BIOLOGIA PLANTARUM 52 (4): 687-694, 2008

Involvement of exopeptidases in dehydration tolerance of spring wheat seedlings

A. MIAZEK[1] and B. ZAGDAŃSKA[1,2]*

Biochemistry Department, Warsaw University of Life Sciences, Nowoursynowska 159, PL-02776 Warszawa, Poland[1]
Plant Physiology and Biochemistry Department, Plant Breeding and Acclimatization Institute, Radzików, POB 1019, PL-00850 Warszawa, Poland[2]

Abstract

The observed inability of 6-d-old seedlings of spring wheat (*Triticum aestivum* L.) to tolerate the same water deficit as compared to the 4-d-old seedlings seems to be associated with the higher carboxypeptidase and lower aminopeptidase activities. Free amino acid pools differentiated also the 4-d-old seedlings from the older ones. Dehydration decreased the amino acid content in 4-d-old seedlings, increased it in 6-d-old seedlings and changed composition of amino acid pool. In tolerant phase of wheat seedling growth carboxypeptidase activity increased in response to water deficit and aminopeptidase activity increased in dehydrated seedlings, independently of their age.

Additional key words: aminopeptidases, carboxypeptidases, *Triticum aestivum.*

Introduction

Proteolysis has been suggested as an important mechanism of regulation of cellular activity. It enables reorganization of plant metabolism under water deficit and thus, improves plant dehydration tolerance (Ingram and Bartels 1996, Bray 1997, Wiśniewski and Zagdańska 2001, Vierstra 2004). Unlimited proteolysis and site-specific, limited proteolysis are selective mechanisms regulating protein processing, half-lives and activities of proteins. In plants, cleavage of peptide bonds is mediated by multitude proteolytic pathways within each cellular compartment (Callis 1995, Schaller 2004, Vierstra 2004). The *MEROPS* peptidase database (http://merops.sanger.ac.uk) lists over 650 proteases for *Arabidopsis* and 148 presumably inactive homologs and about 1300 genes devoted to the ubiquitin/proteasome pathway. However, little is known about the roles they play in the life of plants because functions for only a few of *Arabidopsis* proteinases have been determined genetically (Beers *et al.* 2004).

Aminopeptidases (EC 3.4.11) are exopeptidases that catalyze the hydrolysis of amino acid residues from the N-terminus of protein. They are classified according to hydrolyzed residues, *e.g.*, leucine (Leu) aminopeptidases remove more effectively Leu or other hydrophobic residues at N-termini, although bonds to many other residues are also cleaved. Activities of aminopeptidases have been localized in the cytoplasm, chloroplasts and mitochondria (Walling 2006). The exact roles of aminopeptidases in plant cell metabolism remain to be uncertain, although they are known to be induced after seed germination (Wilson 1986), during senescence (Carrasco and Carbonell 1990), or seed and fruit maturation (Palma *et al.* 2002). The activities of aminopeptidases were correlated with host-pathogen interactions (Schaller and Ryan 1995), wounding (Chao *et al.* 1999), and salinity stress (Dubey and Rani 1990). Many aminopeptidases are certainly involved in protein turnover acting post-proteasomally, others are likely involved in activation/deactivation of biologically active peptides (Walling 2006).

Received 15 November 2006, *accepted* 2 June 2007.
Abbreviations: Ala - alanine, Arg - arginine, Asn - asparagine, Asp - aspartic acid, β-NA - β-naphthylamide, Cys - cysteine, DMSO - dimethyl sulfoxide, EDTA - ethylendiaminetetraacetic acid disodium salt, E64 - (2S,3S)-3-(N-{(S)-1-[N-(4-guanidinobutyl)carbamoyl]3-methylbutyl}carbamoyl)oxirane-2-carboxylic acid , HPLC - high pressure liquid chromatography, Gly - glycine, Gln - glutamic acid, Glu - glutamine, Ile - isoleucine, Leu - leucine, Lys - lysine, Met - methionine, PAGE - polyacrylamide gel electrophoresis, Phe - phenylalanine, pCMB - *p*-chloromercuribenzoate, PMSF - phenylmethylsulfonyl fluoride, Pro - proline, PVP - polyvinylpolypyrrolidone, RWC - relative water content, Ser - serine, Thr - threonine, TNBS - trinitrobenzene sulfonate, Trp - tryptophan, Tyr - tyrosine, Val - valine.
* Corresponding author; fax: (+48) 22 5932562, e-mail: barbara_zagdanska@sggw.pl

Carboxypeptidases (EC 3.4.16-18) sequentially hydrolyze amino acid residues from the C-terminus of proteins. Major carboxypeptidases belong to serine type (Mikola and Mikola 1986). In plants, carboxypeptidases are localized in vacuoles (Mehta *et al.* 1996). These enzymes seem to be responsible for storage protein mobilization and degradation (Parrott *et al.* 2005), and cellular protein turnover (Ramirez-Zavala *et al.* 2004). Serine carboxypeptidases have also been implicated in programmed cell death during the development of the vascular tissue in wheat (Dominguez and Cejudo 1998, Dominguez *et al.* 2002), in brassinosteroid signalling (Li *et al.* 2001) and seed development (Cercós *et al.* 2003).

Degradation of misfolded or denatured proteins under water stress is closely coupled with the synthesis of new proteins from the released amino acids (Schaffer and Fisher 1988, 1990, Guerrero *et al.* 1990, Vincent and Brewin 2000). However, knowledge on the effect of water deficit on proteases, especially exopeptidases is very limited and unequivocal (Grudkowska and Zagdańska 2004, Schaller 2004). In response to water deficit, no increases in aminopeptidase activity in potato (Hildmann *et al.* 1992) and in *Arabidopsis* (Bartling and Nosek 1994) or increase activity in tomato leaves (Chao *et al.* 1999) have been noted. Under severe water deficit, the sensitive cultivar of *Phaseolus vulgaris* exhibited a marked increase in the activity of two different aminopeptidases, whereas the resistant cultivar showed a marked decrease in the activity of these aminopeptidases (Hieng *et al.* 2004). To our knowledge, data on carboxypeptidase response to water deficit are absent.

