DROUGHT-INDUCED LEAF PROTEIN ALTERATIONS IN SENSITIVE AND TOLERANT WHEAT VARIETIES

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Summary. Wheat plants with a fully developed first leaf were subjected to severe but recoverable water stress. Leaves from drought tolerant (Katya and Zlatitza) and drought sensitive (Sadovo and Miziya) varieties in control, drought and recovery conditions were used for the experiments. The physiological response of drought tolerant varieties did not differ from the one of drought sensitive varieties at early seedling stage under these conditions. A comparative study of the ultrathin sections by transmission electron microscopy from control and drought stressed plants revealed prominent changes in mitochondrion fine structures. The relative cell area occupied by mitochondria was reduced in the drought sensitive varieties. An increased quantity of abscisic acid (ABA) was detected in drought stressed wheat plants. The drought sensitive varieties (Miziya and Sadovo) possessed higher azocaseinolytic activity. An immunoblotting analysis was performed for some specificity detection of protein response under drought conditions of Rubisco, Rubisco activase (RA), Rubisco binding protein (RBP), dehydrins (DHN), some heat shock proteins (HSP) and ATP dependent calpain protease (Clp) proteins. The obtained results

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showed that the drought tolerant varieties Katya and Zlatitza had higher levels of these proteins, especially RBP and Clp proteases. Our attention was focused on the coordinated response of Rubisco, RA, RBP, DHNs, HSPs and Clp proteases in stress conditions, such as drought. The biochemical response diversity in susceptible and tolerant wheat varieties is discussed.

***Key words*:** chaperones, dehydrins, drought stress, proteases, Rubisco, leaf ultrastructure.

***Abbreviations*:** Clp - ATP dependent calpain protease, cpn – chaperone, EL - electrolyte leakage, FW - fresh weight, HSP - heat shock protein, MW - molecular weight, Rubisco - ribulose-1,5-bisphosphate carboxylase/oxygenase, RA - Rubisco activase, RBP - Rubisco binding protein, RLS - Rubisco large subunit; RLS-C - C-terminus of Rubisco LS, RLS-N - N-terminus of Rubisco LS, RSS - Rubisco small subunit, RT - room temperature, RuBP - ribulose-1,5-bisphosphate, SDS-PAGE - SDS polyacrylamide gel electrophoresis, TEM - transmission electron microscopy, WD - water deficit.

INTRODUCTION

Drought stress is one of the most widespread environmental stresses when the available water in the soil is reduced and atmospheric conditions cause continuous loss of water by transpiration and evaporation (Kramer, 1980). Many regions of the Earth are often or permanently exposed to drought (Bray, 1997). Up to 26 % from the usable areas of the Earth is subjected to drought (Blum, 1986). Drought is the most severe stress and the main cause of significant losses in growth, productivity of crop plants, and finally their yields (Ludlow and Muchow, 1990). Drought affects morphological, physiological, biochemical and molecular processes in plants resulting in growth inhibition, stomata closure with consecutive reduction of transpiration, decrease in chlorophyll content and inhibition of photosynthesis and protein changes (Lawlor and Cornic, 2002; Yordanov et

al., 2003; Zhu, 2002) to cope with osmotic changes in their tissues. Riccardi et al. (2004) have demonstrated that plant response to water deficit shows some genetic variations.

Crop plants which can use water most efficiently and maintain acceptable yields are perspective regarding their tolerance. Drought tolerance is a complex trait where several characteristics influence plant success during vegetation period (Ingram and Bartels, 1996). It is achieved by modulation of gene expression and accumulation of specific protective proteins and metabolites (Reddy et al. 2004; Zang and Komatsu, 2007). Water stress tolerance has been documented in almost all plants but its extent varies from species to species (Chaitanya et al., 2003).

The phytohormone abscisic acid (ABA) is a stress-induced plant hormone and it has attracted much research attention as a potentially useful trait in selecting for drought tolerance in crops (Zhang et al., 2006). Increasing ABA concentration leads to many changes in development, physiology, and growth. ABA stimulates osmotic adjustment (Ober and Sharp, 1994), induces the synthesis of protective proteins (the *LEA* and related proteins) (Bray, 1993) and it has also been shown to induce the expression of various water stress-induced genes. In a study on ABA accumulation in response to drought stress in the progeny of a cross between two spring wheat (*Triticum aestivum* L.) genotypes contrasting in ABA accumulation, it was demonstrated that the resistant to water stress high ABA selections contained 50% more ABA than low sensitive ABA selections (Quarrie and Lister, 1999).

Generally, drought induces metabolic changes related to protein turnover (alterations in protein synthesis, maintaining the level of some proteins or protein degradation) (Bray, 1997).

