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RESEARCH PAPERS

Drought Tolerance Depends on the Age of the Spring Wheat Seedlings and Differentiates Patterns of Proteinases[1]

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Abstract—The majority of plant species lose their ability to tolerate severe water deficit after germination at the beginning of seedling growth, in the time of emergence of the radical from the seed. The experiment was designed to compare the differences in proteolytic response between 4-and 6-days old spring wheat (*Triticum aestivum* L.) seedlings of Eta cultivar, respectively tolerant and sensitive to severe drought inducing up to 90% water saturation deficit (WSD). In coleoptiles the changes of proteolytic activity had the same trend regardless on the seedlings age and increased about fourfold upon 85% WSD as compared to the control, from about 4 to 19 (U/mg protein h). The dehydration of roots of 4 day old seedlings resulted in sharp, fivefold activity increase at 85% WSD (from 11 to >50 U/mg protein h). In roots of 6 days old seedlings dehydrated to 55% WSD the proteolytic activity raised twofold and was about 2.5 times higher than in roots of 4 days old seedlings dehydrated to the same WSD. In coleoptiles of both the 4- and 6-days old seedlings subjected to drought appearance of new bands of serine proteinases was observed. Presented results indicate that roots are more sensitive to drought than coleoptiles, which brings an argument for breeders showing that programs involving roots phenotyping have a full biochemical rationale.

Keywords: Triticum aestivum, cereals, coleoptile, proteolytic activity, root, water deficit

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INTRODUCTION

Continuously increasing food demand, resulting from a growing human population as well as economical determinants of farming profitability put the permanent requirement for the productivity improvement in front of crop breeders. Maintaining the steady growth rate of yields, parallel to the rate of population increase is possible on the bases of plant biological potential exploration empowered by the knowledge about mechanisms of plant responses to environmental stresses [1]. Since the current statistical data shows, that abiotic stresses like salinity, drought, heat or frost, affect approximately 40% of the world land area and can limit, even by half, the agricultural production worldwide, the scientific investigations on improvement of plant stress tolerance can help in handling that difficult breeding task.

Under environmental conditions inducing tissue dehydration (mainly drought, heat, cold, frost) the proteome rebuilding is an important mechanism regulating the tissues homeostasis and plant adaptation or tolerance as well as plant restoration. The proteome regeneration comprises the degradation of redundant, misfolded or damaged proteins in parallel to modifications of proteins in turn over processes or in the final step of de novo protein synthesis. Proteinases play a key role in those processes. There are 2 main systems of proteinases classification. The older one is based on the types of chemical reactions catalyzed by the enzyme as listed in the Enzyme Classification (EC), published since 1961 by the International Union of Biochemistry and Molecular Biology. According to the newest, 6th edition from 1991 proteolytic enzymes belong to hydrolases with specific activity of proteinases with active centers containing serine and histidine (serine proteinases EC 3.4.21), cysteine (cysteine proteinases EC 3.4.22), two aspartic acid residues (aspartic proteinases EC 3.4.23) or bivalent cation of Zn^{2+} (metalloproteinases EC 3.4.24).

The recent system of proteinases classification comprises only the proteolytic enzymes and theirs inhibitors and is based on statistically significant similarities in theirs amino acid sequences (http://merops.sanger.ac.uk), distinguishing proteinase families,

[1] The article is published in the original.

Abbreviations: E-64—trans-epoxysuccinyl-l-leucylamido-(4-guanidino)butane; HSP—heat stress protein; LEA—late-embryogenesis abundant protein; PMSF—phenylmethanesulfonyl fluoride; WSD—water saturation deficit.

