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Effects of water stress and high-temperature stress on the structure and activity of photosynthetic apparatus of *Zea mays* and *Helianthus annuus*

I. DEKOV, T. TSONEV, and I. YORDANOV

Institute of Plant Physiology, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., bl. 21, Sofia 1113, Bulgaria

Abstract

Effects of high-temperature stress (HTS) and PEG-induced water stress (WS), applied separately or in combination, on the functional activity and ultrastructure of the photosynthetic apparatus (PSA) of maize (*Zea mays* L.) and sunflower (*Helianthus annuus* L.) plants were investigated. In maize plant tissues WS provoked the decrease in RWC by 10.9 %, HTS by 7.0 %, and after simultaneous application of the both treatments the decrease was 32.7 % in comparison with control plants. Similar but more expressed changes were observed in sunflower plants. Sunflower was more sensitive to these stresses. Net photosynthetic rate decreased significantly after all treatments, more in sunflower. In mesophyll chloroplasts after separately applied WS and HTS the number of grana and thylakoids was reduced and electron-transparent spaces appeared. At combined stress (WS+HTS) granal and stromal thylakoids were considerably affected and chloroplast envelope in many of them was partially disrupted.

Additional key words: chloroplast; maize; photosynthesis; polyethylene glycol; relative water content; sunflower; thylakoids; ultrastructure.

Introduction

In the field, plants are often exposed simultaneously to drought and high temperature. Under these stress conditions usually a water deficit in plant tissue develops. In the last years these effects were studied on different levels: from ecophysiology to cell metabolism (Bose and Ghosh 1995, Shinozaki and Yamaguchi 1997). The range and importance of these changes depend on the genetically determined plant capacity and sensitivity, as well as on the intensity and duration of the stress, when applied alone or in combination (Bhadula *et al.* 1998). The photosynthetic apparatus is very sensitive to different stresses (Chaves 1991, Santarius *et al.* 1991, Dua *et al.* 1994, Kramer 1995) and one of the primary sites of injury is photosystem 2 reaction centre (Havaux 1992, Oberhuber and Edwards 1993). Under high temperatures some specific low- and high-molecular stress proteins, localised in the stroma and thylakoids, are synthesised (Pareek *et al.* 1995, Waters *et al.* 1996, Ristic *et al.* 1998, Bhadula *et al.* 1998). The structure and functional activity of chloroplasts are closely associated (Gounaris *et al.* 1984, Ludlow 1987, Ristic and Cass 1991, Kutík 1998, Pastor *et al.* 1999, Stoyanova and Yordanov 1999). Nevertheless, the mechanisms of single and combined action of WS and HTS on photosynthetic apparatus are not elucidated completely.

The aim of this research was to determine the effect of WS and HTS alone and in combination on the structure and function of photosynthetic apparatus of maize and sunflower plants.

Materials and methods

Plants: Maize (*Zea mays* L. cv. Knezha-611, 2L) and sunflower (*Helianthus annuus* L. cv. Peredovik) plants were grown in water culture (hydropony) in the Hellriegel (1898) nutrient solution that contained [mM]: Ca 3; K 2; N 6; P 1; Mg 0.5; Fe 0.09, with addition of A-Z microelements according to Hoagland and Arnon (1950). Iron was supplied as Fe-EDTA prepared from equimolar amounts of $FeCl_3$ and Na-EDTA. Plants were grown in

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Fax: 359-2-73-99-52; e-mail: dekov@bio25.bas.bg

Abbreviations: HTS - high-temperature stress; P_N – net photosynthetic rate; PEG – polyethylene glycol; PG – plastoglobuli; PSA - photosynthetic apparatus; RWC - relative water content; WS - water stress.

greenhouse at a 16-h photoperiod. Nutrient solution was aerated two times a day and changed every 3 d.