Seedlings of spring wheat are extremely tolerant to water deficiency up to the 4th day following imbibition (Blum *et al.* 1980, Guerida *et. al*. 1997, Miazek *et al.* 2001, Bogdan and Zagdańska 2004) and they have been proposed to serve as a model for studying drought tolerance (Farrant *et al.* 2004). Dehydration tolerance, expressed as percentage of seedling survival following severe dehydration of seedlings for 4 d, was 97 - 100 % only up to 4th day following imbibition and then sharply declined to about 50 % on the 5th day following imbibition (Miazek *et al.* 2001). The observed switch to dehydration intolerance, associated with rapid growth of the coleoptile and appearance of the first leaf, is genetically determined (Bogdan and Zagdańska 2004). Dehydration inhibited the mobilization of endosperm reserves, accumulation of dry matter in wheat seedlings and decreased soluble protein content mostly in shoots of both 4-d-old and 6-d-old seedlings (Miazek *et al.* 2001). Thus, the investigation of the exopeptidases activities and their response to dehydration as related to dehydration tolerance should shed a light on involvement of proteolysis in dehydration tolerance. In the comparative examination of exopeptidase responses to dehydration, experiments were carried out on dehydration tolerant (4-d-old) and dehydration sensitive (6-d-old) seedlings of spring wheat.

Materials and methods

Plants: Seeds of spring wheat (*Triticum aestivum* L. cv. Eta) were surface-sterilised with 1 % NaOCl for 20 min and then rinsed several times with distilled water. After soaking in water overnight at 4 ºC, seeds were allowed to germinate and grow for up to 8 d at the day/night temperature of 22/18 °C, relative humidity of 60/70 %, 12-h photoperiod, and photosynthetic photon flux density (PPFD) of 100 μmol $m^{-2}s^{-1}$. Four and 6-d-old seedlings were dehydrated on dry filter paper in the growth chamber for 4 d under the same conditions. This treatment led to the same water deficit (about 20 % RWC), irrespective of the seedling age (Miazek *et al*. 2001). Water deficit in the seedlings was characterized by relative water content (RWC), which was calculated as a ratio of (fresh mass - dry mass) to (water saturated mass - dry mass). After this dry treatment, seedlings were rehydrated and 3 d after rehydration, percentage of survived seedlings was calculated from the number of seedlings resuming growth. Biochemical analyses were performed separately for shoots (coleoptile and the first leaf) and roots.

Free amino acid determination: Samples for free amino acid analysis were prepared according to Tonecki *et al.* (1989). Analyses were carried out on HPLC (*Waters 625 LC System*, Milford, USA) according to Waters Manual.

Enzyme extraction: Each day of the experiment about of 1 g of tissue of shoots (coleoptile and the first leaf) or roots was ground to a fine powder in liquid nitrogen in a cold ceramic mortar. Total protein was extracted from liquid nitrogen powder in 5 cm^3 of cold extraction buffer (50 mM TRIS-HCl pH 7.5) containing 1 % (m/v) insoluble polyvinylpolypyrrolidone (PVP) and 2 mM β-mercaptoethanol for aminopeptidase assays and in 5 cm^3 of cold 50 mM acetate buffer pH 4.6 for carboxy-peptidase assays. The extraction was carried out at 4 °C for 60 min a magnetic stirrer. The extracts were filtered and centrifuged at 20 000 *g* for 20 min at 4 °C and the supernatants were directly used for the enzyme assays and native polyacrylamide gel electrophoresis (PAGE). Soluble proteins were determined according to Bradford (1976), using bovine serum albumine as a standard.

Analysis of exopetidase activities*:* Aminopeptidase activity was assayed in crude extract with L-aminoacyl-β-naphtylamides as substrates according to Kolehmainen and Mikola (1971). The enzymatic hydrolysis of L-aminoacyl-β-naphtylamides was measured by direct coupling of the liberated with stabilized diazonium salt, Fast Garnet GBC. In the routine assays the reaction mixtures contained 0.95 cm^3 50 mM Tris-HCl buffer pH 7.5, 0.025 cm^3 1 mM L-aminoacyl-β-naphtylamide in

DMSO and 0.025 cm^3 of enzyme solution, and they were incubated at 37 ºC for 30 min. The enzymatic reaction was terminated by addition of 0.5 cm^3 of freshly prepared 0.1 % m/v *Tween 20*. The colour developed in 5 min at room temperature and remained constant for several hours. The absorbance was read at 525 nm and compared to a standard curve prepared with free β-naphtylamine. To measure the degree of inhibition of aminopeptidases activities, extract were pre-incubated with the solution of inhibitor in 50 mM Tris-HCl buffer pH 7.5 at 4 °C for 60 min and reaction was started by adding L-aminoacyl-β-naphtylamides as substrates. Reaction was further assayed using the same method as for aminopeptidase activity. One unit of aminopeptidase activity is defined as nmol(liberated amino acid) mg^{-1}(protein) min^{-1}.

Carboxypeptidase activity was assayed according to the method of Mikola and Kolehmainen (1972). The assay of 0.005 cm^3 of N-carboxy-Z-L-Phe-L-Ala or other substrates (2 mM solution in dimethylsulphoxide, DMSO), 0.515 cm^3 of 0.01 M acetate buffer at pH 4.6 and 0.005 cm^3 enzyme extract was incubated for 20 h at 30 ºC. The reaction was stopped after incubation for 60 min with 1 cm^3 freshly prepared trinitrobenzene sulfonate (TNBS) by the addition of 0.5 cm^3 acetic acid. The sample was incubated for 60 min at room temperature. Substrate was omitted for blanks. The absorbance of the liberated C-terminal amino acid reacted with TNBS was measured at 340 nm. One unit of carboxypeptidase activity is expressed as nmol(liberated amino acid) mg^{-1}(protein) min^{-1}.

