BIOLOGIA PLANTARUM 51 (1): 104-109, 2007

Expression of dehydrins under heat stress and their relationship with water relations of sugarcane leaves

A. WAHID* and T.J. CLOSE**

*Department of Botany, University of Agriculture, Faisalabad-38040, Pakistan**
*Department of Botany and Plant Sciences, University of California, Riverside, CA-92521-0124, USA***

Abstract

The heat stress-induced dehydrin proteins (DHNs) expression and their relationship with the water relations of sugarcane (*Saccharum officinarum* L.) leaves were studied. Sugarcane seedlings were subjected to heat stress (day/night temperature of 40/35 °C) under relative humidity 60/65 % to avoid aerial desiccation and determinations made at 4, 12, 24, 36, 48, 60 and 72 h. The leaves showed a sharp decline in the water and osmotic potentials, and relative water content during first 12 h of heat stress, but a regain in their values in 24 h. The pressure potential (ψ_p) decreased initially but increased later and approached control leaves. The increase in ψ_p was tightly correlated to the accumulation of free proline, glycinebetaine and soluble sugars, indicating their possible involvement in the osmotic adjustment under heat stress. Immunological detection revealed the expression of three DHNs with an apparent molecular mass of 21, 23 and 27 kDa under heat stress (48 to 72 h) and their expression was independent of the changes in the water relations of leaves.

Additional key words: compatible osmotica, heat shock proteins, osmotic adjustment, relative humidity, water potential.

Introduction

Heat stress is often defined as a rise in temperature beyond threshold level for a time sufficient to cause irreversible damage to plant development. It is simply a transient elevation in temperature usually about 10 to 15 °C above ambient (Buchanan *et al.* 2000). With a rise in temperature, there is an increase in metabolic activities leading to cellular protection which increases demand for resources (Rawson 1988, Shah and Paulsen 2003). Since leaf photosynthesis is directly impinged upon by heat stress (Camejo *et al.* 2005), a decline in this process limits the supply of photosynthates to keep pace with normal growth (Ebrahim *et al.* 1998, Karim *et al.* 2000). Resultantly, leaf number and its expansion rate, tillering, spike development and grain filling are markedly reduced (Fischer 1985, Fokar *et al.* 1998, Wahid and Shabbir 2005).

Elevated temperature results in rapid loss of water which may cause dehydration. It interacts with soil drought to affect cell and tissue water content and all components of water potential (Machado and Paulsen 2001). Barley seedlings subjected to heat stress initially indicated a severe depression in leaf water potential, which recovered slowly with the time (Wahid and Shabbir 2005). Sudden, not the gradual, heat stress greatly hampered the leaf relative water content and pressure potential. Plants gradually exposed to heat stress indicated an accumulation of several heat stable proteins, which appeared to be associated with heat tolerance in strawberry (Gulen and Eris 2003). Likewise, preconditioned tomato plants with heat indicated a better osmotic adjustment and maintained leaf water potential than non-preconditioned ones (Morales *et al.* 2003).

Most common molecular response of plants submitted to heat stress is the expression of heat shock proteins (HSPs), which have a fairly wide range of molecular masses (10 - 250 kDa). They associate to various cellular structures or organelles to provide protection and act as molecular chaperones (Schoffl *et al.* 1999, Sanmiya *et al.* 2004). The dehydrin proteins (DHNs) are among other proteins that are classified as a group of late embryogenesis abundant (LEA) proteins, referred to as LEA group II, and typically accumulate late in embryo-