WS and HTS: On the 28th day of growth, WS was induced by 20 % (m/v) polyethylene glycol (*PEG-6000, Sigma*, Germany) in the nutrient solution for 48 h (Kawasaki *et al.* 1983). HTS was created by exposing some pots with control plants as well as WS plants at 42±1 °C for 48 h. Thus, the following experimental variants were obtained both for maize and sunflower plants: (*1*) control, 25 °C; (*2*) WS, 25 °C; (*3*) HTS, 42 °C; (*4*) WS+HTS.

Leaf relative water content (RWC) was calculated using the formula: RWC [%] = $(FM_i - DM)/(FM_t - DM) \times 100$, where FM_i - initial fresh mass; DM - dry mass; FM_t - turgid mass after saturation.

Photosynthetic and transpiration rates were measured by portable photosynthesis system *LI-6000* (*Li-Cor*, Lincoln, USA) at leaf temperature 30±2 °C and photosynthetic photon flux density of 800 μmol m^{-2} s^{-1}. The values were obtained from measurements of five plants from each experimental variant.

Transmission electron microscopy (TEM): Samples (1×3 mm) from the middle of the sixth fully developed leaf from all variants were taken and immediately fixed with 5 % (m/v) glutaraldehydehyde (*Sigma*, Germany) in 0.05 M cacodylate buffer (*Serva*, USA) (m/v), pH 7.2 at 0-4 °C for 3 h. After two-fold rinsing in the same buffer the leaf samples were post-fixed in 1.5 % OsO_4 (m/v) (*BDH*, England) for 12 h at room temperature and dehydrated through a graded ethanol series (25, 50, 70, 96, and 100 %), propylene oxide, and embedded in *Durcupan ACM* epoxy resin (*Fluka*, Switzerland). Ultrathin sections were cut on a *Reichert Ultracut* microtome (Austria) and stained with uranylacetate and lead citrate solution according to Reynolds (1963). The specimens were observed on *JEOL* (*JEM 100 CX*, Japan) TEM. In each variant an average of 20 mesophyll chloroplasts was examined.

Statistical analysis: Values obtained were expressed as mean ± SE from measurements of five plants of each variant from three consecutive experiments. The Student's *t*-test was used to evaluate the differences between stressed and control variants.

Results

PEG treatment provoked a 10.9 % decrease in RWC of maize plant tissues, while the HTS reduced it by 7.0 %. After simultaneous application of both stresses (WS+HTS) RWC decrease was 32.7 % in comparison with the control plants (Fig. 1*A*).

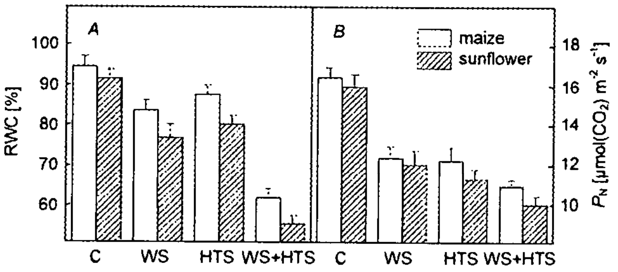

Fig. 1. Relative water content (RWC) (*A*) and net photosynthetic rate, P_N (*B*) of maize and sunflower plants: control (C); water stress (WS); high-temperature stress (HTS), and WS + HTS. Bars represent ±SE.

When sunflower plants were subjected to the respective stresses, similar changes in RWC were observed (Fig. 1*A*). As a result of combined treatment, RWC decreased to 55.4 %. In comparison to maize, sunflower plants were more sensitive to combined stresses.

WS as well as HTS decreased significantly the P_a and when WS+HTS was applied a drastic decrease up to 15 % of the value of control was found (Fig. 1*B*). This influence was more expressed in sunflower plants.

Chloroplasts from the control maize plants (Fig. 2*A*) had a typical bilayer chloroplast envelope. The number of thylakoids per granum ranged from 10 to 40 and more. A small number of plastoglobuli (PG) between the stromal thylakoids was also seen.

In chloroplasts of maize plants subjected to WS (Fig. 2*B*) their length-to-width ratio decreased. The numbers of grana and their constituting thylakoids were reduced. Thylakoid membranes were swollen and lightly disoriented. In some areas the stromal thylakoids were distant and electron-transparent spaces containing homogenous stroma were observed between them.