Aminopeptidase activity gel assay: Analytic electrophoresis under the non-denaturating conditions was performed according to Laemmli (1970) using *Bio-Rad* (*Mini Protean II*., *Bio-Rad Laboratories*, Hercules, USA) Electrophoresis was run in 25 mM Tris-Gly buffer pH 8.3, at constant 30 mA current intensity. Samples for native PAGE were prepared from enzyme extracts by mixing with 10 % sucrose solution and 0.002 cm^3 bromophenol blue. After electrophoresis gels were incubated for 45 min in 1 mM buffered substrate solution (50 mM Tris-HCl buffer, pH 6.8) in 37 °C and aminopeptidases were visualized by the addition of 0.1 % Fast Garnet GBC Salt (Kolehmainen and Mikola 1971). After 10 min the reaction was stopped by washing gels in deionised water.

Statistical analysis: The results represent the average of three independent experiments in three replicates (n = 9). A significance of differences was computed as the Least Significant Difference (LSD) and the mean values obtained were compared by the Tukey′s Honestly Significant Difference Test ($P \leq 0.05$).

Results

Free amino acid content: The total pool of free amino acids was higher in shoots of 4-d-old seedlings than in roots and decreased about 3.5 fold in 6-d-old shoots whereas decreased only slightly in roots (Fig. 1). Under water deficiency the total pool of amino acids increased about 15 % in shoots and about 25 % in roots of 4-d-old seedlings whereas any change has been observed in the content of free amino acids in shoots and roots of 6-d-old seedlings. Changes in the total amino acid content were accompanied by a shift in the composition of the free amino acid pool. The content of the α-ketoglutarate and the pyruvate-derived amino acids in dehydrated 4-d-old seedlings increased. In case of 6-d-old seedlings, the similar shift was only observed in roots.

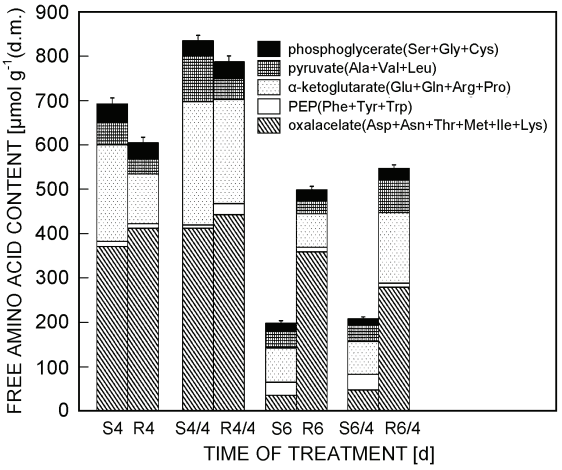

Fig. 1. Effect of dehydration on free amino acids in 4- and 6-d-old shoots (S4 and S6, respectively) and roots (R4 and R6, respectively) of spring wheat seedlings grown under optimum conditions and dehydrated for 4 d (4/4 and 6/4, respectively). Amino acids derived from a common precursor were grouped together. Means of three independent experiments.

Aminopeptidases: The maximum activity of aminopeptidases in crude extracts measured against various substrates was at pH 7.5 with one exception: optimum pH for these enzymes measured against Phe-β-NA was 7.0 (data not shown). β-naphtylamides of ten amino acids were used to compare of aminopeptidase activities. These enzymes preferentially hydrolyzed Phe-β-NA, although the naphtylamides of Leu and Tyr also were substantially cleaved (data not shown). The enzymes scarcely hydrolyzed the Cys-β-NA and Ser-β-NA.

With the progression of seedling growth, aminopeptidase activities increased and attained maximum activity in 4-d-old shoots and then, in case of activity towards Phe-β-NA and Leu-β-NA decreased, whereas in case of Ala-β-NA and Gly-β-NA, activity of these enzymes was stable up to 6 d after imbibition and then decreased (Fig. 2*A*). Dehydration caused a decrease in

aminopeptidase activity towards Phe-β-NA, Leu-β-NA and Arg-β-NA, however activity of aminopeptidases increased considerably towards Ala- and Gly-β-NA both in 4- and 6-d-old shoots (Fig. 3*A*,*B*).

Aminopeptidase activity towards Phe-, Leu-, Ala- and Arg-β-NA decreased in roots of spring wheat seedlings with the progression of growth, while measured against Gly-β-NA increased from 40 to 67 U mg^{-1}(protein) min^{-1} on the 8th day of growth (Fig. 2*B*). Dehydration decreased the activity of aminopeptidases measured towards Phe-, Leu- and Arg-β-NA in roots of both 4 and 6-d-old seedlings, whereas activity towards Ala-β-NA and Gly-β-NA increased twice in both 4- and 6-d-old seedlings (Fig. 3*C*,*D*).

Table 1. The effect of inhibitors on aminopeptidase activities of wheat shoots. Activity assays were carried out with Ala-β-NA and Phe-β-NA as substrates. Values are % of control activity. $LSD_{0.05}$ = 5.02.