The key photosynthetic enzyme in C_3 plants is Rubisco (ribulose-1,5-bisphosphate carboxylase/oxygenase) (EC 4.1.1.39), which takes part in CO_2 fixation and photorespiration (Jensen and Bahr, 1977). This enzyme is localized in the chloroplast stroma. Rubisco accounts for about 30-60 % of the total soluble protein in plants. The enzyme constitutes a large pool of stored leaf nitrogen (20-30 %) that can be quickly remobilized under stress and senescence (Makino et al., 1984; Feller et al., 2008). In accordance with Medrano et al. (1997) the amount of Rubisco protein is slightly affected by

moderate and even prolonged severe drought. Some data about a reduction in Rubisco amount in stressed plants also exist (Majumdar et al., 1991; Parry et al., 2002).

Both the synthesis and assembly of Rubisco depend on two genomes. The RLS are encoded by a single chloroplast gene whereas RSS are encoded by a small family of nuclear genes (Roy, 1989). The correct folding of the RLS requires chaperonin system consisting of Rubisco binding protein (RBP) or cpn60 (the analog of GroL) and cochaperonin cpn10 (the analog of GroES). The Rubisco holoenzyme assembly in chloroplast stroma is an ATP-dependent process (Musgrove et al., 1987). Hemmingsen (1990) indicates that the level of cpn 60 is coordinated positively with that of Rubisco under normal conditions. Very limited data are available to date concerning the response of cpn 60 to stress conditions, especially to drought (Jagtap et al. 1998, Holland et al. 1998).

The activity of Rubisco is regulated by Rubisco activase protein (RA) that possesses ATPase activity (Salvucci et al., 1985). The function of RA is to remove tightly bound sugar phosphates from the active centers of Rubisco. It is considered that RA protein is not a conventional enzyme and belongs to the ATPase family associated with various cellular activities (AAA+ proteins), a class of chaperone-like proteins acting on other macromolecules and catalyzing mechanical processes, such as assembly, operation and disassembly of protein complexes (Sanchez de Jimenes et al., 1995; Portis, 2003; Neuwald et al., 2006). There are converse data about the abundance of RA under drought conditions (Parry et al., 2002; Salekdeh et al., 2002; Haupt-Herting et al., 2001). Rokka et al. (2001) suggest that RA protects chloroplast protein synthesis from drought stress as a chaperone. Chaperons like RBP and RA might associate to each other by protein-protein interactions facilitating directly Rubisco assembly and activation or promoting different processes (Demirevska-Kepova et al., 1999).

The level of proteins, their breakdown and recycling depend on another important protein superfamily AAA^+ (ATPase Associated Activities). Members of this family are involved in a variety of cellular processes controlling the fate of proteins variously facilitating protein folding and unfolding, the assembly and disassembly of protein complexes, protein transport and programmed protein degradation. Energy-dependent Clp

proteases are composed of a proteolytic component as ClpP, and a regulatory ATPase, which can be either ClpA, or ClpC, or ClpX. Homologues of these proteins are found in most bacteria and organelles. ATP-dependent Clp protease, belonging to AAA^+ proteins, is a special protease in the chloroplast stroma as a complex serine type multi-subunit enzyme (Neuwald et al., 2006). The substrates of Clp proteases include abnormal and short-lived regulatory proteins. It is generally accepted that the role of this protease is essential and constitutive (Zheng et al., 2002).

Another group with chaperone-like functions is the one of dehydrins (DHNs). They are a group of heat-stable plant proteins produced during late embryogenesis and believed to play a protective role during cellular dehydration (Close, 1996; Campbell and Close, 1997). Dehydrin proteins and their transcripts have been shown to accumulate during dehydrative stress conditions (drought, low temperature, and salinity) and abscissic acid synthesis and they have a potential *in vivo* role in stabilizing cells under stress (Close, 1996; Suprunova et al., 2004). All observations are consistent with a hypothesis that dehydrins are surfactants capable of inhibiting the coagulation of a range of macromolecules, thereby preserving structural integrity (Close, 1997). Despite their widespread occurrence and abundance in cells under dehydrative conditions, the biochemical role of DHNs remains elusive.

Heat shock proteins (HSPs) increase their expression when cells are exposed to high temperatures or to other stresses (Lindquist, 1986). Heat shock proteins are also specified as molecular chaperones for protein molecules (Schöffl et al., 1998). They perform functions in various intracellular processes and play an important role in protein-protein interactions, folding, assembly, intracellular localization, secretion, prevention of unwanted protein aggregation or degradation and reactivation of damaged proteins (Vierling, 1991; Parsell and Lindquist, 1993). HSPs also help in transporting proteins across membranes within the cell. Their chaperon pathways require energy in the form of ATP hydrolysis for their functioning. Heat shock proteins are present in cells under perfectly normal conditions because of their essential role in protein maintenance. They are induced when a cell undergoes various types of environmental stresses like heat, cold and oxygen deprivation (Feder and Hofmann, 1999; Kregel, 2002).

According to Sørensen et. al. (2003) HSP family and other molecular chaperones play significant roles in relation to stress resistance. Proteins that fail to fold correctly by HSP are generally degraded.