The thylakoid membranes of maize chloroplasts from plants subjected to HTS were swollen and disoriented. A greater number and larger electron-transparent spaces were seen when compared with these in WS-plants. There was a small number of PG. In some chloroplasts the envelope was disrupted (Fig. 2*C*).

In the chloroplasts of maize plants subjected to WS+HTS the number of grana and thylakoids was reduced (Fig. 2*D*). The granal thylakoid membranes were strongly swollen and dilated. Stromal thylakoids were broken, disorganised, and disoriented. There were some PG. Envelope was disrupted in many chloroplasts.

Fig. 2. Transmission electron micrographs of chloroplasts from mesophyll cells of maize plants: *A* - control; *B* - water stress (WS); *C* - high-temperature stress (HTS); *D* - WS+HTS. Bar = 0.5 μm.

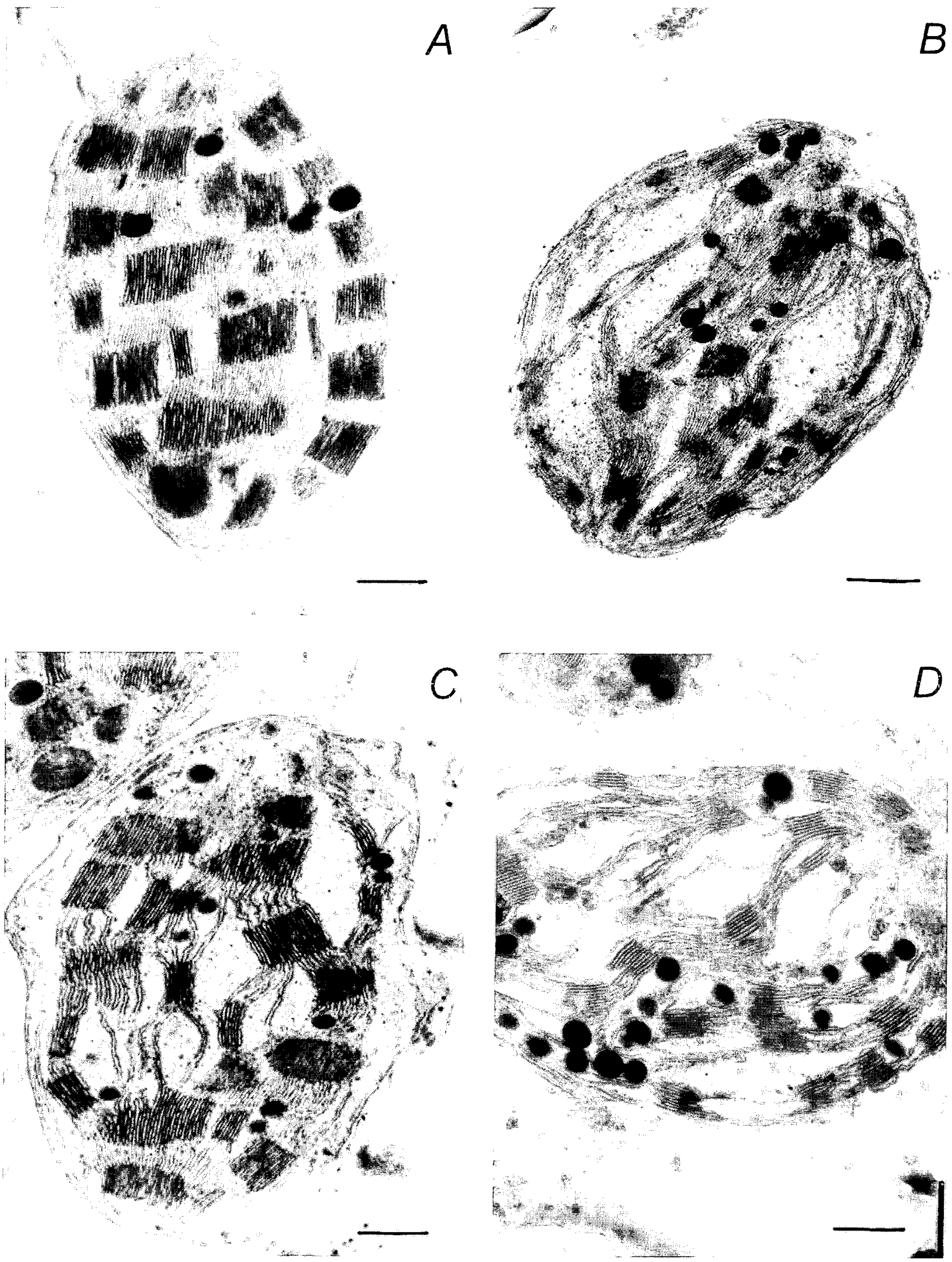

Fig. 3. Transmission electron micrographs of chloroplasts from leaves of sunflower plants: *A* - control; *B* - water stress (WS); *C* - high-temperature stress (HTS); *D* - WS+HTS. Bar = 0.5 μm.

The chloroplasts of control sunflower plants had a smaller length-to-width ratio in comparison to maize plants (Fig. 3*A*). They had well-developed granal and stromal thylakoid system. Under WS, the chloroplasts were significantly affected (Fig. 3*B*). Their shape was more oval and thylakoids were swollen and disoriented. Filled electron-transparent spaces were visible. There were many large PG. The envelope of the chloroplasts was swollen and interrupted.

The chloroplasts of sunflower plants subjected to HTS had an irregular shape (Fig. 3*C*). More thylakoid membranes were swollen and disoriented than after WS. The number of grana and thylakoids was diminished. Electron-transparent areas with significant dimensions appeared. There were also some plastoglobuli.

The changes in ultrastructure of chloroplasts of sunflower plants subjected to WS+HTS were very expressed (Fig. 3*D*). The intactness of chloroplast envelope was interrupted. The chloroplasts were largely vacuolated. There was a great number of large PG. Thylakoid membrane system was strongly disoriented and disorganised. A great part of the stromal thylakoids was broken. Dimensions of grana were strongly reduced as well as the number of thylakoid membranes.

Discussion