Inhibitor	Conc. [mM]	Ala-β-NA	Phe-β-NA
E-64	0.03	60.2	57.7
pCMB	0.05	4.3	5.2
Pepstatin A	0.05	96.8	100.0
PMSF	2.50	87.4	50.2
EDTA	5.00	4.7	93.1
1,10-Phenantroline	10.00	1.9	74.8

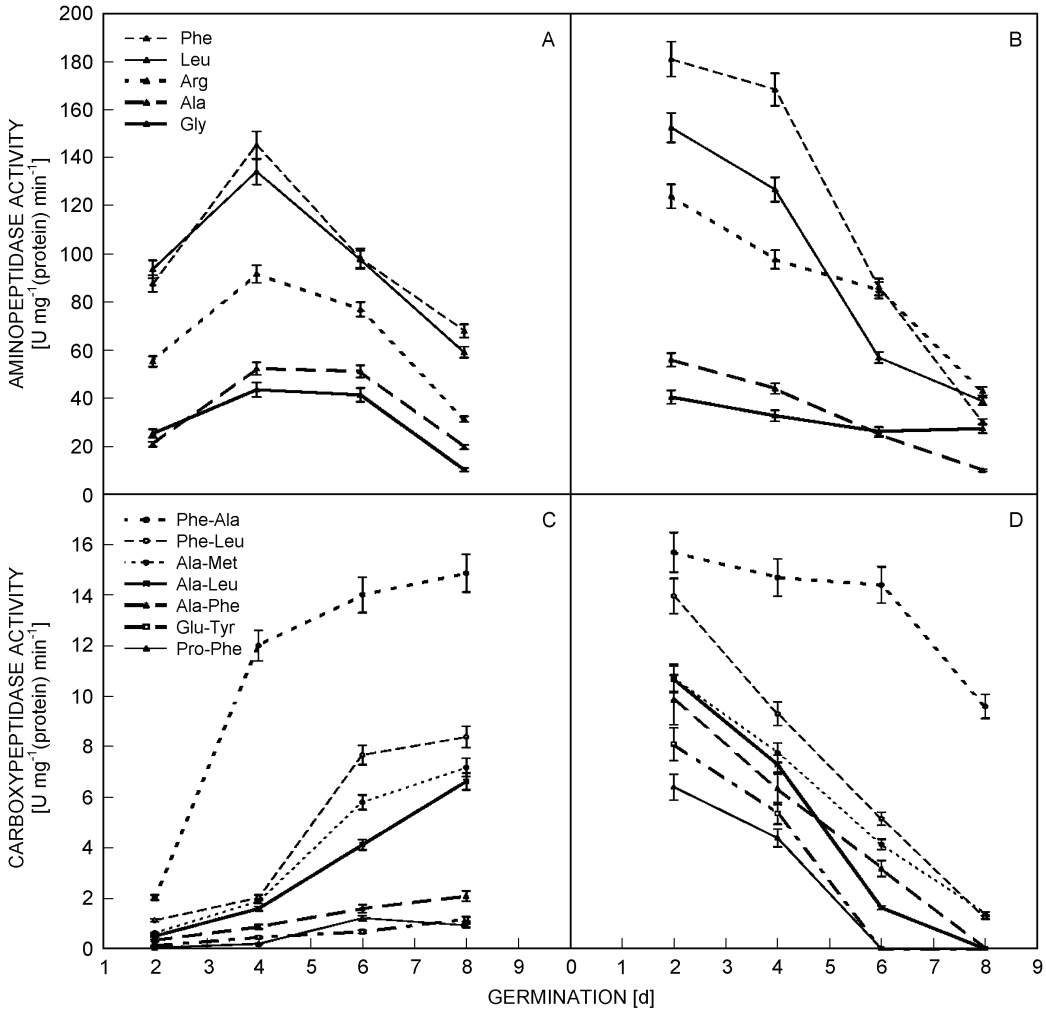

Fig. 2. Changes in aminopeptidase and carboxypeptidase activities in shoots (*A* and *C*) and roots (*B* and *D*) of control wheat seedlings grown under optimal conditions. SD values are indicated.

The effect of potential inhibitors on the catalytic properties of aminopeptidases indicated that consistent inhibition was observed in the presence of 1,10-phenantroline, EDTA and pCMB when measured towards Ala-β-NA (Table 1). However, only pCMB was effective inhibitor towards Phe-β-NA as a substrate.

Using native PAGE two different forms of aminopeptidases were identified (Fig. 4). Aminopeptidase 1, in extracts from both shoots and roots, cleaved Ala-, Arg-, Gly-, Leu- and Phe-β-NA and aminopeptidase 2 cleaved

Leu- and Phe-β-NA only in shoot extract. Dehydration of seedlings intensified the band 2 of shoot aminopeptidases which hydrolyzed Leu-β-NA.

Carboxypeptidases: In preliminary experiments, the maximum carboxypeptidase activities in all crude shoot extracts was observed at pH 4.6, independently of the substrate used (data not shown). The carboxypeptidase activities were found to change with the age of seedlings, showing a gradual increase in shoots and gradual decrease in roots up to 8th day following imbibition (Fig. 2*C*,*D*). Activities of these enzymes were highest with the N-terminally blocked dipeptide, N-CBZ-Phe-Ala in extracts from both shoots and roots. The activities detected with N-carboxy-Z-dipeptides such as Phe-Leu, Ala-Met and Ala-Leu were lower both in shoots and roots and the lowest with the Ala-Phe, Glu-Tyr and Pro-Phe.

In response to dehydration, activity of carboxypeptidases with N-CBZ-Phe-Ala as a substrate decreased sharply both in shoots and roots of 4-d-old seedlings. Carboxypeptidase activities measured towards other substrates increased two-three folds in dehydrated shoots whereas in dehydrated roots they were lower than in control (Fig. 3*E*,*G*). The response of 6-d-old seedlings to