grouped in clans. At present in total about 3000 individual peptidases and inhibitors are deposited there. For wheat (*Triticum aestivum* L.) 60 sequences, grouped in 11 clans: AA, CA, CD, MH, MP, PB, PC, SB, SC, SJ and SK were found (accession date 25.02.2016). Clan AA consists of 11 peptidases of the structure of pepsin, with 2 aspartic residues in the active center and in majority with acidic pH optimum. Clan CA consists of 17 wheat proteolytic enzymes (mostly endopeptidases) of the structure of papain, with cysteine in the active center. That clan comprises RD21 peptidase encoded as MER088854 and MER088855. The family C1 of CA clan groups predominantly exopeptidases. Clan CD counts presently 2 wheat accessions of caspase-like proteinases with histidine and cysteine in their active centers. Clan MH comprises 2 metalloproteinases. Five wheat proteinases are classified in clan PB, which groups self-processing, N-terminal nucleophile (Ntn) hydrolases with endopeptidase activity and cysteine, serine or threonine in the active center. PC clan, which contains cysteine and serine proteinases of specific activity of gamma-glutamyl hydrolases has one wheat deposit. Clan SB contains also one wheat-origin enzyme, by definition, subtilisin like aspartic peptidase. While clan SC contains 4 wheat serine peptidases of endo- or exopeptidase activities. One wheat serine endopeptidase contains SJ clan, whereas SK clan contains 2 wheat serine ClpP peptidases from S14 family and 3 belonging to S41 family. In general wheat aspartyl, serine, cysteine and metalloproteinases deposited in MEROPS database are differentiated by protein tertiary structures, which results in classification to different MEROPS clans [2].

Changes of proteolytic activities under various abiotic stresses were observed for many plant species, for example pea roots [3], common bean and *Vigna* [4, 5], spinach [6] and *Ramonda serbica* [4]. As a reaction to water deficit an increase of vacuolar proteinases activity was detected: in wheat leaves serine proteinases were dominating, twofold increased activity of the cysteine proteinases in parallel to twofold decrease of aspartic ones [7]. Similar induction of cysteine proteinases in pea coleoptile, pea nodule cells [8] and in *Arabidopsis thaliana* [5] has been reported. Serine proteinases are ATP-dependent and act in parallel to CtpA proteinase and thylakoid-bound FtsH metalloproteinase. The DegP serine proteinase is critical for the biogenesis and maintenance of PSII [9]. Serine proteinases in senescence broccoli florets were found [10]; from spinach chloroplasts, two subunits of alkaline serine proteinase with maximal activity in 50°C at pH 8.5 were purified using the HIC–HPLC [6]. Increased activities of serine proteinase [5] in bean leaves had been detected in response to water deficit. In turn the cysteine proteinases are highly resistant and act in acidic pH of apoplasts, vacuoles, or lysosomes during cell aging or apoptosis [11] in an ATP-independent way [12]. Most known cysteine proteinases induced by senescence: SAG12 and RD21 have been structurally characterized [13]. Cysteine proteinases were also purified from germinating barley grains [14], broccoli florets [10], and castor bean leaves [11]. All had the acidic pH. Aspartic proteinases, mainly caspases and metacaspases [13] participate in processes of apoptosis and programmed cell death (PCD). They are well characterized in mammals and are crucial for housekeeping and for recovery from pathological processes [15]. Increased activities of aspartic proteinases [4] in bean leaves had been detected in response to water deficit. In wheat grain, aspartic proteinases were detected and isolated by affinity chromatography in denaturing conditions. Studies of *A. thaliana* dwarf mutant, impaired in carbohydrate metabolism, enabled characterization of NANA, chloroplast-located, aspartic proteinase [1]. Metalloproteinases recently have been identified in ligomeric complexes of plant mitochondrial and chloroplast membranes: in mitochondria, as a part of cytochrome bc1 and in chloroplasts as a part of heterocomplex degrading protein D1, the core protein of photosystem II (PSII) [15]. In *Arabidopsis*, 12 genes encoding proteins homologous to *Escherichia coli* FtsH metalloproteins were identified [16].

This paper contains the data showing proteolytic activities and proteinases profiles in relation to the drought tolerance of coleoptiles and the roots of spring wheat. Seedlings grown for 4 and 6 days were chosen since according to [17] the drought tolerance of younger seedlings in opposite to drought susceptibility of older ones is a good model for such a studies.

