, a fully expanded youngest leaf (second from the top) was excised from each plant at 08:00 and the leaf water potential measurements were made with a Scholander type pressure chamber (*Arimad-2, ELE International,* Tokyo, Japan). For measuring leaf osmotic potential, a proportion of the leaf used for water potential determination was frozen for two weeks, thawed, and the frozen sap was extracted by crushing the material with a metal rod. After centrifugation (8 000×*g*) for 4 min, the sap was used for osmotic potential determination in a vapour pressure osmometer (*Wescor 5520,* Logan, USA). Leaf pressure potential was calculated as the difference between leaf osmotic potential and water potential.

Chlorophyll (Chl) content was determined following the method of Arnon (1949). One gram of fresh leaves was taken from the plants and triturated in 80 % acetone. The absorbance of the extracts was measured at 645, 652, and 663 nm using a spectrophotometer *U-2000* (*Hitachi Instruments*, Tokyo, Japan).

Instantaneous measurements of net photosynthetic rate (P_N), transpiration rate (E), and stomatal conductance (g_s) were made on fully expanded youngest leaf of each plant (second leaf from top) using an open system portable infra-red gas analyser *LCA-4* (*Analytical Development Company*, Hoddesdon, England). Measurements were performed from 09:00 to 11:00 with the following conditions: molar flow of air per unit leaf area 408.5 mmol m^{-2} s^{-1}, atmospheric pressure 97.8 kPa, water vapour pressure inside chamber 1 120-1 220 Pa, PAR at leaf surface was maximum up to 1 280 μmol m^{-2} s^{-1}, temperature of leaf was maximum up to 41.4 °C, ambient temperature 32.3-37.9 °C, ambient CO_2 concentration 351.3 μmol mol^{-1}.

After all these measurements, the plants were harvested. Plant roots were removed carefully from the soil and then were washed for 2-3 min in distilled water. Plants were separated into shoots and roots. Fresh mass of shoots and roots of all the plant samples was recorded. Samples were then dried at 65 °C for one week and dry mass recorded.

Analysis of variance of data for all the parameters was computed using *COSTAT* computer package (*CoHort Software*, Berkeley, USA). The least significant differences between the mean values were calculated following Snedecor and Cochran (1980).

Results

Water deficit conditions (60 % of field capacity) imposed for a period of 30 d to 30 d-old plants of two lines of okra had a significant detrimental effect ($p < 0.001$) on shoot fresh and dry matters, shoot length, and plant leaf area (Fig. 1). Lines differed significantly for these variables ($p < 0.01$) except leaf area per plant. Reduction in shoot fresh and dry biomasses and shoot length in Sabzpari due to drought stress was larger than in Chinese-red (Fig. 1).

Leaf water potential and osmotic potential of both cultivars decreased significantly due to water deficit. Chinese-red had lower leaf water potential as compared to Sabzpari under both well-watered and water limited conditions (Fig. 2). Marked reduction in osmotic potential was observed in Sabzpari under drought stress (Fig. 2). Osmotic adjustment was calculated by subtracting the osmotic potential values at water deficit from those of well-watered plants. Thus Sabzpari had considerably higher osmotic adjustment as compared to Chinese-red. Similar was the pattern of maintenance of pressure potential by the two cultivars (Fig. 2).

Drought stress did not significantly affect Chl *a* content in both cultivars (Fig. 3). In contrast, water deficit caused a marked increase in Chl *b* in both cultivars, however, the increase was slightly higher in Sabzpari than that in Chinese-red. Chl *a/b* ratios decreased significantly in both cultivars under water deficit, but the difference between cultivars was non-significant (Fig. 3).

Watering regime significantly affected P_N, g_s, E, and P_N/E of both okra cultivars (Fig. 4), but in contrast, there was no significant effect of watering on intrinsic water use efficiency (P_N/g_s) and sub-stomatal CO_2/ambient CO_2 ratio (C_i/C_a). On the basis of percent of control values, Chinese-red was better in P_N (50.2 %) as compared to Sabzpari (26.7 %).

