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Influence of ABA and 4PU-30 on the growth, proteolytic activities and protein composition of maize seedlings

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Abstract

The experiments were carried out with germinating maize seeds (*Zea mays* L.), grown 6 d in the dark at 26 °C. Before germination the seeds were soaked for 4 h in solutions containing 1 mM abscisic acid (ABA), 0.1 mM N_1-(2-chloro-4-pyridyl)-N_2 phenylurea (4PU-30) and their combination. The influence of plant growth regulators on the length, fresh (FM) and dry (DM) masses, proteolytic activities and soluble protein fractions in shoots, roots and endosperm were studied. As compared to control the seedlings treated with ABA showed lower length, FM and DM of shoots and roots, and lower proteolytic activities. As a consequence of suppression of both growth and protein breakdown, these seedlings possessed higher protein content in endosperm. 4PU-30 partially decreased the ABA suppressing effects.

Additional key words: germination, glutelins, prolamins, soluble proteins.

Introduction

Abscisic acid (ABA) and cytokinins interfere in many physiological processes as dormancy, seed germination, growth, senescense (Mapelli *et al.* 1984, Nickel 1986). ABA inhibited seed germination (Bhatnagar and Geeta 1980), storage protein degradation and proteolytic activity (Harvey and Oaks 1974a). It could influence also the individual proteases during germination (Segundo *et al.* 1990).

In promoting seed germination some of phenylurea cytokinins were as active as some N^6-substituted purine cytokinins (Thomas *et al.* 1975). The growth of maize seedlings was stimulated after soaking the seed in N_1-(2-chloro-4-pyridyl)-N_2 phenylurea (4PU-30) solutions (Georgiev and Iliev 1996). 4PU-30 increased the total nitrogen content, quantity of the individual protein fractions, photosynthetic activity and dry mass of maize colepotiles (Stefanov *et al.* 1994). Also it could provoke changes in the amino acid composition of some maize polypeptides (Stefanov *et al.* 1998). There were no evidences about 4PU-30 influence on the activity of proteolytic enzymes and storage protein degradation in the early period of seedling growth.

The aim of this study was to investigate the influence of ABA and 4PU-30 on the growth, protein composition and proteolytic activities in an important initial period of maize seedlings development.

Materials and methods

The experiments were carried out with maize (*Zea mays* L., cv. Kneja 530). The seeds were soaked for 4 h in solutions of 1 mM ABA, 0.1 mM 4PU-30 and combination of two, rolled into moist filter paper and

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Abbreviations: ABA - abscisic acid; AP - aminopeptidase; CP - carboxypeptidase; DM - dry mass; FM - fresh mass; GA_3 - gibberellic acid; LSD - least significant difference; ME - mercaptoethanol; NaOAc - sodium acetate; PGR - plant growth regulator; 4PU-30 - N_1-(2-chloro-4-pyridyl)-N_2 phenylurea; PVP - polyvinylpyrolidon; SDS - sodium dodecyl sulphate; UN - unextracted nitrogen.

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grown for 6 d in the dark at 26 °C. The length, fresh (FM) and dry (DM) masses of the shoots, main roots and endosperm were measured.

The enzyme extracts for proteolytic activity determinations were prepared according to Feller *et al.* (1977). Plant tissue was ground in 0.05 M acetate buffer at pH 5.4 containing 1 % insoluble polyvinyl-pyrolidon (PVP) and 0.1 % mercaptoethanol (ME). The homogenates were desalted for one night, by dialysis against the buffer used for homogenization. For determination of endopeptidase activities *L*-leucin-*p*-nitroanilide (Chrispeels and Boulter 1975) for aminopeptidase (AP), and *N*-carbobenzoxy-*L*-phenyl-alanin (Visuri *et al.* 1969) for carboxypeptidase (CP) were used as substrates. Enzyme extracts and substrate solutions were mixed and incubated at 37 °C for 1 h. Endopeptidase activities were determined with 0.5 % solution of casein as substrate in buffers with different pH: 0.05 M acetate at pH 5.4 and 0.05 M Tris-HCl at pH 7.5. They were incubated with enzyme extracts for 3 h at 37 and 45 °C, respectively. The reactions were suppressed by placing the tubes in boiling water for 5 min or by addition of 15 % trichloracetic acid. The free amino groups were determined with ninhydrin method (Moor 1968). The protein content in enzyme extracts was established after the method of Lowry *et al.* (1951).