MATERIALS AND METHODS

Spring wheat seedlings growth and drought treatment. Experiments were carried out on spring wheat (*Triticum aestivum* L., *cv.* Eta). Three days after rehydration, percentage of survived seedlings was calculated as the number of seedlings resuming growth. Grains were surface-sterilized with 1% NaOCl for 20 min and then rinsed several times with distilled water. After germination, fourfold replica of 25 germinated grains were placed side by side between double filter paper strips, rolled up and grown in plastic boxes in a climatic chamber with the day/night temperature of 22/18°C and 12 h photoperiod, relative humidity of 60/70% and photon flux density (PPFD) of 100 μmol/(m^2 s) [18]. Seedlings were fertilized with Knopp solution supplemented with Hoagland's micronutrients. In order to evoke water deficit in seedlings, the nutrient solution was drained off and the containers were left open to dehydrate in the growth chamber for 4 days. After this treatment, seedlings were re-watered. 96 hours after rehydration the percentage of survived seedlings was calculated as the number of seedlings resuming growth. The periods of the seedlings growth and dehydration were determined in separate preliminary experiments. Seedlings

were collected every 24 h for 8 consecutive days and each batch of sampled material was dried for 120 h with continuous 6 hourly check of water saturation deficit (WSD). On that basis, the seedlings tolerance and sensitivity to drought (seedlings grown for 4 and 6 days) as well as the optimal time of seedlings dehydration (96 h) were chosen. The optimal dehydration time induced the same WSD, regardless on seedlings age.

Measurement of water saturation deficit (WSD). The water saturation deficit (WSD) in the seedlings was measured according to Turner method [19] and calculated according to the formula: WSD (%) = (weight of fully turgid leaves—actual fresh weight)/(fully saturated leaf weight-dry weight) × 100%. One hundred plants in 3 repetitions were used. In preliminary experiments the time needed for tissue dehydration to required WSD was determined.

Determination of proteolytic activity. The proteolytic activity was determined spectrophotometrically using azocasein as a substrate. About 1 g of fresh weight of coleoptiles and root tissues were grounded in liquid nitrogen and homogenized in 5 mL of extraction buffer (50 mM HEPES, 1 mM EDTA, 1 mM DTT with 0.2 g PVP at pH 7.5). Extraction was carried for 30 min on magnetic stirrer at 4°C, then homogenate was filtered and centrifuged for 20 min at 20000 *g* (Sigma 3K30). The 1 mL reaction mixture contained: enzymatic extract, 0.25 M citrate/phosphate buffer pH 5.2 and 0.5% azocasein. The pH 5.2, which is the optimal pH of reaction was chosen in previous experiments (data not shown). The reaction was carried out for 2 h at 37°C and stopped by adding of 1 mL of 24% TCA. The acid soluble products were determinate spectrophotometrically at 340 nm. One unit of azocaseinolytic activity was defined as the amount of the enzyme causing a 0.01 increase in A340. The protein content in coleoptiles and root extracts was determined according to [20] using BSA as a standard. Specific diagnostics inhibitors were used to characterize endopeptidases: 10 mM PMSF for serine endopeptidases, 15 μM E-64 for cysteine endopeptidases, 25 mM pepstatin for aspartic endopeptidases and 20 mM EDTA for metallopeptidases. The inhibitors were added to enzymatic extract, then reaction mixture was pre incubated at 4°C for 1 h before 0.5% azocasein was added. The specific proteinase activity was calculated from the difference between the total azocaseinolytic activity and activity measured using specific inhibitor [14].

Classification of proteinases on zymograms. Soluble proteins were extracted by grinding coleoptiles and root tissues in buffer (50 mM sodium acetate pH 4.5, 1 mM EDTA, 2 mM cysteine) and centrifuging at 18000 *g* for 15 min at 4°C. The supernatant was dialyzed against 50 mM sodium acetate (pH 5.0). Electrophoresis gels were prepared according to [21] including 0.1% gelatin as a proteinase substrate. Electrophoresis was carried out in a MiniProtean II system at 25 mA. Loading buffer contained: 50 mM Tris–HCl pH 6.8, 2% SDS, 0.1% bromophenol blue, 10% glycerol. Electrophoresis gels were then washed in 2.5% Triton X-100 for 30 min at room temperature to remove SDS. Gels were incubated at 40°C for 15 h in the assay buffer (50 mM sodium acetate pH 4.5 with 2 mM cysteine). The gels were then stained for 30 min in 0.1% Amido Black dissolved in mixture of 45% methanol and 10% acetic acid to reveal clear bands of proteinase activity on a blue-dark background. For classification of proteinases on zymograms gelatin SDS-PAGE was used. To assign activity bands to proteinases, the class-specific inhibitors were used: 10 mM PMSF for serine proteinases; 10 μM E-64 for cysteine proteinases; 25 mM pepstatin for aspartic proteinases and 20 mM EDTA for metalloproteinases. Prior to electrophoresis, the samples were incubated for 30 min on ice with inhibitor. After electrophoresis and the Triton X-100 wash, the gels were incubated for 30 min at room temperature with the assay buffer containing inhibitor and after buffer exchange into a new assay buffer gels were incubated 6 h at 37°C. Staining and destaining were conducted as described above.