Both the single and simultaneous applications of WS and HTS caused in plants a decrease of RWC that was expressed at different extent in maize and sunflower plants. Maize plants overcome the dehydration under stress better than sunflower plants as indicated by changes of leaf RWC, chloroplast ultrastructure and activity. The decrease of RWC, more expressed in sunflower than in maize under treatments investigated, is probably one of causes for photosynthetic inhibition and ultrastructural damage of chloroplasts. This opinion is supported by Ristic and Cass (1991). Maize plants support higher RWC possibly because they have more closed stomata, better water uptake, and higher water use efficiency (Bethenod *et al.* 1996, our unpublished results). These mechanisms protect them from great water loss and ensure better preservation of the function of photosynthetic apparatus (Scheuerman *et al.* 1991, Oberhuber and Edwards 1993). Evidently, the physiological responses of plants to decreased RWC strongly depend on plant species and on the rate of decrease, duration, and strength of stress as well as on combination with other stresses. WS and HTS applied separately caused a slight decrease in P_N while the simultaneous action of both stresses strongly inhibited photosynthesis. The decrease of P_N may be related to the changes in chloroplast ultrastructure.

WS and HTS influence many cellular processes, and chloroplasts and their membranes are among main targets (Yordanov 1992). Different changes in the structure of chloroplasts of the maize and sunflower plants subjected to WS and HTS may partly result from differences in dehydration developed in them in these conditions.

Severe stress causes an inhibition of protein synthesis and inactivation of some chloroplast enzymes, especially those participating in CO_2 assimilation. The regenerative phase of Calvin cycle, electron transport, and capability for photophosphorylation are disturbed and this may be in close relation with the significant changes in chloroplast ultrastructure. The inhibition of photosynthetic activity at drought stress is partially due to inactivation of photosystem 2 and decrease in non-cyclic photo-phosphorylation (Chaves 1991, Havaux 1992, Oberhuber and Eduards 1993). When leaf desiccation develops, membrane permeability and stroma acidification increase which additionally contributes to enzyme inhibition (Berkowitz and Gibbs 1983, Zuily-Fodil *et al.* 1990).