Soluble protein fractions were extracted according to Landry and Moureaux (1970) as follow: albumins and globulins with 0.5 M NaCl solution, prolamins with 0.5 % sodium acetate (NaOAc) in 70 % ethanol and glutelins with 0.2 M Tris-HCl buffer containing 0.5 % sodium dodecyl sulphate (SDS) and 0.6 % ME. Nitrogen content was measured by a micro-Kjeldahl method.

The present data are a means of at least three independent experiments in three replications each. The data were analysed statistically and the least significant difference (LSD) was used to evaluate differences between the treatment.

Results

ABA strongly inhibited the elongation growth of shoots and roots and also decreased their DM and FM. Seedlings from 4PU-30 treated seeds were characterized with higher DM of the shoots and with lower length, FM and DM of roots. Combination of the two plant growth regulators (PGRs) induced lower inhibition of the length, FM and DM of shoots than this observed after ABA action. The inhibitory effect of the PGR combination in roots was considerably higher than that of every one PGR used independently. As compared with control ABA and ABA plus 4PU-30 treated endosperm retained higher FM and DM (Fig. 1).

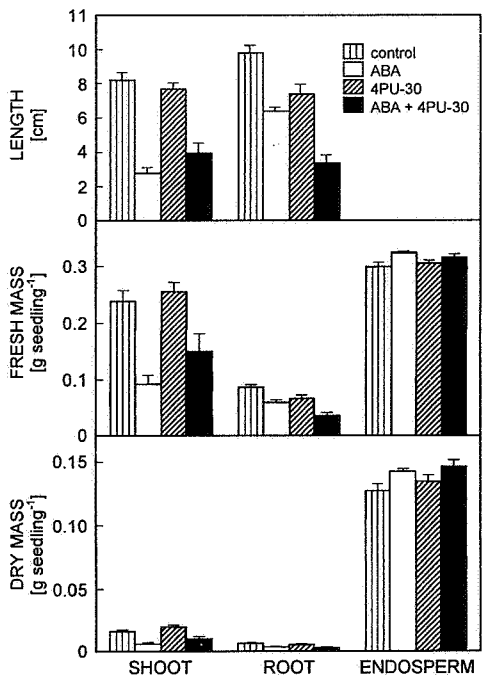

Fig. 1. Effect of 1 mM ABA and 0.1 mM 4PU-30 on the growth of 6-d-old maize seedlings. Means ± SE, $n = 6$.

The most significant differences in proteolytic activities were established in roots and endosperm of 6 d - old maize seedlings (Figs. 2 and 3). Their exo- and endopeptidase activities were considerably suppressed after ABA treatment and slightly inhibited with the combination of the two PGRs. Highest inhibition with ABA and ABA + 4PU-30 was observed in caseolytic activity at pH 5.4. A smaller decrease of carboxypeptidase and caseolytic activities was also observed in shoots of ABA treated seedlings. 4PU-30 increased caseolytic activity in endosperm as well as all proteolytic activities in roots. After other treatments there were no changes in proteolytic activities in shoots and roots.

Significant changes in the total nitrogen content and in protein fractions were observed in shoots and in endosperm. The total nitrogen content was increased only in shoots from 4PU-30 treated seeds (Fig. 4). Increasing total nitrogen content in endosperm was established after ABA or ABA + 4PU-30 treatments.

Highest content of prolamins were observed in endosperm and shoots after all treatments (Fig. 4). This effect was most considerably expressed in endosperm in ABA and ABA + 4PU-30 variants. The content of glutelins in shoots was very similar wheras in endosperm these proteins prevailed significantly in variants ABA and ABA + 4PU-30. After the action of 4PU-30 glutelins slightly diminished in both shoots and endosperm.

Unextracted nitrogen (UN) and non-protein compounds were increased in shoots of all treatments and reached maximum at 4PU-30. Compared with control, ABA and the combination showed strongly elevated quantities of these compounds in endosperm. In endosperm decreasing of UN plus non proteins was measured only after 4PU-30 treatment.