Intracellular proteolysis might have an important role in the reorganization of plant metabolism under stress, however these processes remain poorly understood compared to the role of proteases in germination and senescence (Feller, 2004; Grudkowska and Zagdanska, 2004). The proteolytic response under drought was found to be different from that of natural senescence (Khanna-Chopra et al., 1999). The contribution of cysteine proteases to total proteolytic activity increases drastically in response to water deficit in wheat (Zagdanska and Wisnievski, 1996) and some experimental evidence suggests that drought-sensitive species and varieties have higher proteolytic activity compared to the resistant ones (Roy-Macauley et al., 1992; Hieng et al., 2004), however, data on proteolytic activity relation to drought sensitivity or resistance are still quite limited. Zagdanska and Wishnewski (1998) showed that ATP-dependent proteolysis contributed to the acclimation-induced drought resistance in spring wheat.

Nowadays Rubisco still remains an enzyme with a very complex and poorly elucidated regulation of its activity and quantity (Houtz and Portis, 2003). During the past few years the investigations concerning Rubisco and its changes under different stress conditions were reconsidered with a special emphasis to the important role of RA and RBP (Portis, 2003; Haupt-Herting et al., 2001; Rokka et al., 2001; Demirevska-Kepova et al., 2005). Data about RBP response as chaperone and the common response of Rubisco, RA and RBP as well as HSPs and DHNs under stress conditions are quite limited. Different classes of proteases are involved in the response to drought, especially those located in plant vacuoles (Roy-Macauley et al. 1992; Martinez et al., 2007). Very little is known about the functioning of Clp proteolytic system and its physiological role in higher plants under stress conditions.

Our working hypothesis suggests the existence of close relationship and coordination of functions among chaperonins, HSPs, DHNs and proteases under drought stress, as well as certain diversity in the expression of drought inducible proteins comparing sensitive and tolerant varieties.

The main purpose was to study changes of Rubisco and some stress-

inducible proteins (chaperons, HSPs, DHNs and proteases) and their complex interrelationships under drought and control conditions and their ABA responsiveness by comparison of selected wheat varieties with different drought tolerance. Our study was focused on the expression of specific plant proteins responsible for stress tolerance and searching of specific protein or non-protein drought stress markers. A combination of biochemical, immunochemical methods and electron microscopy was used to trace the changes under severe but recoverable drought at cellular and sub-cellular levels.

MATERIAL AND METHODS

Plant material

The experiments were carried out mainly with four winter wheat varieties (Katya, Sadovo, Zlatitza and Miziya). In some experiments wheat varieties Prelom and Pobeda were also used. All varieties are high yielding under optimal water supply. Variety Katya was considered as drought resistant (Kalapos et al., 1996). Variety Sadovo was less resistant to drought than Katya (Simova-Stoilova et al., 2006). The varieties Zlatitza and Miziya in field conditions showed a drought sensitivity index according to Fisher and Maurer (1978) of 0.657 and 1.282, respectively (unpublished data). Thus, Zlatitza was a drought-tolerant while Miziya was a drought-sensitive variety.

Plants were grown in a growth chamber in pots containing 400 g leached meadow cinnamonic soil (pH 6.2) at day/night temperatures of 25/21 °C, 150 μmol m^{-2} s^{-1} photosynthetically active radiation and 16-h photoperiod (for more details see Demirevska et al., 2008). Soil moisture was controlled daily by gravimetric measurements of the pots. Water was added daily to maintain relative soil humidity of 70 % of the maximal soil moisture capacity. Drought treatment was imposed on 8-day-old plants (fully developed first leaf and expanding second one) for a period of 7 days followed by a 3-d period of recovery by optimal watering. The control plants were watered daily during the whole period. Leaf samples were taken from the control and stressed plants after 7-day drought (14-day-old plants) and from age

control and recovery plants (17-day-old plants). The experimental scheme is shown in Fig. 1. All analyses were performed on the first leaf, which was fully expanded at the beginning of the treatment. Leaf material was frozen in liquid nitrogen.

Experimental Scheme

Water supply	Watering							Watering							Watering		
Days	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Control														C			CR

Water supply	Watering							Water withholding							Leaf sampling - C, D, R, CR
Days	1	2	3	4	5	6	7	8	9	10	11	12	13	14	
Drought														D	

Water supply	Watering							Water withholding							Re-watering		
Days	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Recovery																	R

Fig. 1. Experimental scheme. Leaf sampling was made from control (C), drought treated plants (D), recovered plants (R) and control plants of the same age as recovered plants (CR).

ABA quantification

ABA quantity in wheat leaf extracts was measured using OLCHEMIM Enzyme Immunoassay Kit according to the included instruction.