Under WS and HTS the plants are subjected to oxidative injuries as a result of overproduction of oxygen radicals in chloroplasts (Price *et al.* 1989, Sgherri *et al.* 1996). These findings are used to explain the significant injuries of some enzymes with active SH-groups, chloroplast pigments, and membrane structure integrity during stress (Navari-Izzo *et al.* 1989, Mishra and Singhal 1993). Accumulation of PG observed in chloroplasts under stress may be a result of destructive processes from the damage of thylakoid membranes (Ivanova *et al.* 1993). We observed that the destructive processes were more expressed at simultaneous action of WS and HTS than at separate application.

We found that the preservation of membrane structural integrity is an important factor of plant capability to overcome different stresses. Estimation of functional and structural damages of chloroplasts at WS and HTS is important for determining the extent of adaptivity and resistance, respectively, of plant tolerance to these stress factors acting individually or simultane-ously as it happens very often in nature.

References

Berkowitz, G.A., Gibbs, M.: Reduced osmotic potential effects on photosynthesis. Identification of stromal acidification as a mediating factor. - Plant Physiol. **71**: 905-911, 1983.

Bethenod, O., Tardieu, F., Katerji, N.: Relationship between net photosynthetic rate and stomatal conductance in leaves of field-grown maize subjected to soil compaction or soil drying. - Photosynthetica **32**: 367-379, 1996.

Bhadula, S.K., Yang, G.P., Sterzinger, A., Ristic, Z.: Synthesis of a family of 45 ku heat shock proteins in a drought and heat resistant line of maize under controlled and field conditions. – J. Plant Physiol. **152**: 104–111, 1998.

Bose, A., Ghosh, B.: Effect of heat stress on ribulose 1,5-bisphosphate carboxylase in rice. - Phytochemistry **38**: 1115-1118, 1995.

Chaves, M.M.: Effects of water deficits on carbon assimilation. - J. exp. Bot. **42**: 1-16, 1991.

Dua, A., Talwar, G., Singal, H.R., Singh, R.: CO_2 exchange, primary photochemical reactions and enzymes of photosynthetic carbon reduction cycle in *Brassica* pods during water stress and recovery. - Photosynthetica **30**: 261-268, 1994.

Gounaris, K., Brain, A.R.R., Quinn, P.J., Wiliams, W.P.: Structural re-organisation of chloroplasts thylakoid membranes in response to heat-stress. - Biochim. biophys. Acta **766**: 198-208, 1984.

Havaux, M.: Stress tolerance of photosystem II *in vivo*. Antagonistic effects of water, heat, and photoinhibition stresses. - Plant Physiol. **100**: 424-432, 1992.

Hellriegel, H.: Die Methode der Sandkultur. - Arb. deutsch. landwirtsch. Ges. **34**: 7–19, 1898.

Hoagland, D.R., Arnon, D.I.: The water-culture method for growing plants without soil. - Cal. Agr. Exp. Stat. Circ. **347**: 32, 1950.

Ivanova, A.P., Yordanov, I.T., Stefanov, K.L., Seizova, K.A., Popov, S.S.: Effect of heat stress on the lipid and fatty acid composition of the thylakoid membranes in two cultivars of pea acclimated to elevated temperatures. - Photosynthetica **29**: 131–137, 1993.

Kawasaki, T., Akiba, T., Maritsugu, M.: Effect of high concentrations of sodium chloride and polyethylenglycol on the growth and ion absorption in plants. I. Water culture experiments in a greenhouse. - Plant Soil **75**: 75-85, 1983.

Kramer, P.J., Boyer, J.S.: Water Relations of Plants and Soils. – Acad. Press, San Diego 1995.

Kutík, J.: The development of chloroplast structure during leaf ontogeny. – Photosynthetica **35**: 481-505, 1998.