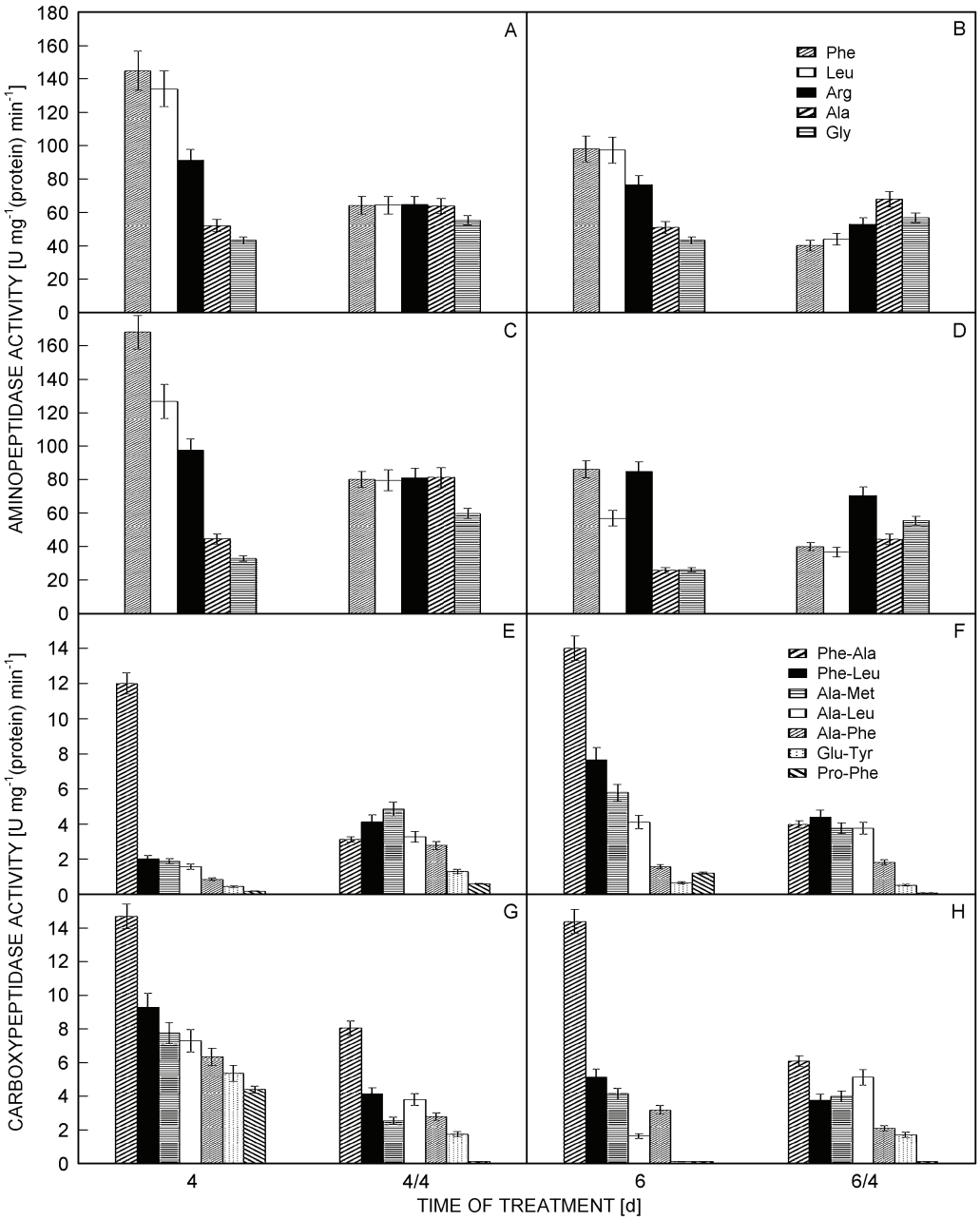

Fig. 3. Changes in aminopeptidase and carboxypeptidase activities in shoots (*A*,*B*,*E*,*F*) and roots (*C*,*D*,*G*,*H*) of control wheat seedlings and 4-d-old and 6-d -old seedlings subjected to dehydration. SD values are indicated.

Table 2. The effect of inhibitors on wheat shoot carboxypeptidase activities. Activity assays were carried out with N-CBZ-Ala-Phe and N-CBZ-Phe-Ala as substrates. Values are % of control activity. $LSD_{0.05} = 4.03$.

Inhibitor	Conc. [mM]	N-CBZ-Ala-Phe	N-CBZ-Phe-Ala
E-64	0.01	93.2	100.0
	0.05	77.3	94.6
Pepstatin A	0.01	100.0	100.0
	0.05	97.9	99.8
PMSF	1.00	37.8	18.2
	5.00	19.0	20.3
EDTA	1.00	99.1	100.0
	10.00	89.4	96.3

severe water deficiency was substrate-dependent: the enzyme activities in shoots decreased in the presence of N-CBZ-Phe-Ala, N-CBZ-Phe-Leu, N-CBZ-Ala-Met and N-CBZ-Pro-Phe (Fig. 3F). In dehydrated roots, the activities measured with N-CBZ-Ala-Leu and N-CBZ-Glu-Tyr were higher than in well watered roots, with N-CBZ-Ala-Met, N-CBZ-Ala-Phe and N-CBZ-Pro-Phe remained at the same level but with N-CBA-Phe-Leu and N-CBZ-Phe-Ala diminished in comparison with control (Fig. 3*H*).

A potent inhibitor of various Ser enzymes, PMSF inhibited strongly carboxypeptidase activities measured with N-CBZ-Ala-Phe and N-CBZ-Phe-Ala as substrates (Table 2). E-64, a specific inhibitor of cysteine peptidases activity, pepstatin A and EDTA (the aspartate and metallopeptidases inhibitors, respectively) has been found to be ineffective as inhibitors of carboxypeptidase activity.

Discussion

Loss of dehydration tolerance by the 6-d-old of the seedlings of spring wheat was accompanied by reduction in total soluble protein content (Miazek *et al.* 2001) and lower pool of free amino acids. Decrease in the soluble protein content did not correspond to the level of dehydration tolerance because similar content was observed in both tolerant and sensitive phase of seedling growth Miazek *et al.* 2001). However, the content of amino acids both in shoots and roots of wheat seedlings differentiated the 4-d-old seedlings from the older ones.