Statistical analysis. The results presented here are the mean values with standard deviations of n ($n = 9$) measurements from 3 to 5 independent experiments. Differences between treatments were tested with a one-factor analysis of variance (ANOVA) to make comparisons to control values. The mean values were compared by the Tukey's Honestly Significant Difference (HSD) Test ($P < 0.05$) and marked with different letters on the figures. Statistical analysis was performed using SAS system 9.2 software (SAS Institute, 2009).

RESULTS AND DISCUSSION

The Impact of the Seedling's Age on Its Ability to Tolerate Dehydration

The majority of plant species lose their drought tolerance after germination at the beginning of seedling growth, in time of emergence of the radical from the seed. The first 4 days of germination and growth are the period of intensive cell division and differentiation, followed by an elongation growth phase which is the most sensitive to the lack of water [4]. In our experiment spring wheat seedlings were drought tolerant till the first leaf appeared and they then began to lose tolerance at the 5th day of growth. Up to the 4th day more than 90% of them re-grew after re-watering. On the 5th day only 62% re-grew and this ability decreased with each consecutive day down to 17% for seedlings grown for 8 days in optimal conditions (Fig. 1a). For seedlings dehydrated for 96 h, WSD increased above the level of 85% for both coleoptiles (Fig. 1b) and roots (Fig. 1c) independently on seedlings age. Considering this, the dehydration intolerance seems to be associated more with the stage of

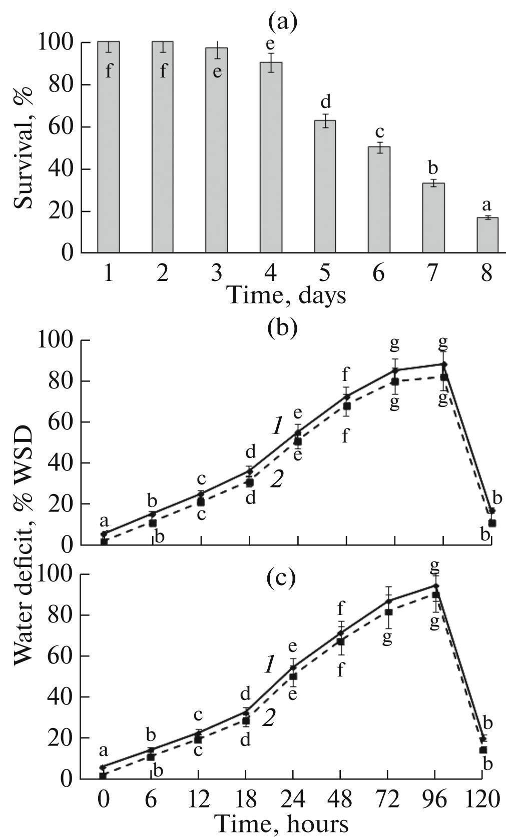

Fig. 1. Changes in ability to drought toleration in spring wheat seedlings grown from 1 to 8 consecutive days, expressed as a percentage of seedlings survival rate (a) and evidence of its relation to the growth stage by presentation of the changes in WSD in coleoptiles (b) and roots (c) of seedlings grown for 4 (*1*) and 6 days (*2*).

seedling growth, as it was previously suggested. A similar loss of tolerance was observed also for other cultivars of spring wheat [22].

Table 1. Changes in total proteolytic activity [U/(mg protein h)] in coleoptiles and roots of 4- and 6-day old spring wheat seedlings grown under optimal conditions (control) and subjected to deepening WSD

	Coleoptile		Root	
	4-day	6-day	4-day	6-day
Control	4.25a	4.90a	11.34a	17.90b
55% WSD	6.87ab	10.93c	15.24b	39.54e
70% WSD	7.50b	9.76bc	18.77bc	44.79f
85% WSD	19.89e	18.50e	57.40g	43.80f
90% WSD	13.30d	12.30d	30.27d	32.45d

Proteolytic activity was measured spectrophotometrically using azocasein as a substrate. Different letters mean significance at $P < 0.05$ according to Tukey`s test.