Received 19 May 2005, *accepted* 7 January 2006.
Abbreviations: DHNs - dehydrin proteins; FP - free proline; GB - glycinebetaine; RWC - relative water content; SS - water soluble sugars; ψ_p - pressure potential; ψ_s - osmotic potential; ψ_w - water potential.
Acknowledgements: First author acknowledges the Ministry of Science and Technology, Government of Pakistan for postdoctoral award, Terrance Donovan for providing the sugarcane material and R.D. Fenton for excellent help in the laboratory work.
* Corresponding author; fax: (+92) 41 9200764, e-mail: drawahid2001@yahoo.com

genesis or in vegetative tissues in response to environmentally imposed dehydrative forces, such as drought, salinity and freezing (Close *et al.* 1997, Svensson *et al.* 2002). DHNs are water-soluble proteins, rich in glycine and charged polar amino acids, and free of cysteine and tryptophan. They display repeated sequence motifs. The highly conserved domain in all DHNs is a lysine-rich domain (K-segment) characterized by the consensus EKKGIMDKIKEKLPG. Other domains are a track of serine residues (S-segment), less conserved domains that are usually rich in polar amino acids (Ö-segments) and Y-segments with consensus T/VDEYGNP, located near the N-terminus (Yin *et al.* 2004). They are thought to protect the cellular membranes and organelles during cellular dehydration induced by salinity, water deficit and low temperature or in response to ABA treatment (Borovskii *et al.* 2002, Svensson *et al.* 2002, Koag *et al.* 2003, Goyal *et al.* 2005). There is no report of the expression of DHNs under heat stress, although some evidence exists on the accumulation of DHN transcripts in citrus under high temperature (Porat *et al.* 2004).

Sugarcane (*Saccharum officinarum* L.) is a major source of sucrose. As a C_4 plant, sugarcane has high temperature optimum for growth, but needs to be frequently irrigated (Qureshi *et al.* 2002). Albeit its origin from sub-tropics and tropics, sugarcane shows significant reduction in growth and sugar yield at supra-optimal temperature (Ebrahim *et al.* 1998, 1999, Robertson *et al.* 1998). It shows the expression of HSPs in the cultured cells as a heat tolerance response (Moisyadi and Harrington 1989), but the data on the expression of other stress-related proteins, particularly the DHNs is lacking.

Changes in the relative humidity (RH) of the air is one of the major factors that greatly modulate the high temperature tolerance ability of plant (Perdomo *et al.* 1996), low RH of the air results in rapid loss of water and causes a state of dehydration and disrupts membrane functions (Taiz and Zeiger 2002). Very few studies deal with high temperature stress in which RH of air has been monitored. Under such circumstances, it becomes hard to precisely determine the heat stress tolerance of any plant species. The objective of this study was to determine the short-term effect of heat stress on sugarcane to monitor *a*) DHNs expression, *b*) changes in the leaf water relations, and *c*) possible relation of DHNs expression with leaf osmotic potential when heat stress was the only variable and other conditions including RH were kept uniform both for control and stressed plants.

Materials and methods

Experimental details and growth conditions: Single noded sets of sugarcane (*Saccharum officinarum* L. cv. NCO-310) were sown in pots containing well-manured loam and kept in a greenhouse. Experimental design was completely randomized with three replications. The pots were watered every alternate day to keep the soil moisture up to required levels. Thirty day after sprouting one half of the pots were shifted to one growth chamber set at day/night temperature of 28/23 ± 1 °C (control) while the other half to another chamber that was brought to day/night temperature of 40/35 ± 1 °C (heat stress) in about 4 h. Other growth conditions in both the chambers were: soil moisture at field capacity, 13-h photoperiod, irradiance of 500 - 550 µmol m^{-2} s^{-1} and day/night RH 60/65 %. Samples were taken 4, 12, 24, 36, 48, 60 and 72 h after submitting the plants to heat stress.

Water relations: Leaf water potential (ψ_w) was determined with pressure chamber (*Soil Moisture Equipment Corp*., Santa Barbara, CA, USA). Frozen leaf tissues were thawed, sap expressed, centrifuged at 5 000 *g* and leaf osmotic potential (ψ_s) was determined with a vapour pressure osmometer (*Wescor 5100-C*, Logan, UT, USA). Leaf pressure potential (ψ_p) was computed as a difference of ψ_w and ψ_s. For the determination of RWC, the leaf discs were cut, determined for fresh mass (FM) and floated on distilled water in Erlenmeyer flasks. After 4 h the discs were taken out, excess water removed, determined for water saturated mass (TM) while their dry mass (DM) was taken after drying for 3 d in an oven. RWC was calculated using the formula:

RWC = [(FM-DM/TM-DM) × 100].