A significant reduction in E and g_s was observed in both cultivars under water deficit (Fig. 4). Cultivars differed significantly in these attributes mainly due to large difference in the two cultivars under well-watered conditions. P_N/E decreased considerably in both cultivars due to water deficit, but the decrease was more marked in Chinese-red than in Sabzpari (Fig. 4). However, P_N/g_s and C_i/C_a remained unaffected by drought stress and the cultivars did not differ significantly in these attributes (Fig. 4).

Discussion

Chinese-red was superior to Sabzpari in tolerating drought stress. Both the cultivars are high yielding under normal soil and environmental conditions but information on their degree of drought tolerance is not known.

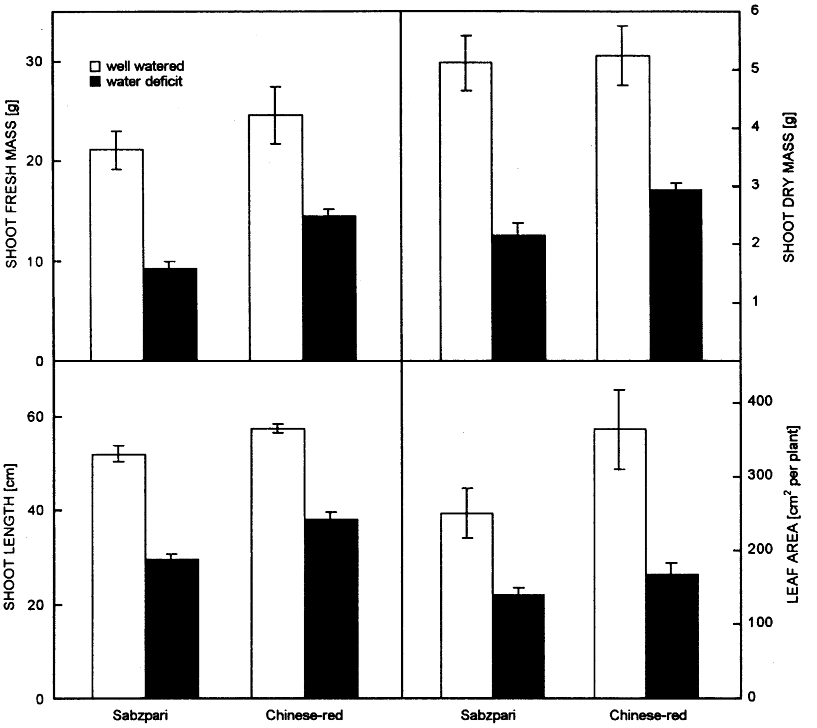

Fig. 1. Growth attributes of two *Hibiscus* cultivars when 30-d-old plants were subjected for 30 d to water deficit. Means ± SE.