SDS-PAGE, immunoblot analysis and protein quantification

The soluble leaf proteins were extracted from 0.5 g leaf material in ice-cold 100 mM Tris-HCl buffer (pH 8.0), containing 20 mM $MgCl_2$, 10 mM

$NaHCO_3$, 1 mM EDTA (disodium salt), 2 mM phenylmethanesulfonyl fluoride, 12.5 % glycerol (v/v), 20 mM ß-mercapthoethanol and 2 % (w/v) Polyclar. After centrifugation (15 min, 15 000 *g*, 4 °C), the supernatant was boiled in sample buffer for SDS-PAGE. The proteins were separated by 12 % SDS-PAGE with a *Mini Protean II Dual Slab Cell* (Bio-Rad) according to Laemmli (1970). Samples with protein quantity equivalent to the same FW were loaded for all variants. Gels were stained with Coomassie brilliant blue R-250 or transferred into nitrocellulose membrane (Bio-Rad) as described by Mitsuhashi and Feller (1992) using Trans Blot system (Bio-Rad). RLS, RA, RBP, N-terminus or C-terminus of RLS, ClpP and ClpA were identified using antibodies against the corresponding proteins (Demirevska-Kepova et al., 2008). Polyclonal antibodies against sHSP, HSP110 (Stressgen), monoclonal antibodies against HSP70 (Stressgen) and polyclonal antibodies against DHN (kindly sent from Prof. Close, Department of Botany and Plant Sciences, Riverside, California, USA) were used. Goat-anti-rabbit-IgG (for bridging) and peroxidase-anti-peroxidase soluble complex were used to enhance the sensitivity of the antigen-antibody reaction as described earlier (Mitsuhashi and Feller, 1992). The peroxidase reaction was developed with 4-chloro-alpha-naphtol (Sigma).

The content of total soluble proteins was measured by the method of Bradford (1976) at 595 nm with bovine serum albumin as a standard.

Proteolytic activity

Leaf material (0.5 g FW) was homogenized in 2.5 ml (for controls and recovered) or 3 ml (for drought treated) ice-cold 50 mM Tris-HCl buffer (pH 7.5) containing 2 mM $MgCl_2$, 2 mM $CaCl_2$, 10 mM β-mercaptoethanol, 0.005 % Triton X 100, 50 mg Polyclar AT and centrifuged at 14 000 *g* for 40 min at 4 °C. Proteolytic activity was assayed spectrophotometrically using azocasein as a substrate according to Fisher and Feller (1993). The pH optima were determined preliminarily.

Heat stable proteins

Aliquots from the above mentioned protein extracts were heated at 95 °C

for 10 min, allowed to cool on ice, centrifuged and the protein remaining in solution was precipitated with trichloracetic acid and acetone. Precipitated protein was pelleted by centrifugation, dried, dissolved in SDS-PAGE sample buffer and polypeptides were separated using 12 % SDS-PAGE.

Transmission electron microscopy and morphometric analysis

Samples were prepared according to the standard procedure and embedded in Durcupan resin. The ultrathin sections were stained with uranyl acetate and lead citrate, and examined by a transmission electron microscope (Jeol JEM 1010) operating at 100 kV. The morphometric analysis was performed using the software ImageJ (version 1.36b, National Institutes of Health, USA).

Statistical analysis

Results were based on at least three replicates from three independent experiments. Standard deviations are indicated by vertical bars.

RESULTS AND DISCUSSION

Progressive water stress was induced in plants with a fully developed first leaf by withholding irrigation for 7 days, followed by 3 days of recovery. The effect of 7-d water deprivation followed by a recovery on plant growth is presented in Fig. 2. According to our previous results the growth of the 2nd leaf was inhibited in drought treated plants in the 14-day-old plants of all varieties studied and they did not develop the 3rd leaf (Demirevska-Kepova et al., 2008). In the recovered 17-day-old plants the growth was resumed, and the 3rd leaf appeared. Development of the age controls of 17-day-old recovered plants was more advanced than the one of 14-day-old control plants.

The severity of water stress was determined by analysing changes in shoot fresh weight (FW), water deficit (WD), and electrolyte leakage (EL) (Demirevska-Kepova et al., 2008). The data obtained showed that the increased WD (to 55-60 % at day 7) caused a significant reduction of