The free proline (FP) content was determined from the fresh leaves extracted with sulphosalicylic acid and the extracts reacted with acid-ninhydrin, as described by Bates *et al*. (1973). The contents of soluble sugars (SS) were determined from fresh leaf tissue that was chopped and extracted in water at 80 °C with continuous shaking for 4 h and filtered. An aliquot of the filtrate was reacted with anthrone reagent by heating in a water bath at 100 °C for 20 min and absorbance of coloured complex taken at 620 nm (Yoshida *et al.* 1976) using a spectrophotometer (*DU 650*, *Beckman*, Coulter, USA). The glycinebetaine (GB) content was determined using the method of Grieve and Grattan (1983). Leaf extracts prepared by vigorous shaking in 2 M H_2SO_4 were cooled and mixed with equal volume of periodide, vortexed and kept at 0 - 4 °C for 16 h. The mixture was centrifuged at 10 000 *g* at 4 °C for 15 min and aspirated the supernatant while cool. The periodide crystals were dissolved in 1,2-dichloroethane to take the absorbance at 365 nm.

Proteins extraction and immuno-blotting: The leaf tissue was ground in Tris-buffer (pH 7.2) in the presence of Cocktail protease inhibitor and centrifuged. Total amount of proteins in the supernatant was determined by Bradford assay. Heat stable proteins were obtained by heating an aliquot from above at 95 °C for 5 min,

centrifuged and determined from the supernatant. Proteins (5 μg per lane) were separated by SDS-PAGE using 13 % (m/v) acrylamide gels and before being electrophoretically transferred to nitrocellulose membrane (Close 1993). The membrane was blocked in 3 % (m/v) gelatin in Tris-buffered NaCl and then incubated with anti-peptide IgG (1 μg mg^{-1}) for 2 h at room temperature. Goat anti-rabbit IgG (GAR) conjugated to alkaline phosphatase was used as secondary antibody (1:1000 dilution) with membrane incubated for 45 min at room temperature. Alkaline phosphatase activity was detected by using NBT and BCIP as substrates (Harlow and Lane 1988).

Statistical analysis: Statistical analysis of the data was performed using *COSTAT* software. LSD values were determined after performing DMR test and significant levels of temperature treatments and time points were ascertained. Parallels were drawn between compatible osmolytes and ψ_p and RWC of leaves. Alphabets have been shown in the figures where there is an interaction of temperature treatments and time points.

Results

Data regarding leaf water relations revealed that heat stressed plants initially decreased leaf ψ_w, which attained a steady state after 12 h and slightly increased later on (Fig. 1). Leaf ψ_s also decreased and became steady with the passage of time, showing an interaction ($P < 0.001$) of temperature treatments and time points. Changes in leaf ψ_w and ψ_s were accompanied by an increase in the leaf ψ_p after initial slump, implying that plants were able to adjust osmotically (Fig. 1). Likewise, leaf RWC also indicated an initial loss but later increased almost approaching the control leaves (Fig. 1).