Changes of Total Proteolytic Activity in Response to Water Deficit

Successive dehydration of seedlings grown under optimal conditions for 4 and 6 days induced proteolytic activity in both organs. In coleoptiles changes of activity had the same trend regardless of the seedlings age. Up to a strong dehydration inducing 70% WSD the activity was low. Then it increased about fourfold as compared to the control, independently of the seedlings age (Table 1). Further dehydration, up to 90% WSD, caused the decrease of proteolytic activity in coleoptiles. The dynamics of changes of the proteolytic activity in roots was different than in coleoptiles. The dehydration of seedlings grown for 4 days and dehydrated to 85% WSD resulted in a sharp increase of proteolytic activity in roots. The 55% WSD dehydration of seedlings grown for 6 days, induced about twofold increase of the proteolytic activity in roots, which was about 3.5 times higher than in the 4 days roots. The activity remained unchanged in further experiment to drop down to the same activity as in the seedlings grown for 4 days at 90% WSD (Table 1). The changes in activity can be related to the loss of drought tolerance in emerging seedlings. The increase of proteolytic activity in plants at vegetative growth stage under drought conditions is a well-known phenomenon. Drought induced proteinases were found in the aerial organs of *Arabidopsis*, pea, common bean and in the coleoptiles of spring wheat [5, 23]. Our experiments have shown similar proteolytic responses in coleoptiles and roots. As long as the young seedlings were not strongly desiccated the proteolytic activity in the roots increased slightly, in opposite to older seedlings in which the roots reacted rapidly from the beginning of the stress duration. Such type of reactions is characteristic for tolerant and sensitive individuals, respectively [4]. The drought induced proteinases were detected mostly as vacuolar ones with optimal acidic pH and in our previous studies the maximum activity was also detected in a range of pH 5.0–6.0 [18].

Comparison of Proteinases Profiles from Control and Drought Treated Seedlings

The zymography reveals also the differences between studied seedlings. To assign the zymography bands to appropriate proteinase the preliminary experiments, with different concentrations of specific inhibitors, were run (data not shown). In extracts from control coleoptiles of seedlings grown for 4 days 2 bands were detected: aspartic proteinase and cysteine proteinase, whereas in older seedlings 3 bands were found: aspartic proteinase, serine proteinase and cysteine proteinase. Water deficit induced 6 more isoforms in coleoptiles of younger seedlings: 1 aspartic proteinase and 5 serine proteinases bands. The extracts from coleoptiles of the control seedlings grown for 6 days were characterized by an additional band of serine proteinase as compared to relevant

younger control. Under drought conditions 3 additional serine proteinases were induced (Fig. 2a).

In the extracts from the roots of control seedlings grown for 4 days 9 bands were detected: 2 aspartic proteinases, 3 serine proteinases and 4 cysteine proteinases. In older seedlings 4 bands disappeared: aspartic proteinase, serine proteinase and 2 cysteine proteinases. Due to the dehydration of younger seedlings 1 aspartic proteinase, 1 serine proteinase and 2 cysteine proteinases bands disappeared, while in older seedlings the number of enzyme isoforms visible on zymograms did not change (Fig. 2b).

There is little work about the zymographic studies of proteinases from coleoptiles and roots. In literature two bands of cysteine proteinases (38 and 36 kD) in control drought sensitive wheat seedlings as well as the appearance of additional three cysteine isoforms upon drought were reported, whereas for triticale and rye subjected to 40% dehydration, only 36 kD and 38 kD bands activity increase was detected [23]. Cysteine proteinases are attributed to house-keeping functions removing the abnormal and misfolded proteins [8] and associated with leaf senescence [11]. In seedlings grown for 4 days a decrease in the number of cysteine proteinases bands was observed, which could be associated with the process of carbonylation of those proteins and the loss of proteolytic activity [24].