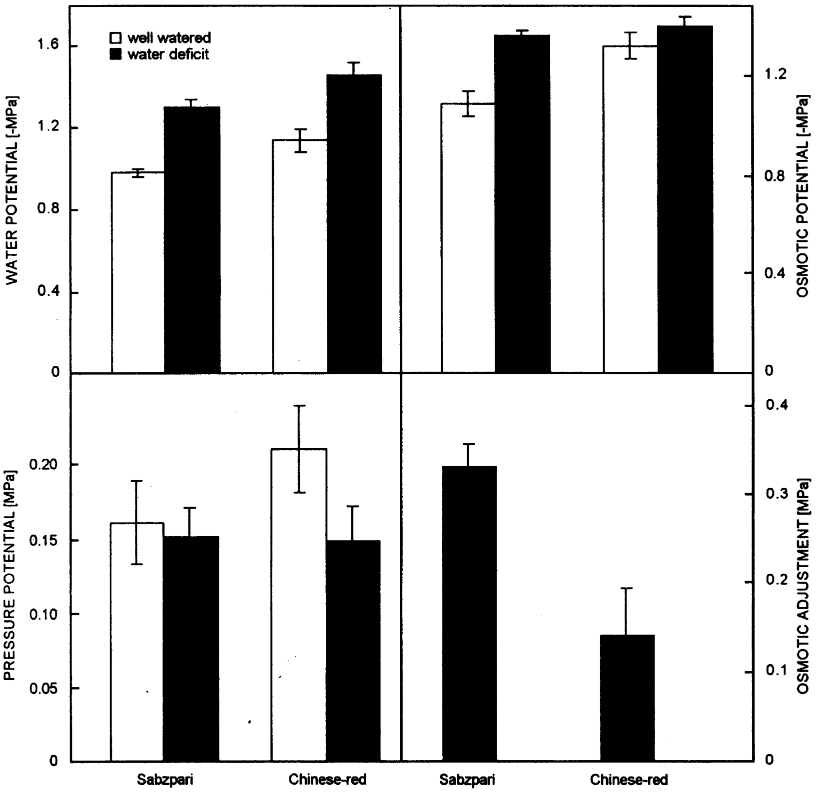

Fig. 2. Water relations of two *Hibiscus* cultivars when 30-d-old plants were subjected for 30 d to water deficit. Means ± SE.

Of many physiological traits responsible for tolerance of plants to water deficit, osmotic adjustment is significant in regulating cellular metabolic activities in plants subjected to drought stress by adequate absorption of water from the growth medium thereby maintaining pressure potential (Morgan 1992, Wright *et al.* 1997, Serraj and Sinclair 2002). However, the drought tolerant Chinese-red had considerably lower capability of osmotic adjustment as compared to Sabzpari. Similar was the pattern of leaf pressure potential in the two cultivars. Such a lack of positive relationship of drought tolerance with osmotic adjustment in the two okra cultivars has been found in *Brassica carinata* (Ashraf and Sharif 1998), *Macroptilium atropurpureum* (Wilson *et al.* 1980), or *Brassica napus* and *B. juncea* (Niknam and Turner 1999). In addition, some other reports show no or little relationship between osmotic adjustment and yield or growth in different crop species (Shackel and Hall 1983, Munns 1988, Blum 1989).

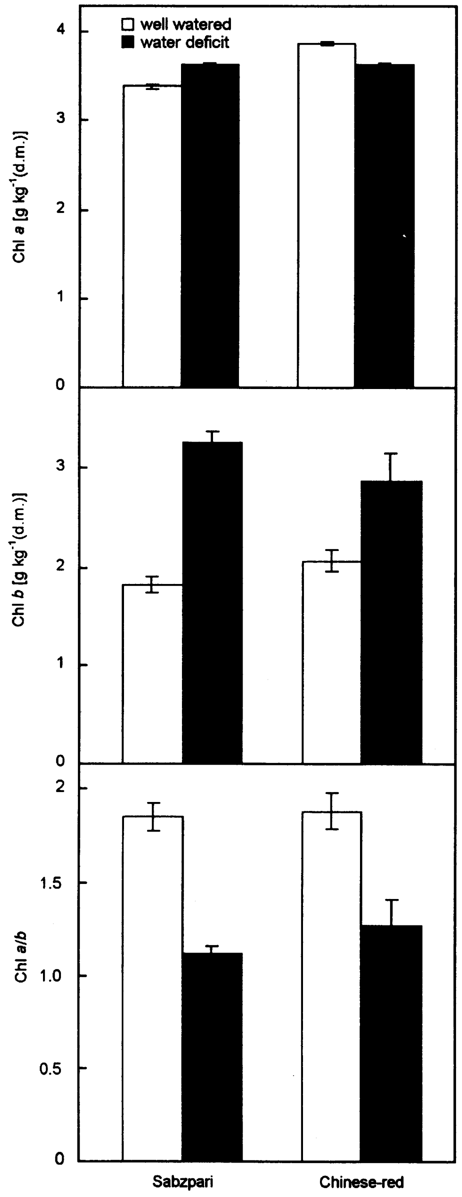

Fig. 3. Chlorophyll contents of two *Hibiscus* cultivars when 30-d-old plants were subjected for 30 d to water deficit. Means ± SE.