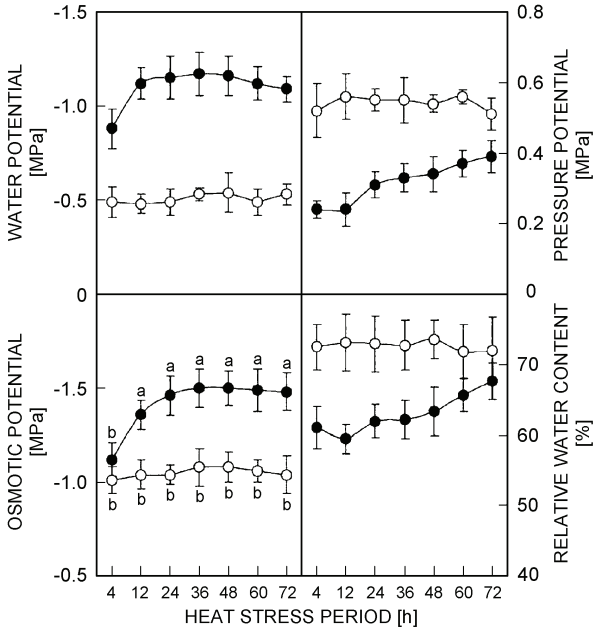

Fig. 1. Water relations parameters of sugarcane (clone NCO-310) leaves under heat stress (40/35 °C) measured after 4 to 72 h (*closed symbols*). Control plants (*open symbols*) were maintained at 28/23 °C. Means ± SD, $n = 3$.

Among the compatible osmotica, FP and GB of leaves indicated a time dependent sharp accumulation under heat stress, but not in leaves of control plants, with an interaction ($P < 0.001$) of temperature treatments and time points (Fig. 2). Although both the osmolytes indicated a similar pattern of accumulation, the leaf FP accumulation started earlier than GB accumulation and attained a steady state at 72 h. Leaf GB content showed a slight increase between 60 and 72 h in contrast to FP. Leaf SS although showed accumulation under heat stress with an interaction ($P < 0.001$) of temperature treatments and time, but this accumulation was lesser than both FP and GB (Fig. 2). Heat stable proteins, on the other hand, indicated a small but gradual increase due to heat stress ($P < 0.01$). However, there was no interaction of temperature treatments and time points. Parallels drawn between ψ_p and RWC of leaves and the contents of compatible osmotica indicated that both ψ_p and RWC were strongly correlated with the accumulation of compatible solutes under heat stress but not under control conditions (Table 1). This indicated the

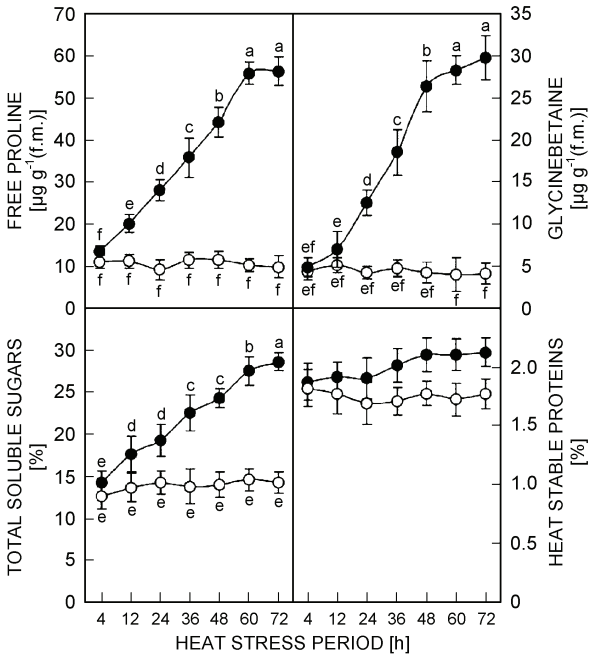

Fig. 2. Changes in some compatible osmolytes and heat stable proteins of sugarcane (clone NCO-310) leaves under heat stress (40/35 °C) measured after 4 to 72 h (*closed symbols*). Control plants (*open symbols*) were maintained at 28/23 °C. Means ± SD, $n = 3$.

Table 1. Correlations coefficients (r) of compatible solutes with changes in leaf pressure potential (ψ_p) and relative leaf water content (RWC) of sugarcane leaves under control (28/23 °C) and heat stress (40/35 °C) conditions. ** - $P < 0.01$, *** - $P < 0.001$, ns - non-significant.