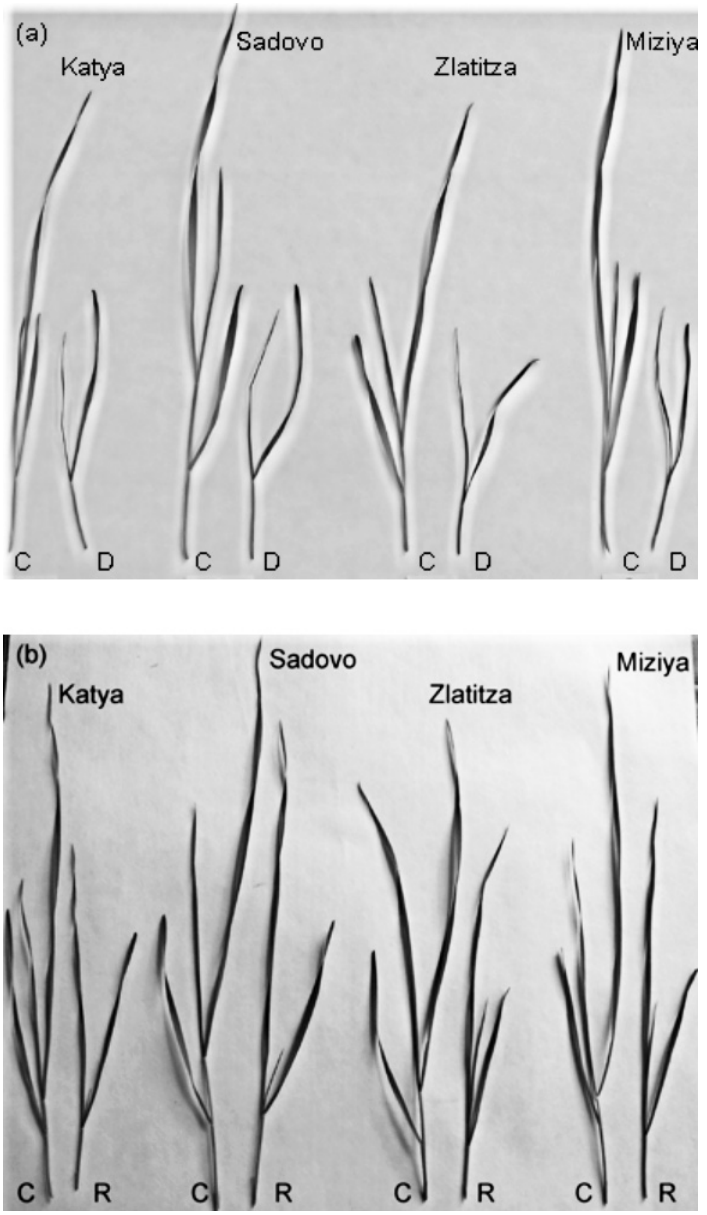

Fig. 2. Shoots of 14-day-old plants from wheat varieties Katya, Sadovo, Zlatitza and Miziya grown in control (C) and drought (D) conditions (a), and 17-day-old plants after recovery (R) with control (CR) of recovery (b).

shoot FW (about 40 %) and a sharp increase of EL (2-3 times). The growth response to drought treatment did not differ among the varieties. Plants restored completely their water status and the parameters studied after re-watering, thus indicating that severe but recoverable drought stress was applied. The physiological response of drought tolerant varieties did not

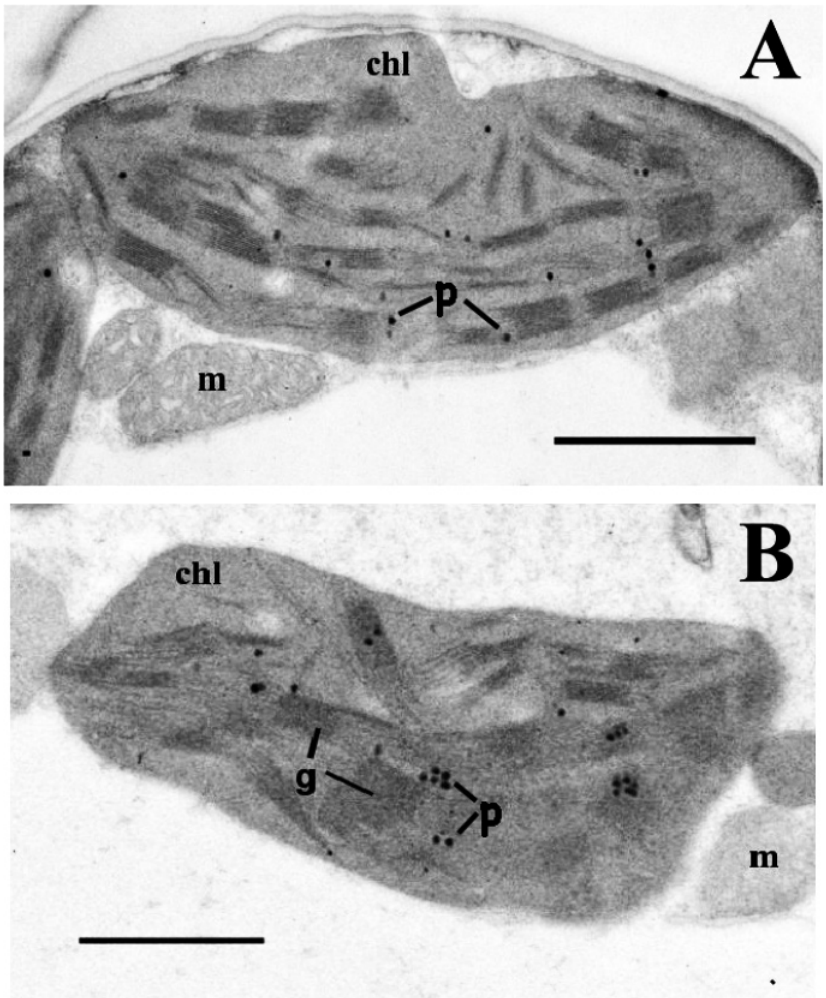

Fig. 3. Transmission electron micrographs of leaf sections from control (A) and drought-stressed (B) wheat plants. Note: m – mitochondria, chl – chloroplasts, g – chloroplast grana, p - plastoglobuli. Scale bar (A) = 2 μm, (B) = 1.5 μm.

differ from the one of drought sensitive varieties at early seedling stage under these conditions.