In plants, higher g_s increases CO_2 diffusion into leaf thereby favouring higher P_N. Higher P_N could in turn favour a higher biomass and higher crop yields (Taiz and Zeiger 1998, Ashraf *et al.* 2001). The results for P_N and g_s presented here for two lines of okra did not show a significant relationship because P_N was higher in Chinese-red as compared to that in Sabzpari, but in contrast, the lines did not differ significantly in leaf g_s under drought stress. Winter *et al.* (1988) considered that g_s was not a good selection criterion for drought resistance.

E and P_N/E declined considerably in both lines of okra under water limiting conditions, but the reduction in the former attribute was more in Sabzpari than that in Chinese-red, and the lines did not differ significantly in the latter parameter. This seemed to be due to similar pattern of reduction in P_N and E under water limiting conditions. WUE and P_N were not positively correlated in the two lines of okra. This is in contrast to what has already been found in the C_4 dicot *Amaranthus retroflexus* (Sage and Pearcy 1987) and sugarcane (Ranjith and Meinzer 1997).

Chl *b* content in both lines of okra increased markedly after the water deficit treatment, whereas Chl *a* remained unaffected in both lines. The differential pattern of accumulation of Chl *a* and Chl *b* caused a significant reduction in Chl *a/b* in both cultivars under water limiting regime. This is in contrast to some earlier studies reflecting that Chl *a/b* ratio under water deficit increases in most crop species (Garcia *et al.* 1987, Estill *et al.* 1991). Decline in Chl *a/b* under water deficit may have been due to a significant effect on LHC2 complexes containing most Chl *b* in the mature thylakoid membranes (Green 1988). Increase in Chl *b* contents in okra due to water stress may have been due to either enhanced synthesis or slow breakdown of Chl *b*. For instance, if slow breakdown of Chl *b* had taken place, it would have been due to slow activity of chlorophyllase, but this is in contrast to what has earlier been reported (Garcia *et al.* 1987, Majumdar *et al.* 1991).

In conclusion, the growth of two okra cultivars examined in the present study was strongly associated with P_N, but not with g_s or WUE under drought stress. There was a significant negative relationship between growth and osmotic adjustment. Since the conclusions drawn in the present study are based on only two cultivars, further work must be carried out involving a large number of cultivars/lines of okra to affirm such a negative relationship between growth and different physiological attributes including osmotic adjustment.