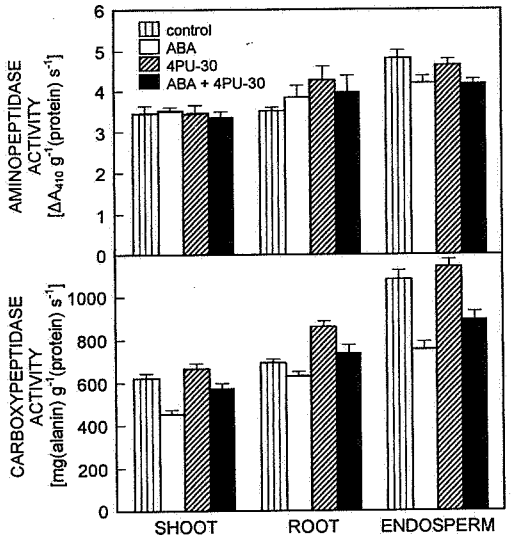

Fig. 2. Effect of 1 mM ABA and 0.1 mM 4PU-30 on the exopeptidase activities in 6-d-old maize seedlings. Means ± SE, $n = 6$.

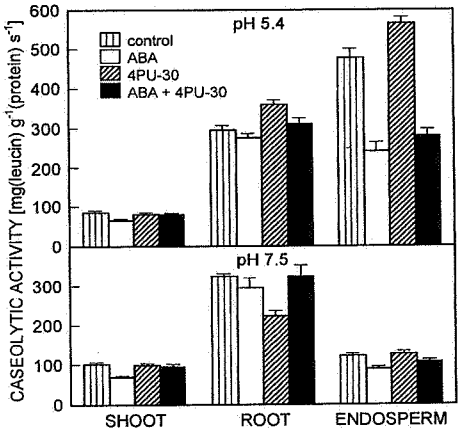

Fig. 3. Effect of 1 mM ABA and 0.1 mM 4PU-30 on the endopeptidase activities in 6-d-old maize seedlings. Means ± SE, $n = 6$.

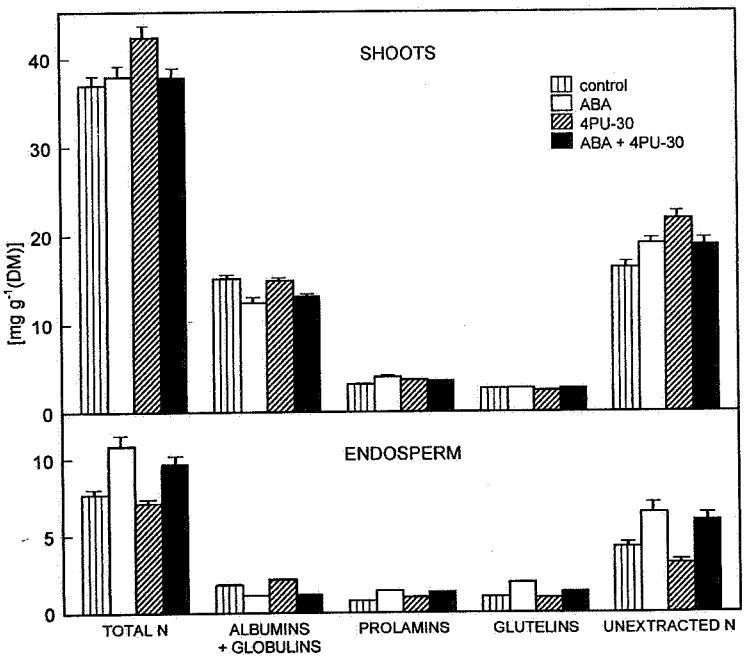

Fig. 4. Effect of 1mM ABA and 0.1 mM 4PU-30 on the nitrogen content and individual protein fractions in both 6-d-old maize shoots and 6-d-old endosperm. Means ± SE, $n = 6$.

Discussion

When maize seeds germinated, storage proteins were degraded to supply the embryo with amino acids to support seedling growth. This hydrolytic process involved the production of proteolytic enzymes from cells of the aleurone layer and was controlled by PGRs (Torrent *et al.* 1989). There were data that ABA affected biochemical processes in aleurone layer of barley seeds (Mapelli *et al.* 1984) as well as the synthesis of m-RNA or proteins