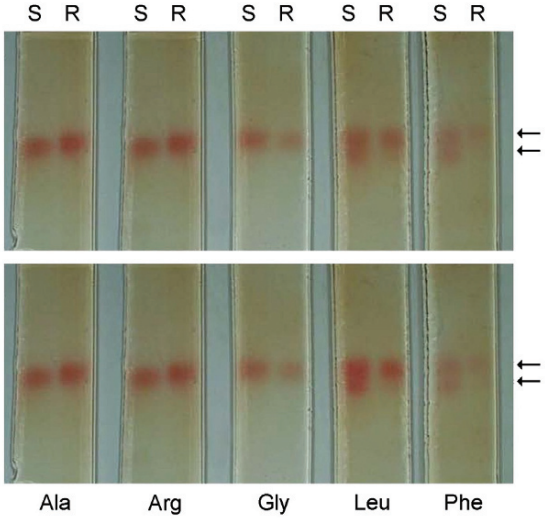

Fig. 4. Changes in aminopeptidase activities (*arrows*) in shoots (S) and roots (R) of control (*above*) and dehydrated (*below*) wheat seedlings determined by native gel electrophoresis. After electrophoresis gels were incubated with various substrates as shown in the figure.

Dehydration-induced accumulation of α-ketoglutarate and pyruvate derived amino acids in shoot and roots of 4-d-old wheat seedlings and in roots of 6-d-old wheat seedlings implies changes in amino acid metabolism. Although similar shift in amino acid metabolism was described for the water stressed wheat (Zagdańska and Neuman 1984), maize (Navari-Izzo *et al.* 1990) and sunflower (Foyer *et al.* 1998) the possible mechanism for the stress-induced alteration in amino acid pathways remains unclear and physiological meaning disputable (Cherian *et al.* 2006, Simon-Sarkadi *et al.* 2006).

Exoproteolytic activity changed differently during growth and development of shoot of young wheat seedlings. The resistant phase of growth of wheat seedlings was associated with the higher carboxypeptidase and lower aminopeptidase activities in comparison to the seedlings in dehydration sensitive phase of growth. Due to the fact that aminopeptidase activities decreased with the age of seedlings of barley (Mikola and Kolehmainen 1972) and maize (Feller *et al.* 1978) and in leaves of barley (Thayer *et al.* 1988) and wheat (Feller *et al.* 1978, Vodkin and Scandalios 1980), the observed differences in activities of examined enzymes seem to be associated with the seedling age and not with he level of dehydration tolerance. This is consistent with the finding that different bean cultivars did nor revealed relation between activity of aminopeptidases and dehydration tolerance (Roy-Macauley *et al.* 1992, Cruz de Carvalho *et al.* 2001, Hieng *et al.* 2004).

In young wheat seedlings two aminopeptidases are present: the first exhibited wide substrate specificity (Phe-, Leu-, Arg-, Ala- and Gly-β-NAs) and second exhibited narrow substrate specificity (Phe- and Leu-β-NAs). The Ala-β-NA specific aminopeptidase seems to be metalloenzyme that is inhibited by metal chelators (1,10-phenantroline and EDTA). The efficient inhibitory effect obtained with pCMB with concomitant lack of inhibitory effect of E-64 on alanine aminopeptidase implicated the involvement of Cys residues in retain the appropriate enzyme conformation (El-Amrani *et al.* 1995, Ogiwara *et al.* 2005). The second wheat seedling aminopeptidase seems to be cysteine enzyme, as measured by the inhibitory effect of pCMB and E-64. This is consistent with the finding that bean leaf amino-

peptidases, hydrolyzing L-Leu-p-nitroanilide and L-Ala-*p*-nitroanilide, are different enzymes (Hieng *et al.* 2004). In response to water deficit, aminopeptidase activities towards Gly-β-NA and Ala-β-NA increased both in shoots and roots of wheat seedlings, independently of the seedling age. It should be stressed out that amino-peptidases hydrolyzing Leu-β-NA and Phe-β-NA remained more active in the resistant phase of growth. Leucine aminopeptidase activity increased under water deficit in *Lycopersicon esculentum* (Chao *et al.* 1999) and *Phaseolus vulgaris* (Hieng *et al.* 2004).

The increase in carboxypeptidase activities observed during shoot development of young seedlings and decrease in activities with the age of roots possibly reflects different regulation of proteolytic processes in sink tissues (roots) than in source tissues (shoots). However, little is known about the expression of carboxypeptidase genes and their role(s) in plant development.

Carboxypeptidases of wheat seedlings hydrolyzed N-CBZ-Phe-Ala with the highest rate. Generally the rate of hydrolysis of N-terminally blocked dipeptides with Phe and Ala at the penultimate position was preferred. These results may indicate that plant carboxypeptidases exist in multiple molecular forms differing in substrate specificity (Mikola and Mikola 1986, Mehta *et al.* 1996). Carboxypeptidases characterised the highest rate of hydrolysis towards N-CBZ-Ala-Phe and N-CBZ-Phe-Ala seem to be serine enzymes as judged on the inhibitory effect of PMSF. The response of carboxypeptidase activities to water deficit was both, organ and substrate specific. Generally, in resistant phase of growth the carboxypeptidase activity increased in shoots (with the exception of activity towards N-CBZ-Phe-Ala) and decreased in roots (with the exception towards the same substrate). The reverse was observed in sensitive phase of growth of wheat seedlings.

The question has been raised to what extent examination of size and composition of amino acid pool referring to exopeptidase activities in the seedlings of wheat could provide information on regulation of dehydration tolerance. The main difference in the size of amino acid pool between 4-d-old and 6-d-old seedlings concerned both fully hydrated and dehydrated seedlings. This was significantly higher in the 4-d-old seedlings but reduced to a low value in the 6-d-old-seedlings. The higher carboxypeptidase activities in younger seedlings suggest that plant carboxypeptidases are a part of regulated increase in intracellular protein turnover. Since the N-terminal residue of proteins determines their half-lives (Varshavsky 1996, Walling 2006) aminopeptidases may modulate protein levels or activate of many regulatory proteins and facilitate turnover of damaged proteins under dehydration. In this way aminopeptidases acting probably in concert with carboxypeptidases may profoundly influence plant responses to stresses.