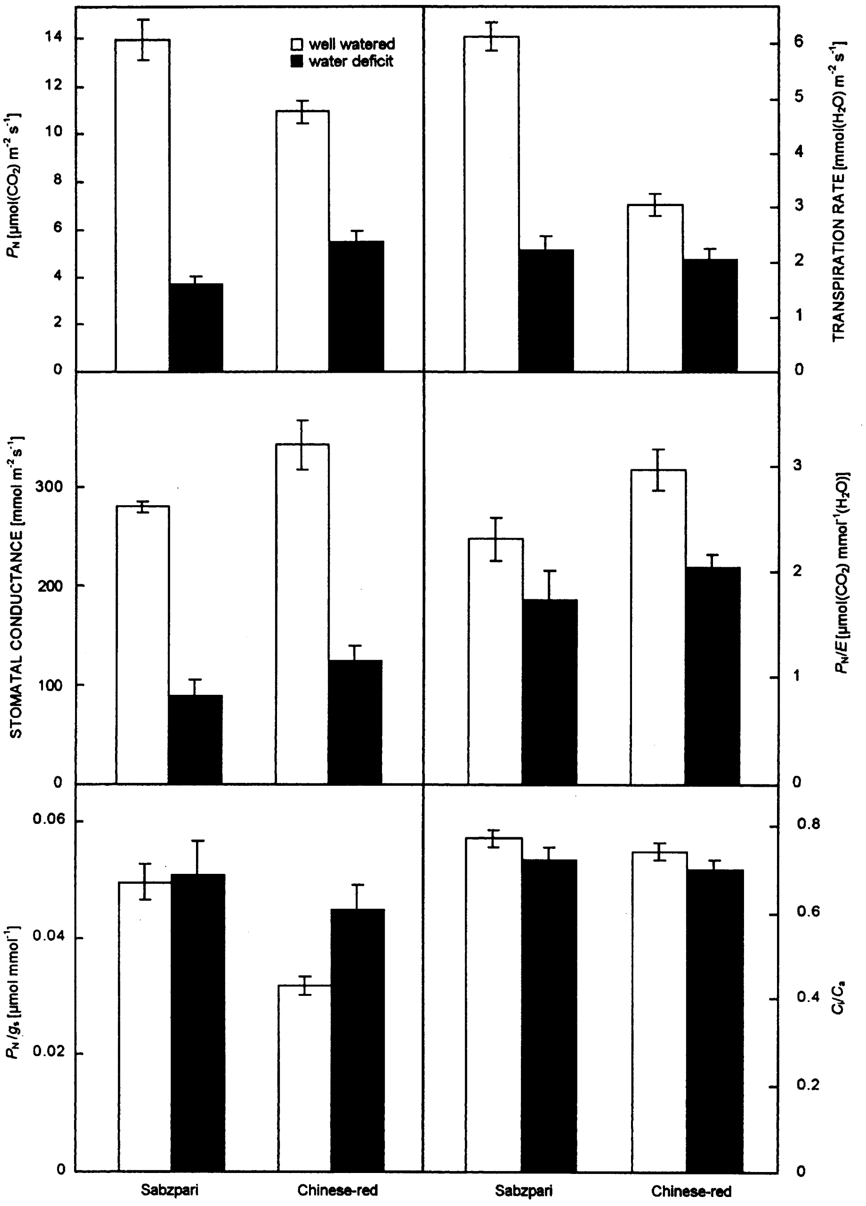

Fig. 4. Gas exchange parameters of two *Hibiscus* cultivars when 30-d-old plants were subjected for 30 d to water deficit. Means ± SE.

References

Arnon, D.I.: Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. – Plant Physiol. **24**: 1-15, 1949.

Ashraf, M., Arfan, M., Ahmad, A.: Salt tolerance in okra: ion relations and gas exchange characteristics. – J. Plant Nutr. (in press), 2003.

Ashraf, M., Bokhari, M.H., Chishti, S.N.: Variation in osmotic adjustment of accessions of lentil (*Lens culinaris* Medic.) in response to drought stress. – Acta bot. neerl. **41**: 51-62, 1992.

Ashraf, M., Shahbaz, 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. **134**: 131-144, 2001.

Ashraf, M., Sharif, R.: Assessment of inter-cultivar/line variation of drought resistance in a potential oil-seed crop, Ethiopian mustard (*Brassica carinata* A.Br.). – Arch. Acker. Pflanz. Boden. **43**: 251-165, 1998.

Blum, A.: Osmotic adjustment and growth of barley genotypes under drought stress. – Crop Sci. **29**: 230-233, 1989.

Bray, E.A.: Plant responses to water deficit. – Trends Plant Sci. **2**: 48-54, 1997.

Estill, K., Delaney, R.H., Smith, W.K., Ditterline, R.L.: Water relations and productivity of alfalfa leaf chlorophyll variants. – Crop Sci. **31**: 1229-1233, 1991.

Fukai, S., Cooper, M.: Development of drought-resistant cultivars using physio-morphological traits in rice. – Field Crops Res. **40**: 67-86, 1995.

Garcia, A.L., Torrecillas, A., León, A., Ruiz-Sánchez, M.C.: Biochemical indicators of the water stress in maize seedlings. – Biol. Plant. **29**: 45-48, 1987.

Green, B.R.: The chlorophyll-protein complexes of higher plant photosynthetic membranes or Just what green band is that? – Photosynth. Res. **15**: 3-32, 1988.