Two serine proteinases were detected in the soybeans [25] and were also associated with PCD in *Avena sativa* [26]. The same share of serine proteinases in the roots of seedlings, independent on seedlings age and growth conditions (Table 2) could demonstrate the different biochemical response of roots as compared to coleoptiles and might suggest the importance of serine proteinases for basic metabolism in roots. Additionally, their multiple isoforms, visualized on zymograms (Figs. 2a and 2b), differ in coleoptiles and roots and are differentiated by tissue dehydration, which strengthens the argument about the different biochemical pathways induced in response to drought in both organs. Also the recent transcriptomic studies on drought-treated 11-day-old rice seedlings reinforced the question about different biochemical pathways despite the common genes expressed in leaves and roots [27]. In roots of maize vacuolar serine proteinase (RSSI) was strongly induced as a consequence of sugar starvation [28].

The metalloproteinases activity decreased upon drought in coleoptiles as well as in roots and represented less than 1/10 of the total activity regardless of the seedlings age (Table 2) and were not detected on zymograms (Fig. 2).

Contribution of Specific Proteinases in Total Proteolytic Activity

The share of cysteine proteinases activity in the total activity increased more in the coleoptiles of the drought sensitive older seedlings, than in the drought tolerant younger ones (Table 2). Since cysteine proteinases are highly resistant at acidic or neutral pH, they can operate in proteolytically harsh environments of apoplasts, vacuoles, or lysosomes during cell aging or apoptosis [11]. The higher share in total proteolytic activity of dehydrated drought sensitive coleoptiles may be interpreted as a consequence of the worse physiological state of those seedlings, as indicated by weaker re-growth after re-watering. Such reaction is in accordance with a reported negative correlation between the activities of cysteine proteinases and wheat tolerance to drought [8]. While the increased share of cysteine proteinases in coleoptiles in the response to drought was significant, their participation in the root was not so spectacular, most probably due to the fact of earlier stress occurrence in roots, entailing earlier and different biochemical reaction in coleoptiles [27].

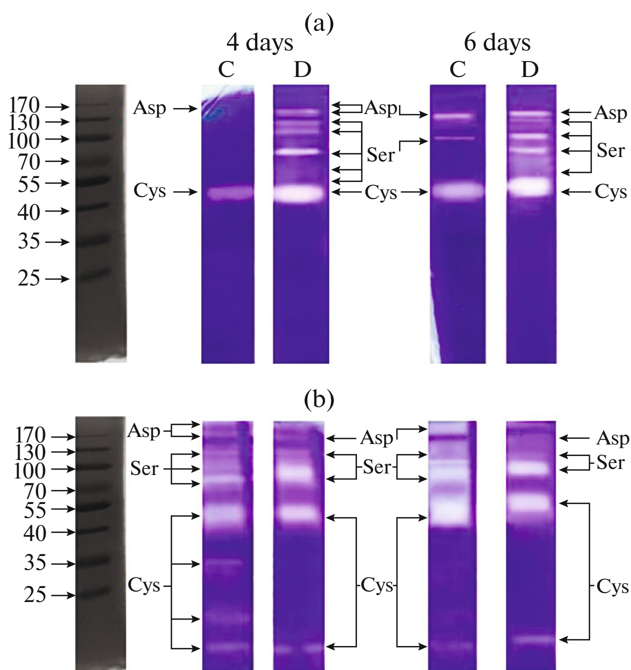

Fig. 2. Proteinases profiles in coleoptiles (a) and roots (b) of 4-day old spring wheat seedlings grown in control conditions (marked C) and subjected to dehydration (marked D), as detected by SDS-PAGE electrophoresis.

In opposite to cysteine proteinases the share of serine proteinases in total proteolytic activity decreased upon drought, in coleoptiles significantly and scarcely in roots, enhancing the image of different reactions to drought in those organs (Table 2). Serine proteinases are supposed to participate in many physiological processes: senescence as well as hypersensitive response to pathogen attack (including the regulation of Rubisco proteolysis) and also in processes of early germination and in differentiation of specialized plant tissues, like