References

Bartling, D., Nosek, J.: Molecular and immunological characterization of leucine aminopeptidase in *Arabidopsis thaliana*: a new antibody suggests a semi-constitutive regulation of a phylogenetically old enzyme. - Plant Sci **9**: 199-209, 1994.

Beers, E.P., Jones, A.M., Dickerman, A.W.: The S8 serine, C1A cysteine and A1 aspartic protease families in *Arabidopsis*. - Phytochemistry **65**: 43-58, 2004.

Blum, A., Sinmena, B., Ziv, O.: An evaluation of seed and seedling drought tolerance screening test in wheat. - Euphytica **29**: 727-36, 1980.

Bogdan, J., Zagdańska, B.: Drought resistance of spring wheat during germination and seedling growth. - Bull. IHAR **233**: 73-80, 2004.

Bradford, M.M.: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. - Anal. Biochem. **72**: 248-254, 1976.

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

Callis, J.: Regulation of protein degradation. - Plant Cell **7**: 845-57, 1995.

Carrasco, P., Carbonell, J.: Changes in the level of peptidase activities in pea ovaries during senescence and fruit set induced by gibberellic acid. - Plant Physiol. **92**: 1070-1074, 1990.

Cercós, M., Urbez, C., Carbonell, J.: A serine carboxypeptidase gene (PsCP), expressed in early stages of reproductive and vegetative development in *Pisum sativum,* is induced by gibberellins. - Plant mol. Biol. **51**: 165-174, 2003.

Chao, W.S., Gu, Y.-Q., Pautot, V., Bray, E.A., Walling, L.L.: Leucine aminopeptidase RNAs, proteins, and activities increase in response to water deficit, salinity, and the wound signals - systemin, methyl jasmonate, and abscisic acid. - Plant Physiol. **120**: 979-992, 1999.

Cherian, S., Reddy, M.P., Ferreira, R.B.: Transgenic plants with improved dehydration- stress tolerance: progress and future prospects. - Biol. Plant. **50**: 481-495, 2006.

Cruz de Carvalho, M.H., d'Arcy-Lameta, A., Roy-Macauley, H., Gareil, M., El Maarouf, H., Pham Thi, A.T., Zuily-Fodil, Y.: Aspartic protease in leaves of common bean (*Phaseolus vulgaris* L.) and cowpea (*Vigna unguiculata* L. Walp): enzymatic activity, gene expression and relation to drought susceptibility. - FEBS Lett. **492**: 242-246, 2001.

Dominguez, F., Cejudo, F.J. Germination-related genes encoding proteolytic enzymes are expressed in the nucellus of developing wheat grains. - Plant J. **15**: 569-574, 1998.

Dominguez, F., Gonzalez, M.C., Cejudo, F.J.: A germination-related gene encoding a serine carboxypeptidase is expressed during the differentiation of the vascular tissue in wheat grains and seedlings. - Planta **215**: 727-734, 2002.

Dubey, R.S., Rani, M.: Influence of NaCl salinity on the behavior of protease, aminopeptidase and carboxypeptidase in rice seedlings in relation to salt tolerance. - Aust. J. Plant Physiol. **30**: 133-145, 1990.

El-Amrani, A., Suire, C., Camara, B., Gaudillère, J.-P., Couée, I.: Purification and characterization of a novel amino-peptidase, plastidial alanine-aminopeptidase, from the cotyledons of etiolated sugar beet seedlings. - Plant Physiol. **109**: 87-94, 1995.

Farrant, J.M., Bailly, C., Leymarie, J., Hamman, B., Côme, D., Corbineau, F.: Wheat seedlings as a model to understand desiccation tolerance and sensitivity. - Physiol. Plant. **120**: 563-574, 2004.

Feller, U.K., Soong, T.-T., Hageman, R.H.: Patterns of proteolytic enzyme activities in different tissues of germinating corn (*Zea mays* L.). - Planta **140**: 155-162, 1978.

Foyer, C., Valadier, M.-H., Migge, A., Becker, T.W.: Drought-induced effects on nitrate reductase activity and mRNA and on the coordination of nitrogen and carbon metabolism in maize leaves. - Plant Physiol. **117**: 283-292, 1998.

Grudkowska, M., Zagdańska, B.: Multifunctional role of plant cysteine proteinases. - Acta Biochim. Polon. **51**: 609-624, 2004.

Guerida, M., Shroyer, J.P., Kirkham, M.B., Paulsen, G.M.: Wheat coleoptile and root growth and seedling survival after dehydration and rehydration. - Agron. J. **89**: 822-826, 1997.

Guerrero, F.D., Jones, J.T., Mullet, J.E.: Turgor-responsive gene transcription and RNA levels increase rapidly when pea shoots are wilted and expression of three inducible genes. - Plant mol. Biol. **15**: 11-26, 1990.

Hieng, B., Ugrinović, K., Šuštar-Vozlič, J., Kidrič, M.: Different classes of proteases are involved in the response to drought of *Phaseolus vulgaris* L. cultivars differing in sensitivity. - J Plant Physiol. **161**: 519-530, 2004.

Hildmann, T., Ebneth, M., Pena-Cortes, H., Sanchez-Serrano, J.J., Willmitzer, L., Prat, S.: General roles of abscisic and jasmonic acids in gene activation as a result of mechanical wounding. - Plant Cell **4**: 1157-1170, 1992.