Majumdar, S., Ghosh, S., Glick, B.R., Dumbroff, E.B.: Activities of chlorophyllase, phosphoenolpyruvate carboxylase and ribulose-1,5-bisphosphate carboxylase/oxygenase in the primary leaves of soybean during senescence and drought. – Physiol. Plant. **81**: 473-480, 1991.

Morgan, J.M.: Osmoregulation and water stress in higher plants. – Annu. Rev. Plant Physiol. **35**: 299-319, 1984.

Morgan, J.M.: Osmotic components and properties associated with genotypic differences in osmoregulation in wheat. – Aust. J. Plant Physiol. **19**: 67-76, 1992.

Morgan, J.M., Tan, M.K.: Chromosomal location of a wheat osmoregulation gene using RFLP analysis. – Aust. J. Plant Physiol. **35**: 803-806, 1996.

Munns, R.: Why measure osmotic adjustment? – Aust. J. Plant Physiol. **15**: 717-726, 1988.

Niknam, S.R., Turner, D.W.: Physiological aspects of drought tolerance in *Brassica napus* and *B. juncea*. – In: New Horizons for an Old Crop. Proceedings of the 10th International Rapeseed Congress, Canberra 1999.

Ranjbarfordoei, A., Samson, R., Van Damme, P., Lemeur, R.: Effects of drought stress induced by polyethylene glycol on pigment content and photosynthetic gas exchange of *Pistacia khinjuk* and *P. mutica*. – Photosynthetica **38**: 443-447, 2000.

Ranjith, S.A., Meinzer, F.C.: Physiological correlates of variation in nitrogen-use efficiency in two contrasting sugarcane cultivars. – Crop Sci. **37**: 818-825, 1997.

Sage, R.F., Pearcy, R.W.: The nitrogen use efficiency of C_3 and C_4 plants. II. Leaf nitrogen effects on the gas exchange characteristics of *Chenopodium album* (L.) and *Amaranthus retroflexus* (L.). – Plant Physiol. **84**: 959-963, 1987.

Serraj, R., Sinclair, T.R.: Osmolyte accumulation: can it really help increase crop yield under drought conditions? – Plant Cell Environ. **25**: 333-341, 2002.

Shackel, K.A., Hall, A.E.: Comparison of water relations and osmotic adjustment in sorghum and cowpea under field conditions. – Aust. J. Plant Physiol. **10**: 423-435, 1983.

Snedecor, G.W., Cochran, W.G.: Statistical Methods. 7th Ed. – Iowa State University Press, Ames 1980.

Srinivasa Rao, N.K., Bhatt, R.M., Sadashiva, A.T.: Tolerance to water stress in tomato cultivars. – Photosynthetica **38**: 465-467, 2000.

Tabaeizadeh, Z.: Drought-induced responses in plant cells. – Int. Rev. Cytol. **182**: 193-247, 1998.

Taiz, L., Zeiger, E.: Plant Physiology. 2nd Ed. – Sinauer Associates Publ., Sunderland 1998.

Turner, N.C.: Crop water deficits: a decade of progress. – Adv. Agron. **39**: 1-51, 1986.

Wilson, J.R., Ludlow, M.M., Fisher, M.J., Schulze, E.D.: Adaptation to water stress of leaf water relations characteristics of some tropical forage grasses and legume in a semi-arid environment. – Aust. J. Plant Physiol. **7**: 207-220, 1980.

Winter, S.R., Musick, J.T., Porter, K.B.: Evaluation of screening techniques for breeding drought-resistant winter wheat. – Crop Sci. **28**: 512-516, 1988.

Wright, P.R., Morgan, J.M., Jessop, R.S.: Turgor maintenance by osmoregulation in *Brassica napus* and *B. juncea* under field conditions. – Ann. Bot. **80:** 313-319, 1997.

Zhu, J.-K., Hasegawa, P.M., Bressan, R.A.: Molecular aspects of osmotic stress in plants. – Crit. Rev. Plant Sci. **16**: 253-277, 1997.