Plant mitochondria and chloroplasts are involved in many metabolic processes implicated in cell adaptation to abiotic stress. They act as cellular power plants, being involved in the processes of formation of carbohydrates and production of ATP and NADPH (McCabe et al., 2000). Our previous investigations on the structure and functioning of leaf mitochondria from three varieties of winter wheat with different level of drought tolerance showed variety-specific response to dehydration (unpublished data). Examination at a high level of resolution with a transmission electron microscope revealed a marked loss of the internal mitochondrial structure in all three varieties upon drought treatment. However, after the rehydration period, the ultrastructure of drought tolerant variety mitochondria was completely recovered, whereas in the drought-susceptible genotypes some typical damage symptoms still existed. The preliminary comparison of

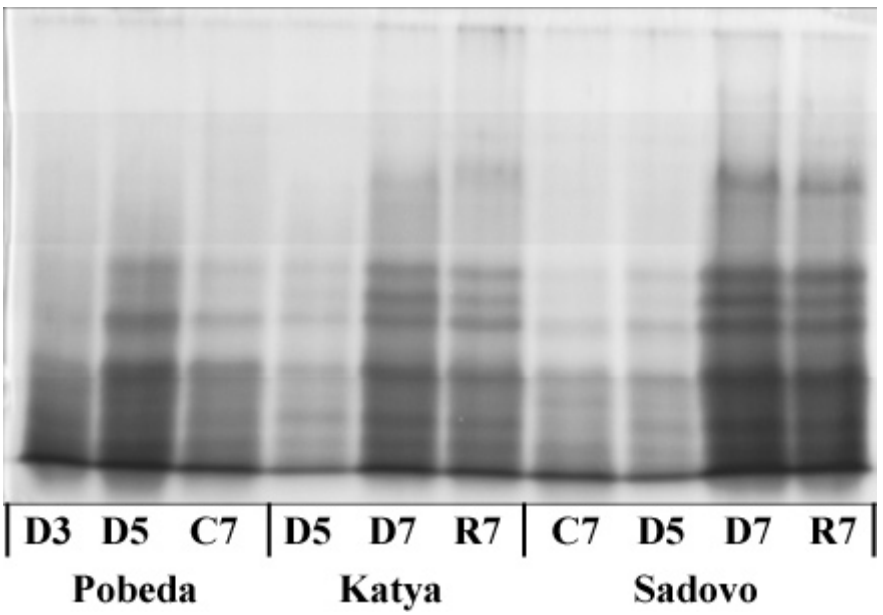

Fig. 4. Heat stable proteins level in wheat leaves from varieties Katya, Sadovo and Pobeda. C – control conditions, D – drought, R – recovery, 3, 5, 7 – days of treatment.

ultrathin sections from control and drought-stressed (Fig. 3) plants showed prominent ultrastructural alterations in the photosynthetic apparatus, such as irregularly shaped chloroplasts, accumulation of plastoglobules and disorganization of the granal stacks.

Abscisic acid levels in the first fully developed leaves of the wheat varieties Pobeda, Katya, and Sadovo were determined during periods of drought and recovery as a part of the study on the physiological basis of differences in drought tolerance (data not shown). Generally, the levels of ABA rose throughout the drought treatment. ELISA determination of ABA quantity in wheat leaves showed that ABA level was elevated during 7 days drought treatment approximately 1.5-fold in variety Pobeda, 2.5-fold in variety Katya and 5-fold in variety Sadovo. Varieties Katya and Pobeda had initially higher ABA levels in non-stressed plants (between 2 – 4 times), which could be regarded as a prerequisite for drought tolerance.

Heat stable proteins (DHN) level was elevated under drought stress,

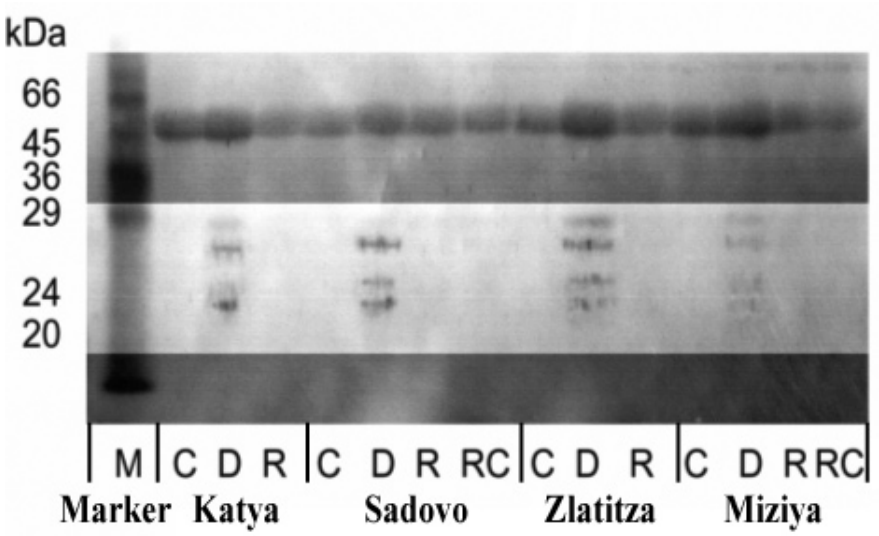

Fig. 5. Immunoblot analysis of extracts from wheat varieties Katya, Sadovo, Zlatitza and Miziya in control conditions (C), drought (D), recovery after drought (R) and age control for recovery (CR) using polyclonal antibodies against DHNs. Presstained marker (M). Samples with protein quantity equivalent to 5 mg FW were loaded per lane.