Osmoticum	ψ_p		RWC	
	control	heat stress	control	heat stress
FP	0.159ns	0.967***	0.446ns	0.919**
GB	0.380ns	0.963***	0.476ns	0.898**
SS	0.350ns	0.961***	0.234ns	0.895**

involvement of compatible solutes with the maintenance of ψ_p and improved water status of leaves under heat stress. Heat-stable proteins increased linearly in heat stressed leaves only (Fig. 2). Sugarcane leaves studied for the expression of DHNs by immunoblotting with antisera raised against consensus peptide indicated no expression of DHNs in control leaves and up to 24 h in heat stressed leaves. There was an expression of a 21 kDa protein after 48 h of stress. This 21 kDa protein was strongly expressed after 72 h, together with two more proteins with apparent molecular mass of 23 and 27 kDa (Fig. 3).

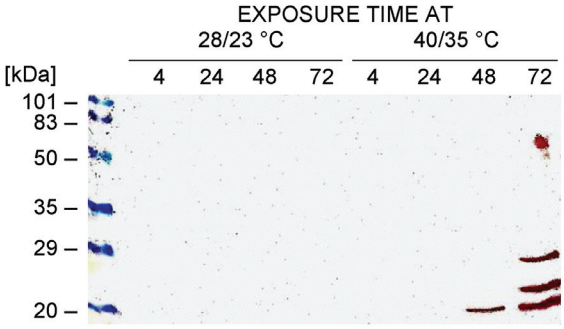

Fig. 3. Immunoblot of dehydrins from sugarcane (clone NCO-310) leaves under heat stress (40/35 °C) determined after 4 to 72 h. Control plants were maintained at 28/23 °C. Molecular mass markers on the *left*.

Discussion

Like most mesophytic species, sugarcane also shows sensitivity to supra-optimal growth temperatures despite the fact that it has higher temperature optimum compared to C_3 species (Qureshi *et al.* 2002). Increased evapotranspiration of water due to high temperature is a major cause for growth suppression of this high water requiring crop species (Ebrahim *et al.* 1998). This study conducted under high day/night temperature but relatively high RH as well as optimum soil moisture indicated an initial depression in water status of leaves (during first 12 h). The increased temperature during initial phase hampered the water status of leaves even though the RH was sufficiently high indicating that root hydraulic conductivity was readily affected. It is plausible that immediately after exposure, heat stress affected the membrane permeability making them more permeable to water and solutes (Marcum 1998, Jiang and Huang 2001). However, both the ψ_w and RWC shortly became steady and RWC nearly approached control leaves (Fig. 1). Furthermore, leaves were able to adjust osmotically by the increased synthesis of compatible solutes, FP > GB > SS and some amount of heat stable proteins with the passage of time under heat stress (Fig. 2). Occurrence of close relationships between increased synthesis of FP and GB and SS and those of ψ_p and RWC (Table 1) revealed that these compatible osmolytes not only enabled the sugarcane to improved the water status of cells adjusted osmotically but also improved the integrity of cellular membranes and gradually helped the leaves to escaped from heat induced stress damage (Jain *et al.* 2001, Xing and Rajashekar 2001, Wahid and Shabbir 2005).