Ingram, J., Bartels, D.: The molecular basis of dehydration tolerance in plants. - Annu. Rev. Plant Physiol. Plant mol. Biol. **47**: 377-403, 1996.

Kolehmainen, L., Mikola, J.: Partial purification and enzymatic properties of an aminopeptidase from barley. - Arch. Biochem. Biophys. **145**: 633-642, 1971.

Laemmli, U.K.: Cleavage of structural proteins during the assembly of the head bacteriophage T4. - Nature **227**: 680-685, 1970.

Mehta, R.A., Warmbardt, R.D., Mattoo, A.K.: Tomato fruit carboxypeptidase. Properties, induction upon wounding, and immunocytochemical localization. - Plant Physiol. **110**: 883-892, 1996.

Miazek, A., Bogdan, J., Zagdańska, B.: Effect of water deficit during germination of wheat seeds. - Biol. Plant. **44**: 397-403, 2001.

Li, J., Lease, K.A., Tax, F.E., Walker, J.C. BRS1, a serine carboxypeptidase, regulates BRII signaling in *Arabidopsis*. - Proc. nat. Acad. Sci USA **98**: 5916-5921, 2001.

Mikola, J., Kolehmainen, L: Localization of activity of various peptidases in germinating barley. - Planta **10**4: 167-177, 1972.

Mikola, L., Mikola, J.: Occurrence and properties of different types of peptidases in higher plants. - In: Dalling, M.J. (ed.): Plant Proteolytic Enzymes. Vol. 1. Pp. 97-117. CRC Press, Boca Raton 1986.

Navari-Izzo, F., Quartacci, M.F., Izzo, R.: Water-stress induced changes in protein and free amino acids in field-grown maize and sunflower. - Plant Physiol. Biochem. **28**: 531-537, 1990.

Ogiwara, N., Amano, T., Satoh, M., Shioi, Y.: Leucine aminopeptidase from etiolated barley seedlings: characterization and partial purification of isoforms. - Plant Sci **168**: 575-581, 2005.

Palma, J.M., Sandalio, L.M., Corpas, F.J., Romero-Puertas, M.C., McCarthy, I., Del Rio, L.A.: Plant proteases, protein degradation, and oxidative stress: role of peroxisomes. - Plant Physiol. Biochem. **40**: 521-30, 2002.

Parrott, D., Yang, L., Shama, L., Fischer, A.M.: Senescence is accelerated, and several proteases are induced by carbon "feast" conditions in barley (*Hordeum vulgare* L.) leaves. - Planta **222**: 989-1000, 2005.

Ramirez-Zavala, B., Mercado-Flores, Y., Hernandez-Rodriguez, C., Villa-Tanaca, L.: Purification and characterization of a serine carboxypeptidase from *Kluyveromycetes marxianus*. - Int. J. Food Microbiol. **91**: 245-252, 2004.

Roy-Macauley, H., Zuily-Fodil, Y., Kidrič, M., Pham Thi, A.T., Veira da Silva, J.: Effect of drought stress on proteolytic activities in *Phaseolus* and *Vigna* leaves from sensitive and resistant plants. - Physiol. Plant. **85**: 90-96, 1992.

Schaffer, M.A., Fischer, R.L.: Analysis of mRNA that accumulates in response to low temperature identifies a thiol protease gene in tomato. - Plant Physiol. **87**: 431-436, 1988.

Schaffer, M.A., Fischer, R.L.: Transcriptional activation by heat and cold of a thiol protease gene in tomato. - Plant Physiol. **93**: 1486-1492, 1990.

Schaller, A., Ryan, C.A.: Systemin: a polypeptide defense signal in plants. - BioEssays **18**: 27-33, 1995.

Schaller, A.: A cut above the rest: the regulatory function of plant proteases. - Planta **220**: 183-197, 2004.

Simon-Sarkadi, I., Kocsy, G., Varhegyi, A., Galiba, G., De Ronde, J.A.: Stress-induced changes in the free amino acid composition in transgenic soybean plants having increased proline content. - Biol. Plant. **50**: 793-796, 2006.

Thayer, S.S., Choe, H.T., Rausser, S., Huffaker, R.C.: Characterization and subcellular localization of aminopeptidases in senescing barley leaves. - Plant Physiol. **87**: 894-897, 1988.

Tonecki, J., Gorin, N., Verghagen, W.: Quantitative high-performance liquid chromatography and enzymatic estimation of four free amino acids in powders of anthers from bulbs of tulip. - J. Liquid Chrom. **12**: 2321-2331, 1989.

Varshavsky, A.: The N-end rule: functions, mysteries, uses. - Proc. nat. Acad. Sci USA **93**: 12142-12149, 1996.

Vierstra, R.D.: Proteolysis in plants: mechanisms and functions. - Plant mol. Biol. **32**: 275-302, 2004.

Vincent, J.L., Brewin, N.J.: Immunolocalization of a cysteine protease in vacuoles, vesicles and symbiosomes of pea nodule cells. - Plant Physiol. **123**: 521-530, 2000.

Vodkin, L., Scandalios, J.G.: Comparative properties of genetically defined peptidases in maize. - Biochemistry **19**: 4660-4667, 1980.

Walling, L.L.: Recycling or regulation? The role of amino-terminal modifying enzyme. - Curr. Opin. Plant Biol. **9**: 227-233, 2006.

Wilson, K.A.: Role of proteolytic enzymes in the mobilization of protein reserves in the germinating dicot seed. - In: Dalling, M.J. (ed.): Plant Proteolytic Enzymes. CRC Press, Boca Raton 1986.

Wiśniewski, K., Zagdańska, B.: Genotype-dependent proteolytic response of spring wheat to water deficiency. - J. exp. Bot. **52**: 1455-66, 2001.

Zagdańska, B., Neumann, M.: Amino acids changes in drought-affected spring wheat. - Bull. Pol. Acad. Sci. **32**: 229-235, 1984.