Ludlow, M.M.: Light stress at high temperature. – In: Kyle, D.J., Osmond, C.B., Arntzen, C.J. (ed.): Photoinhibition. Pp. 89–109. Elsevier, Amsterdam 1987.

Mishra, R.K., Singhal, G.S.: Photosynthetic activity and peroxidation of thylakoid lipids during photoinhibition and high temperature treatment of isolated wheat chloroplasts. - J. Plant Physiol. **141**: 286-292, 1993.

Navari-Izzo, F., Quartacci, M., Izzo, R.: Lipid changes in maize seedlings in response to field water deficits. – J. exp. Bot. **40**: 675–680, 1989.

Oberhuber, W., Edwards, G.E.: Temperature dependence of the linkage of quantum yield of photosystem II to CO_2 fixation in C_4 and C_3 plants. - Plant Physiol. **101**: 507-512, 1993.

Pareek, A., Singla, S., Grover, A.: Immunological evidence for accumulation of two high-molecular-weight (104 and 90 kDa) HSPs in response to different stresses in rice and in response to high temperature stress in diverse plant genera. – Plant mol. Biol. **29**: 293–303, 1995.

Pastor, A., López-Carbonell, M., Alegre, L.: Abscisic acid immunolocalization and ultrastructural changes in water-stressed lavender (*Lavandula stoechas* L.) plants. - Physiol. Plant. **105**: 272-279, 1999.

Price, A.H., Atherton, N.M., Hendry, G.A.F.: Plants under drought stress generate activated oxygen. - Free Radical Res. Commun. **8**: 61-66, 1989.

Reynolds, E.S.: The use of lead citrate at high pH as an electron opaque stain in electron microscopy. - J. Cell Biol. **17**: 208-213, 1963.

Ristic, Z., Cass, D.D.: Chloroplast structure after water shortage and high temperature in two lines of *Zea mays* L. that differ in drought resistance. - Bot. Gaz. **152**: 186-194, 1991.

Ristic, Z., Yang, G., Martin, B., Fullerton, S.: Evidence of association between specific heat-shock protein(s) and the drought and heat tolerance phenotype in maize. - J. Plant Physiol. **153**: 495–505, 1998.

Santarius, K.A., Exner, M., Thebut-Lassak, R.: Effects of high temperature on the photosynthetic apparatus in isolated mesophyll protoplasts of *Valerianella locusta* (L.) Betcke. - Photosynthetica **25**: 17-26, 1991.

Scheuerman, R., Biehler, K., Stuhlfauth, T., Fock, H.P.: Simultaneous gas exchange and fluorescence measurements indicate differences in the response of sunflower, bean and maize to water stress. - Photosynth. Res. **27**: 189-197, 1991.

Sgherri, C.L.M., Pinzino, C., Navari-Izzo, F.: Sunflower seedlings subjected to increasing stress by water deficit. Changes in $O_2^{\cdot-}$ production related to the composition of thylakoid membranes. - Physiol. Plant. **96**: 446-452, 1996.

Shinozaki, K., Yamaguchi-Shinogzaki, K.: Gene expression and signal transduction in water-stress response. - Plant Physiol. **115**: 327-334, 1997.

Stoyanova, D., Yordanov, I.: Influence of drought, high temperature, and carbamide cytokinin 4-PU-30 on photosynthetic activity of plants. 2. Chloroplast utrastructure of primary bean leaves. – Photosynthetica **37**: 621-625, 1999.

Waters, E., Lee, G., Verling, E.: Evolution, structure and function of the small heat shock proteins in plants. - J. exp. Bot. **47**: 325–338, 1996.

Yordanov, I.: Response of photosynthetic apparatus to temperature stress and molecular mechanisms of its adaptations. - Photosynthetica **26**: 517–531, 1992.

Zuily-Fodil, Y., Vazquez-Tello, A., Vieira da Silva, J.: Effect of water deficit on cell permeability and on chloroplast integrity. - Bull. Soc. Bot. Fr. Actual. bot. **137**: 115–123, 1990.