Role of stress proteins has been well established in view of the protection they provide against different stresses including high temperature (Maestri *et al.* 2002, Sung *et al.* 2003). Among the various stress proteins, HSPs are ubiquitously expressed to perform a variety of functions in rescuing the cells from adverse effects of elevated temperatures (Schoffl *et al.* 1999, Sanmiya *et al.* 2004). DHNs, a unique class of proteins, are expressed under a variety of stresses including water deficits, salinity, chilling and freezing temperatures, *etc*. (Close 1997, Rinne *et al.* 1999, Svensson *et al.* 2002, Allagulova *et al.* 2003, Yin *et al.* 2004). There is hardly any study documenting the expression of DHNs under heat stress, although a report of the accumulation of dehydrin transcripts in citrus exists under heat stress (Porat *et al.* 2004). Here, for the first time, we report heat stress induced expression of three DHNs in sugarcane with apparent molecular masses of 21, 23 and 27 kDa, appearing between 48 - 72 h (Fig. 3). The former one matched with the molecular mass of DHN4 of barley cv. Morex and the latter two with DHN11 and DHN8 of cv. Dicktoo, which were expressed under freezing stress (Zhu *et al.* 2000). It is important to note that the expression of all these proteins occurred despite the fact that leaves began to adjust osmotically by regain of water associated with substantial accumulation of compatible osmolytes (Figs. 1, 2). This revealed that the expression of these DHNs was remotely related to the changes in leaf water relations and support our view that their accumulation was due to sole effect of heat stress.

Although DHNs are distinct group of proteins, they show some commonalities with HSPs with respect to physiological properties, structure and functions. Both are water soluble and show the maintenance of structural stability under temperature extremes and are expressed under a variety of stresses (Schoffl *et al.* 1999, Svensson *et al.* 2002). Structural resemblance is the presence of

conserved domains in both (Chen and Vierling 1991, Close 1997). As regards their putative functions, both act as molecular chaperones (Schoffl *et al.* 1999, Maestri *et al.* 2002, Goyal *et al.* 2005) and associate to the cytoplasmic membranes and organelles (Borovskii *et al.* 2002, Koag *et al.* 2003, Sanmiya *et al.* 2004). These similarities of DHNs with HSPs provide clues about their possible role in the heat stress tolerance of sugarcane. It appears that DHNs by virtue of their property of associating to the cytoplasmic membranes stabilized their structure and enable the sugarcane to withstand heat stress.

In conclusion, despite well-defined humidity conditions, initial effect of heat stress is the hampered water relations of leaves. Increased earlier synthesis of compatible solutes and later expression of DHNs improved the integrity of cellular membranes and enabled the sugarcane to maintain ψ_p. Results further suggest that expression of DHNs is independent of dehydration stress and have a definitive protective role like other heat stress proteins.

References

Allagulova, C.R., Gimalov, F.R., Shakirova, F.M., Vakhitov, V.A.: The plant dehydrins: structure and putative functions. - Biokhimiya **68**: 945-951, 2003.

Bates, I.S., Waldren, R.P., Teare, I.D.: Rapid determination of free proline for water stress studies. - Plant Soil **39**: 205-207, 1973.

Borovskii, G.B., Stupnikova, I.V., Antipina, A.I., Valdimerova, S.V., Voinikov, V.K.: Accumulation of dehydrins-like proteins in the mitochondria of cereals in response to cold, freezing, drought and ABA treatment. - BioMed Centr. Plant Biol. **2**: 5-11, 2002.

Buchanan, B.B., Gruissem, W., Jones, R.L.: Biochemistry and Molecular Biology of Plants. - American Society of Plant Physiologists, Rockville 2000.

Camejo, D., Rodriguez, P., Morales, M.A., Dell'Amico, J.M., Torrecillas, A., Alarcon, J.J.: High temperature effects on photosynthetic activity of two tomato cultivars with different heat susceptibility. - J. Plant. Physiol. **162**: 281-289, 2005.

Chen, Q., Vierling, E.: Analysis of conserved domains identifies a unique structural feature of a chloroplast heat shock protein. - Mol. gen. Genet. **226**: 425-431, 1991.

Close, T.J.: Dehydrins: a commonality in the response of plants to dehydration and low temperature. - Physiol. Plant. **100**: 291-296, 1997.

Close, T.J., Fenton, R.D., Moonan, F.: A view of plant dehydrins using antibodies specific to the carboxy terminal peptide. - Plant mol. Biol. **23**: 279-286, 1993.