(Mozer 1980). ABA inhibited germination in a wide range of species (Wareing and Saunders 1971). Different effects, often contradictory, of cytokinins on germination and seedling growth were observed. Benzyladenine and kinetin at high concentrations inhibited the growth of barley coleoptiles (Khan 1969), but 4PU-30 in similar concentrations stimulated the growth of maize coleoptiles (Stefanov *et al.* 1994, Georgiev and Iliev 1996). In our experiments 4PU-30 induced a slight increase of shoot growth and strong inhibition of root growth. When the two PGRs were applied together, 4PU-30 decreased in some extent ABA inhibitory effect on shoot growth (Fig. 1). These results were similar to data of Khan (1969) who confirmed that benzyladenine and kinetin reversed ABA inhibition of growth and α-amylase activity in barley. However, cytokinins did not reverse ABA-induced inhibition of α-amylase and protease in maize endosperm (Harvey and Oaks 1974a). During the first two days of germination of maize seeds proteases activity was low. It increased 4- to 5-fold from day 2 to 4 and thereafter declined (Moureaux 1979). We compared proteases activity at day 6 of germination when the main quantities of storage proteins were degraded (Feller *et al.* 1978, Segundo *et al.* 1990, Hay *et al.* 1991). At that time proteases activity was not at maximum rate but was constant (Moureaux 1979, Mohammad and Esen 1990). No important changes were observed in total nitrogen (TN) content and in different protein fractions of shoots (Fig. 4). However in endosperm only ABA treated variants possessed highest TN and highest contents of prolamins and glutelins. In combination (ABA + 4PU-30) the values for TN, prolamins and glutelins slightly diminished but nevertheless were higher than the parallel values in 4PU-30 variant. We assumed that this could be explained with depression of storage proteins breakdown as a consequence of PGRs action. Prolamin and glutelin degradation in maize endosperm occurred 3 d after germination and coincided with the appearance of a group of proteases with an acidic pH optimum (Mohammad and Esen 1990, Barros and Larkins 1990).

As a result of ABA treatment growth of maize seedlings and degradation of storage proteins in endosperm were depressed. It was observed by Abe *et al.* (1977), Fujimaki *et al.* (1977) and Moureaux (1979) that proteases activity increased gradually with time of germination. This prompted us to propose that the depression of growth and protein degradation established in our experiments after ABA action was connected with retaining of the activity of proteases increasing at germination. There were reports that the acid endopeptidases played a major role in the seed protein mobilization (Harvey and Oaks 1974 b, Feller *et al.* 1978, Hay *et al.* 1991). As a substrate for endopeptidase casein was used because it has a similar pH optimum like zein. Our data showed that endopeptidase activity was depressed by ABA in shoots and endosperm in both pH investigated, but the depression was especially considerable in endosperm at pH 5.4 (Fig. 3).

The decrease in AP and CP activities by ABA was established, but not strongly expressed (Fig. 2). AP activity in endosperm slightly prevailed the activity in shoots and roots which means that probably AP played no significant role in the storage proteins mobilization in the early periods of seed germination. Similar results have been reported previously (Blätler and Feller 1988, Segundo *et al.* 1990). CP activity was higher in all investigated parts of 4PU-30 treated seedlings. There were evidences that CP participated in the first steps of protein degradation (Feller *et al.* 1978, Mikkonen 1986). This point of view correlated with relatively faster growth and more rapidly degradation of storage proteins in endosperm of 4PU-30 treated seedlings observed by us.

Investigation of proteins degradation and of activities of proteases involved in the first steps of breakdown of seed proteins is very essential for understanding of this complicated and not yet satisfactory resolved process. Characterizing of the effects of ABA and 4PU-30 comes to supplement the elucidation of this process as well as the capacity of these PGRs to influence plant growth, protein synthesis and proteases activity.

References

Abe, M., Arai, S., Fujimaki, M.: Purification and characterization of a protease occurring in endosperm of germinating corn. - Agr. biol. Chem. **41**: 893-899, 1977.

Barros, E., Larkins, B.: Purification and characterization of zein degrading proteases in endosperm of germinating maize seeds. - Plant Physiol. **94**: 297-303, 1990.

Bhatnagar, V.K., Geeta, R.: Effect of some growth regulators on *Zea mays*. - Geobios **7**: 183-184, 1980.

Blättler, R., Feller, U.: Identification and stability of aminopeptidases in extracts from bean seeds. - Aust. J. Plant Physiol. **15**: 613-619, **1988**.

Chrispeels, M.J., Boulter, D.: Control of storage protein metabolism in cotyledons of germinating mung beans. Role of endopeptidase. - Plant Physiol. **55**: 1031-1037, 1975.

Feller, U.K., Soong, T.-S.T., Hageman, R.N.: Leaf proteolytic activities and senescence during grain development of field grown corn (*Zea mays* L.). - Plant Physiol. **59**: 290-294, 1977.