Table 2. Level and percentage contribution of specific proteinases making up the total proteolytic activity [U/(mg protein h)] in coleoptiles and roots of 4- and 6-days old spring wheat seedlings grown under optimal conditions (control) and subjected to 85% WSD (dehydrated)

		control		dehydrated	
		units	%	units	%
Coleoptile					
4 days	Ser	1.66	39	5.19	28
	Asp	0.81	19	2.53	19
	Met	0.51	12	1.60	8
	Cys	1.28	30	3.99	44
6 days	Ser	1.81	37	2.58	21
	Asp	0.88	18	2.34	19
	Met	0.74	14	0.98	8
	Cys	1.47	30	5.41	44
Root					
4 days	Ser	4.54	40	11.20	37
	Asp	3.06	27	10.90	37
	Met	2.04	18	2.12	7
	Cys	1.70	15	6.05	20
6 days	Ser	6.80	38	11.68	36
	Asp	4.48	25	10.71	33
	Met	3.76	21	2.60	8
	Cys	3.04	17	7.46	23

stomata or secondary cell wall. In general, they participate in ATP-dependent proteolysis, however some evidence indicated that they may act exclusively as chaperones, independently from their proteolytic function [24]. Our results are in accordance with studies of drought sensitive common bean [4] and pea varieties [5]. It has been reported that serine proteinase DegP is critical for the biogenesis and maintenance of photosystem II [9] and that *Arabidopsis* mitochondrial ATP-dependent serine proteinases (Lon1 and ClpP) participate in the protection of mitochondrial proteome against the accumulation of carbonylated proteins [9]. Lon1 serine proteinase was also suggested as crucial for the post-germinative growth of seedlings [29]. The gene encoding C1 serine proteinase showed expression in soybean leaves and seedlings, and its expression was intensified by light [25]. Similarly, MCA serine proteinase activity was related to the expansion of tobacco tissues [28]. In our experiments greater decline in the share of serine proteinases in total proteolytic activity in coleoptiles of older seedlings may be indicated for example by a stronger disintegration of photosynthetic apparatus in seedlings which are drought sensitive, which is a common reaction to drought [9]. In younger seedlings their activity could be explained by contribution to tissue growth.

The share of aspartic proteinases in the total proteolytic activity in all types of studied coleoptiles did not change. Simultaneously, a similar increase of share of aspartic proteinases in the total proteolytic activity in the roots was found (Table 2). Aspartic proteinases are involved in processes of plant senescence, programmed cell death (PCD), reproduction and responses to both biotic and abiotic stresses. An increase in the aspartic proteinases activity due to water deficit was detected in drought sensitive cowpea leaves [4]. Their transcriptional and post-transcriptional regulation, faster and stronger in sensitive cultivars of common bean was suggested [4]. The functional analysis of transgenic *A. thaliana* overexpressing VlAP17 gene encoding aspartic proteinase showed an enhanced dehydration tolerance in germinating seeds, seedlings and mature plants as well as the enhanced resistance of plasma membranes. It was also suggested that the VlAP17 gene may be involved in the ABA biosynthetic pathway or may function upstream of it [30].

More studies are needed for full explanation of relations between root to shoot signals in response to soil drought and aspartic proteinase activity [27, 30]. Presented results describe only one of many aspects of plant response to water deficit and on the other hand one of many aspects of proteolytic enzymes function, since proteolytic enzymes play important role in many physiological processes, both under normal conditions as well under stressful ones.

In conclusion, comparative examination of the proteolytic response of spring wheat seedlings subjected to increasing dehydration clearly demonstrated the participation of different proteinases in reorganization of plant metabolism to allow plants to attain a new homeostasis based on metabolic adaptation.

The loss of drought tolerance of wheat seedlings grown for 6 days was connected with higher cysteine proteinases and lower serine proteinases activities in coleoptiles. Presented results indicated that roots are more sensitive to drought than coleoptiles, which brings an argument for breeders showing that programs involving roots phenotyping have a full biochemical rationale. Since the understanding of the phenomena of crop drought tolerance is crucial for the breeding industry, further studies on participation of proteolytic enzymes in processes of plant adaptation are needed.

Abiotic and biotic stresses force degradation of misfolded, damaged or redundant proteins. Proteinases play one of the key roles in those processes. The results obtained in our experiment with spring wheat

seedlings as well as results of other species confirmed, that proteinases participate in plant metabolism reorganization, which allows the plant to reach new homeostasis under stress conditions. Authors are aware, that those results describe only one of the many various possible plant`s response to water deficit. Further studies, covering determination of the gene sequence and expression, the protein accumulation including its localization (both plant and subcellular) are needed to fully understand the mechanisms of plant`s responses to stresses.

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