Ebrahim, M.K., Zingsheim, O., El-Shourbagy, M.N., Moore, P.H., Komor, E.: Growth and sugar storage in sugarcane grown at temperatures below and above optimum. - J. Plant Physiol. **153**: 593-602, 1998.

Ebrahim, M.K.H., Zingsheim, O., Veith, R., Abo-Kassem, E.E.M., Komor, E.: Sugar uptake and storage by sugarcane suspension cells at different temperatures and high sugar concentrations. - J. Plant Physiol. **154**: 610-616, 1999.

Fischer, R.: Number of kernels in wheat crops and the influence of solar radiation and temperature. - J. agr. Sci. **108**: 447-461, 1985.

Fokar, M., Nguyen, H.T., Blum, A.: Heat tolerance in spring wheat. II. Grain filling. - Euphytica **104**: 9-15, 1998.

Goyal, K., Walton, L.J., Tunnacliffe, A.: LAE proteins prevent protein aggregation due to water stress. - Biochem. J. **388**: 151-157, 2005.

Grieve, C.M., Grattan, S.R.: Rapid assay for determination of water soluble quaternary ammonium compounds. - Plant Soil **70**: 303-307, 1983.

Gulen, H., Eris, A.: Some physiological changes in strawberry (*Fragaria* × *ananassa* 'Camarosa') plants under heat stress. - J. hort. Sci. Biotechnol. **78**: 894-898, 2003.

Harlow, E., Lane, D.: Antibodies: A Laboratory Manual. - Cold Spring Harbor Laboratory Press, Cold Spring Harbor - New York 1988.

Jain, M., Mathur, G., Koul, S., Sarin, NB.: Ameliorative effects of proline on salt stress-induced lipid peroxidation in cell lines of groundnut (*Arachis hypogaea* L.). - Plant Cell Rep. **20**: 463-468, 2001.

Jiang, Y., Huang, B.: Osmotic adjustment and root growth associated with drought preconditioning-enhanced heat tolerance in Kentucky bluegrass. - Crop Sci. **41**:1168-1173, 2001.

Karim, M.A., Fracheboud, Y., Stamp, P.: Effect of high temperature on seedling growth and photosynthesis of tropical maize genotypes. - J. Agron. Crop Sci. **184**: 217-223, 2000.

Koag, M.-C., Fenton, R.D., Wilkens, S., Close, T.J.: The binding of maize DHN1 to lipid vesicles. Gain of structure and lipid specificity. - Plant Physiol. **131**: 309-316, 2003.

Machado, S., Paulsen, G.M.: Combined effects of drought and high temperature on water relations of wheat and sorghum. - Plant Soil **233**: 179-187, 2001.

Maestri, E., Klueva, N., Perrotta, C., Gulli, M., Hguyen, H.T., Marmiroli, N.: Molecular genetics of heat tolerance and heat shock proteins in cereals. - Plant mol. Biol. **48**: 667-81, 2002.

Marcum, K.B.: Cell membrane thermostability and whole plant heat tolerance of Kentucky bluegrass. - Crop Sci. **38**: 1214-1218, 1998.

Moisyadi, S., Harrington, H.M.: Characterization of the heat shock response in cultured sugarcane cells. I. Physiology of the heat shock response and heat shock protein synthesis. - Plant Physiol. **90**: 1156-1162, 1989.

Morales, D., Rodríguez, P., Dell'Amico, J., Nicolás, E., Torrecillas, A., Sánchez-Blanco, M.J.: High-temperature preconditioning and thermal shock imposition affects water relations, gas exchange and root hydraulic conductivity in tomato. - Biol. Plant. **47**: 203-208, 2003.

Perdomo, P., Murphy, J.A., Berkowitz, G.A.: Physiological changes associated with performance of Kentucky bluegrass cultivars during summer stress. - HortScience **31**: 1182-1186, 1996.