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

Fujimaki, M., Abe, M., Arai, S.: Degradation of zein during germination of corn. - Agr. biol. Chem. **41**: 887-891, 1977.

Georgiev, G.Ts., Iliev, L.K.: Influence of the phenylurea cytokinins 4PU-30 on the growth of corn seedlings after seed treatment. - Comp. rend. Acad. bulg. Sci. **49**: 75-78, 1996.

Harvey, B.M., Oaks, A.: The role of gibberellic acid in the hydrolysis of endosperm reserves in *Zea mays*. - Planta **121**: 64-74, 1974a.

Harvey, B.M., Oaks, A.: The hydrolysis of endosperm protein in *Zea mays*. - Plant Physiol. **53**: 453-457, 1974b.

Hay, P., Ramsaur, T., Smith, C., Miernyk, J.: Storage protein mobilization during germination and early seedling growth of *Zea mays*. - Physiol. Plant. **81**: 377-384, 1991.

Khan, A.: Cytokinin inhibitor antagonism in the hormonal control of 2-amylase synthesis and growth in barley seed. - Physiol. Plant. **22**: 91-103, 1969.

Landry, J., Moureaux, T.: Hétérogéneité des glutelines du grain de mais: Extraction sélective et composition en acides aminés des trois fractions isolées. - Bull. Soc. Chem. Biol. **52**: 1021-1037, 1970.

Lowry, O.H., Rossenbrough, N., Farr, A., Randall, R.: Protein measurement with the Folin phenol reagent. - J. biol. Chem. **193**: 265-275, 1951.

Mapelli, S., Lombardi, L., Rocchi, P.: Gibberellin and abscisic acid effects on the activity of hydrolytic enzymes in de-embryonated barley seeds. - Plant Growth Regul. **2**: 31-40, 1984.

Mikkonen, A.: Activities of some peptidases and proteinases in germinating kidney bean, *Phaseolus vulgaris*. - Physiol. Plant. **68**: 282-286, 1986.

Mohammad, K., Esen, A.: Zein degradation the endosperm of maize seeds during germination. - Amer. J. Bot. **77**: 973-980, 1990.

Moor, S.: Amino acid analysis: aqueous dimetyl sulfoxide as solvent for the ninhydrin reaction. - J. biol. Chem. **243**: 6281-6283, 1968.

Moureaux, T.: Protein breakdown and protease properties of germinating maize endosperm. - Phytochemistry **18**: 1113-1117, 1979.

Mozer, T. J.: Control of protein synthesis in barley aleurone layers by the plant hormones gibberellic acid and abscisic acid. - Cell **20**: 479-485, 1980.

Nickell, I.G.: Effects of N_1-(2-chloro-4-pyridyl)-N'-phenylurea on grapes and other crops. - In: McLaren, J.S. (ed.): Proceedings of 13th Annual Meeting of the Plant Growth Regulator Society of America. Pp. 236-241. Academic Press, New York 1986.

Segundo, B., Casacuberta, J., Puigdomenech, P.: Sequential expression and differential hormonal regulation of proteolytic activities during germination in *Zea mays*. - Planta **181**: 467-474, 1990.

Stefanov, B., Iliev, L., Popova, N.: Effect of the phenylurea cytokinin 4PU-30 on the growth and protein composition of maize seedlings. - J. Plant Growth Regul. **13**: 231-234, 1994.

Stefanov, B., Iliev, L., Popova, N.: Influence of GA_3 and 4PU-30 on leaf protein composition, photosynthetic activity and growth of maize seedlings. - Biol. Plant. **41**: 57-63, 1998.

Thomas, T.H., Palevitch, D., Biddington, N.L., Austin, R.B.: Growth regulators and the phytochrome-mediated dormancy of celery seeds. - Physiol. Plant. **35**: 101-106, 1975.

Torrent, M., Geli, M., Ludevid, D.: Storage protein hydrolysis and protein - body breakdown in germinated *Zea mays* L. seeds. - Planta **180**: 90-95, 1989.

Visuri, J., Mikula, J., Enari, T.: Isolation and partial characterization of a carboxypeptidase from barley. - Eur. J. Biochem. **7**: 193-199, 1969.

Wareing, P.F., Saunders, P.F.: Hormones and dormancy. - Annu. Rev. Plant Physiol. **22**: 261-288, 1971.