especially at day 7 and after recovery (Fig. 4). The expression of dehydrins with MW between 20-30 kDa was observed under drought conditions (Fig.5). The DHN protective mechanism regarding plant drought tolerance remains still unclear.

Drought applied to wheat plants resulted in increasing HSPs levels (HSP110, HSP70, HSP60 and sHSPs), which were higher, especially for HSP60 (Fig. 6). Obviously the up-regulation of these chaperone proteins is

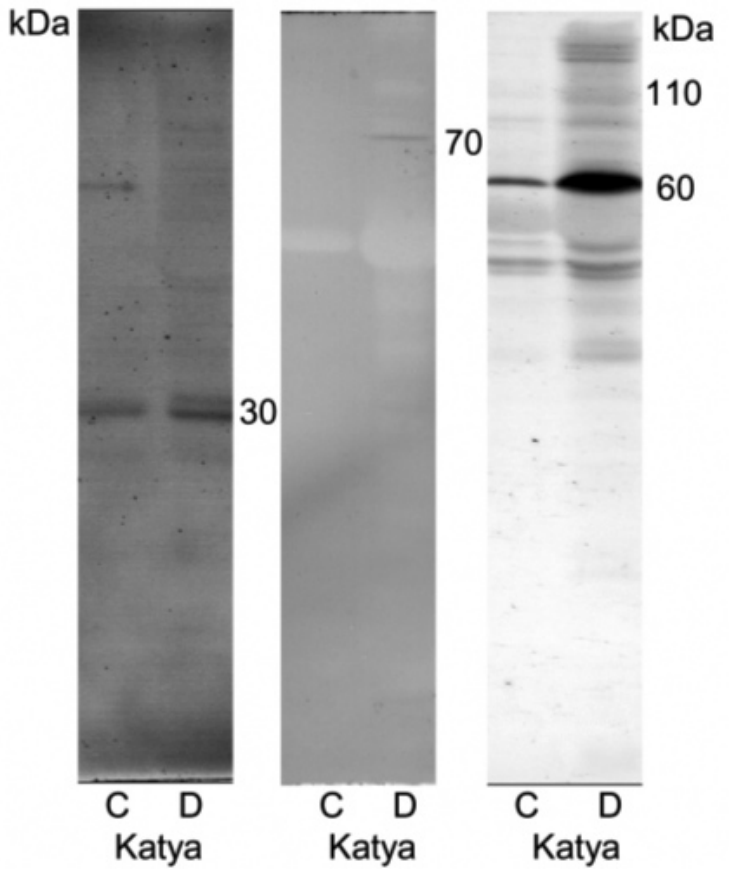

Fig. 6. Immunoblot analysis of extracts from wheat variety Katya in control conditions (C) and drought (D) using polyclonal and monoclonal antibodies against HSPs. Samples with protein quantity equivalent to 5 mg FW were loaded per lane.

very important for wheat plants subjected to drought.

Immunoblot analysis showed more specific changes in the abundance of some individual proteins in wheat leaves under treatment and control conditions (see Demirevska-Kepova et al., 2008). Wheat seedlings subjected to severe but reversible drought stress maintained Rubisco protein quantity and even enhanced it. Similar tendency was observed in other studies, as well (Pääkkönen et al., 1998; Pancović et al., 1999; Pelloux et al., 2001). RA was only slightly affected, while RBP content increased approximately 3 times. The intensities of RBP bands increased by about 50 % under drought stress conditions in the drought tolerant varieties Katya and Zlatitza. These results suggest that RBP plays an important role as a chaperone in plant cells under drought stress, maintaining Rubisco at the appropriate level, necessary for quick recovery. Water deficit, as a result of osmotic stress, probably provokes production of molecular chaperons (Zang and Komatsu, 2007) including RBP.

Both N-terminus and C-terminus of RLS were revealed in weaker intensities in the controls of the drought tolerant varieties. Some degradation products of RLS were also detected (see Demirevska-Kepova et al., 2008). Their intensities increased under stress conditions and diminished during recovery. They may be a consequence of non-enzymatic Rubisco degradation (Ishida et al., 1997).