Porat, R., Pasentsis, K., Rozentzvieg, D., Gerasopoulos, D., Falara, V., Samach, A., Lurie, S., Kanellis, A.K.: Isolation of a dehydrin cDNA from orange and grapefruit citrus fruit that is specifically induced by the combination of heat followed by chilling temperatures - Physiol. Plant. **120**: 256-264, 2004.

Qureshi, S.A., Mandramootoo, C.A., Dodds, G.T.: Evaluation of irrigation schemes for sugarcane in Sindh, Pakistan using SWAP93. - Agr. Water Manage. **54**: 37-48, 2002.

Rawson, H.M.: Effect of high temperatures on the development and yield of wheat and practices to reduce deleterious effects.

- In: Klatt, A.R. (ed.): Wheat Production Constraints in Tropical Environments. Pp. 44-62. CIMMYT, Mexico City 1988.

Rinne, P.L., Kaikuranta, P.L., Van der Plas, L.H., Van der Schoot, C.: Dehydrins in cold-acclimated apices of birch (*Betula pubescens* Ehrh.): production, localization and potential role in rescuing enzyme function during dehydration. - Planta **209**: 377-388, 1999.

Robertson, M.J., Bonnett, G.D., Hughes, R.M., Muchow, R.C., Campbell, J.A.: Temperature and leaf area expansion of sugarcane: Integration of controlled-environment, field and model studies. - Aust. J. Plant Physiol. **25**: 819-828, 1998.

Sanmiya, K., Suzuki, K., Egawa, Y., Shono, M.: Mitochondrial small heat-shock protein enhances thermotolerance in tobacco plants. - FEBS Lett. **557**: 265-268, 2004.

Schoffl, F., Prandl, R., Reindl, A.: Molecular responses to heat stress. - In: Shinozaki, K., Yamaguchi-Shinozaki, K. (ed): Molecular Responses to Cold, Drought, Heat and Salt Stress in Higher Plants. Pp. 81-98. K.R.G. Landes Co., Austin 1999.

Shah, N.H., Paulsen, G.M.: Interaction of drought and high temperature on photosynthesis and grain filling of wheat. - Plant Soil **257**: 219-226, 2003.

Sung, D.-Y., Kaplan, F., Lee, K.-J., Guy, C.L.: Acquired tolerance to temperature extremes. - Trends Plant Sci. **8**: 179-187, 2003.

Svensson, J., Ismail, A.M., Palva, E.T., Close, T.J.: Dehydrins. - In: Storey, K.B., Storey, J.M. (ed.): Cell and Molecular Responses to Stress. Vol. 3. Sensing, Signalling and Cell Adaptation. Pp. 155-171. Elsevier Science, Amsterdam 2002.

Taiz, L., Zeiger, E.: Plant Physiology, 3rd Edition. - Sinauer Associates Inc. Publishers, Massachusetts 2002.

Wahid, A., Shabbir, A.: Induction of heat stress tolerance in barley seedling by pre-sowing seed treatment with glycinebetaine. - Plant Growth Regul. **46**: 133-141, 2005.

Xing, W., Rajashekar, C.B.: Glycine betaine involvement in freezing tolerance and water stress in *Arabidopsis thaliana*. - Environ. exp. Bot. **46**: 21-28, 2001.

Yin, Z., Pawlowicz, I., Bartoszewski, G., Malinowski, R., Malepszy, S., Robat, T.: Transcriptional expression of a *Solanum sogarandinum* pGT::*Dhn10* gene fusion in cucumber, and its correlation with chilling tolerance in transgenic seedlings. - Cell mol. Biol. Lett. **9**: 891-902, 2004.

Yoshida, S., Forno, D.A., Cock, J.H., Gomaz, K.U.: Laboratory Manual for Physiological Studies of Rice. - IRRI, Los Baños, 1976.

Zhu, B., Choi, D.-W., Fenton, R., Close, T.J.: Expression of the barley multigene family and the development of freezing tolerance. - Mol. gen. Genet. **264**: 145-153, 2000.