An enhanced response of Clp proteases under severe drought stress and after recovery was observed (see Demirevska-Kepova et al., 2008). The drought resistant variety Katya exhibited the strongest ClpP proteases induction under drought stress and during the recovery period. Clp proteases function in plant stress response and their physiological substrates are far from being elucidated. In our experiments Clp proteases were more abundant under severe drought. Our studies are in agreement with the findings of Nakashima et al. (1997), who showed up-regulation of Clp protease subunit homolog in *Arabidopsi*s under water stress and senescence. Physiological substrates of Clp proteases under dehydration still remain to be elucidated.

Azocaseinolytic activity increased significantly under severe drought and diminished after recovery (Fig. 7). Dynamics of protease activity followed inversely the changes in leaf protein content. The drought resistant

varieties Katya and Zlatitza had higher protease activity in the controls but a negligible increase in proteolytic activity under severe drought. In the drought-sensitive varieties Miziya and Sadovo, decreased protein content was observed under drought in agreement with higher azocaseinolytic activity at pH 5 and pH 8.5 on the 7th day of drought, whereas in the drought-resistant varieties the increase in azocaseinolytic activity was comparatively weak. The major contribution of the proteolytic activity at pH 5 was the one

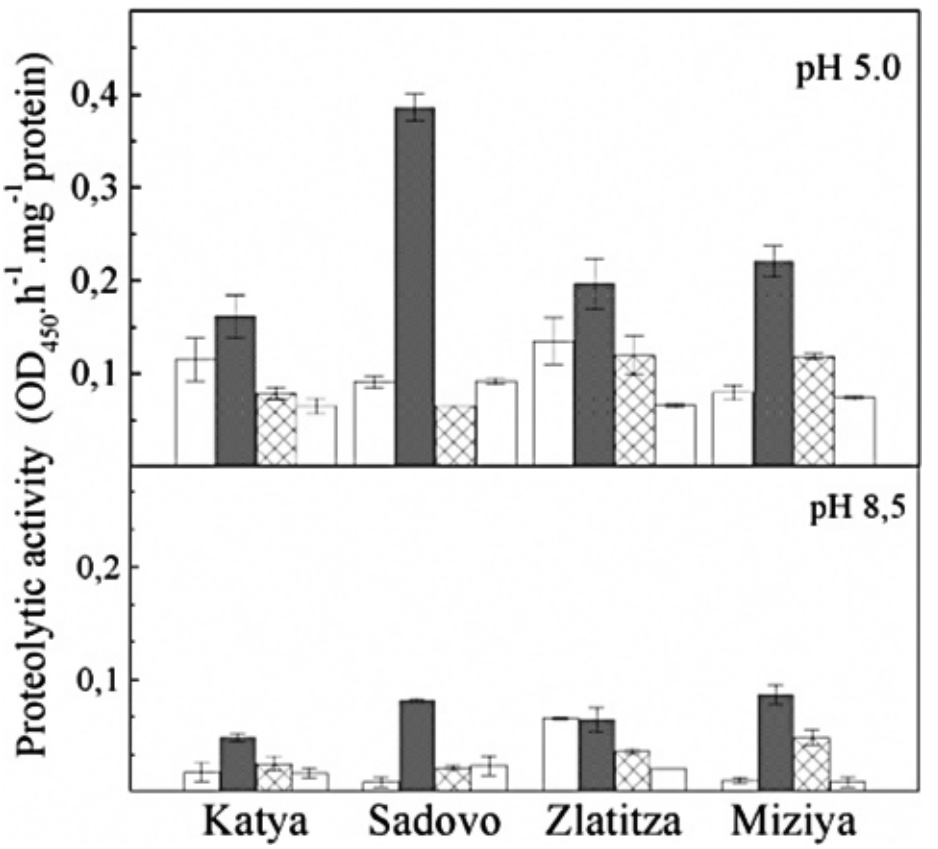

Fig. 7. Leaf proteolytic activity at pH 5.0 and pH 8.5 with substrate azocasein. White columns – control plants; grey columns – plants after 7 days drought; checked columns – recovered plants; white columns – age control of recovery. Values are means of three replicates. Vertical bars indicate standard deviations. Abscissa - varieties under study.

related to vacuolar proteases. It seems that vacuolar proteases are involved in drought sensitivity rather than in drought resistance mechanisms.

CONCLUSION

Progressive water stress in wheat was induced by withholding irrigation for seven days at a fully expanded first leaf developmental stage, followed by a three-days recovery period. The physiological response of drought tolerant varieties did not differ from the one of drought sensitive varieties at early seedling stage under these conditions. The comparison of ultrathin sections from control and drought-stressed plants revealed prominent changes in mitochondrion and chloroplast fine structures. The drought tolerant varieties had higher levels of RBP and Clp proteases. Variety specific differences in protease response were observed. The results obtained point to the role of the predominant endogenous proteolytic activities in the mechanisms of drought sensitivity. The drought tolerant varieties Katya and Zlatitza showed higher level of RBP in the control and drought stressed plants compared to the sensitive varieties. The high level of this protein could be useful as a marker for drought tolerance.

Consequently, the application of severe but recoverable drought stress to wheat varieties with different drought tolerance led to a coordinated response of Rubisco, RA, RBP, HSPs and Clp proteases, which could contribute to plant stress tolerance.

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