The effect of plant growth regulators and chlorsulfuron on electrophoretic profiles of soluble proteins, polypeptides and antioxidant enzymes in maize seedlings

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ABSTRACT

Investigations were carried out with maize (*Zea mays* L.) seedlings. The effect of chlorsulfuron (CHF) on the electrophoretic profiles of the total and thermostable proteins, polypeptides and some antioxidant enzymes was compared to the effect of abscisic acid (ABA), benzylaminopurine (BAP) and N^1-(2-chloro-4-pyridyl)-N^2-phenylurea (4PU-30) applied in concentrations that inhibit the growth of seedlings. It was established that the effect of CHF on seedling growth as well as on the studied biochemical parameters was most significant. The reduction of growth by CHF was accompanied by a significant decrease in the quantity of main proteins and specific qualitative changes in the electrophoretic patterns for coleoptiles. The appearance of a new native protein with Rm 0.22 and polypeptide with MW 67 kDa was demonstrated. Some similarity of the effect on protein band with Rm value 0.22 was observed under the influence of 4PU-30 as well. In the endosperm the effect of CHF was manifested as a delay of degradation. CHF and 4PU-30 influenced the activity of basic peroxidases in the opposite manner. The strong inhibiting effect of both cytokinins BAP and 4PU-30 on the activity of slow migrating superoxide dismutase (SOD) isoenzymes was observed. Each of the tested substances shows a differential effect on the proteins and antioxidant enzymes, despite the fact that all of them were applied at concentrations that inhibit the growth of seedlings.

Keywords: abscisic acid; benzylaminopurine; catalase; chlorsulfuron; maize; peroxidase; proteins; N^1-(2-chloro-4-pyridyl)-N^2-phenylurea; superoxidedismutase

Germinating seeds in soil are exposed to many unfavourable environmental conditions like flooding, drought, low and high temperatures, residual amounts of herbicides, plant growth regulators etc. Studies on plant responses to herbicide and plant growth regulator application are important because the appropriate treatments are essential for the proper growth of plants and high quality of crop production. These treatments cause a wide variety of morphological, physiological and biochemical effects. On the molecular level they are manifested as changes in the pattern of gene expression (Skriver and Mundy 1990, Guan and Scandalios 1998, Guan et al. 2000). Recent investigations showed that CHF frequently retarded growth and caused yield losses at crop harvest (Fletcher et al. 1995, Rengel and Wheal 1997). Biochemical and genetic studies have demonstrated that in plants treated by CHF the inhibition of enzyme acetolactate synthase (ALS) was the primary reason for the observed effects (LaRossa and Falco 1984, Ray 1984) but less attention has been given to other proteins and enzymes (Zabalza et al. 2002). Benzylaminopurine (BAP) and N^1-(2-chloro-4-pyridyl)-N^2-phenylurea (4PU-30) are cytokinins classified into different groups according to their chemical structure. They exhibit similar physiological effects, but 4PU-30 has higher biological activity and selectivity in certain bioassays (Karanov et al. 1992). 4PU-30 has a protective effect and caused an alleviation of the herbicide-induced inhibition of growth (Karanov et al. 2000).

It is well known that environmental stresses exert their effect by reactive oxygen species (ROS) accumulation (Yu and Rendel 1999). A tight correlation exists between ABA and ROS production. Environmental stresses and growth regulators alternate the expression of many genes encoding protective enzymes – antioxidants and proteins helping the cells to cope with the stress as well as supraoptimal concentrations of plant regulators.

In the present study the effect of CHF on electrophoretic profiles of the total and thermostable soluble proteins, polypeptides and some antioxidant

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enzymes were compared to the effect of abscisic acid (ABA), BAP and 4PU-30. All substances were applied at concentrations that inhibit growth of maize seedlings.

MATERIAL AND METHODS

Investigations were carried out with 5-day old maize (*Zea mays* L.) seedlings, hybrid Kneja 530. Dry mature seeds were soaked for 4 hours in solutions of 500μM BAP, 500μM ABA, 500μM 4PU-30 and 500μM CHF (Petkova et al. 2003). The seeds were washed with distilled water after treatment and placed on moist filter paper roles in the dark in vermiculate at temperature 24–25°C. Seeds, soaked in distilled water were used as a control. Coleoptiles and endosperms were analysed.

Soluble proteins were extracted with 0.5M Tris-HCl buffer, pH 7.5 (plant material:buffer 1:3 w/v) and the extract was centrifuged at 12 000 g for 30 min. All steps of extraction were performed at 4°C. The supernatant was used as a crude extract for protein, polypeptide, peroxidase, catalase and superoxide dismutase (SOD) electrophoretic investigations. Protein content of the extracts was determined by the method of Lowry et al. (1951). The fraction of termostable proteins was obtained by the procedure of Close et al. (1993) The supernatants with soluble proteins were boiled at 100°C for 10 min, kept in ice and then centrifuged at top speed in a microcentrifuge for 15 min at 4°C. The supernatante contained termostable proteins. Native total and thermostable soluble proteins and antioxidant enzymes were separated electrophoretically in 7.5% PAGE according to the method of Davis (1964). The protein bands were stained with Coomassie Brilliant Blue G 250. Equal amounts of protein (100 or 200 μg) were run on gels. Polypeptides were resolved by electrophoresis of proteins under denaturing (SDS) and reducing (β-mercaptoethanol) conditions in 12.5% PAGE (Laemmli 1970). Peroxidase isoenzymes were stained on the gels with benzidine as H-donor by the method of Ornstein (1964). Catalase isoenzymes were visualized on the gels according to Woodbury et al. (1971). Superoxide dismutase isoenzymes were stained by the method of Greneche et al. (1991). Quantitative differences between enzyme and protein spectra were evaluated by the intensity of the staining of bands and qualitative differences were estimated by the number and Rm (relative mobility – ratio between distance in cm from the start of the gel to the place of the protein [isoenzyme] band on the gel and the distance from the start of the gel to the front [marker dye bromphenol blue]) values of the bands. Electrophoretic patterns were scanned densitometrically (ERT-10DDR). All chemicals used were purchased by Sigma-Aldrich (Germany).

RESULTS

For maize coleoptiles 9–11 protein bands were visualized on the gels (Figure 1A) after native electrophoresis. A similar electrophoretic picture was observed for the control, ABA, BAP and 4PU-30 treated variants. The main quantity of protein was localized in the bands with Rm values 0.05, 0.35 and 0.50. The electrophoretic profile of CHF treated plants varied significantly. First of all the protein quantity of bands with Rm values 0.35 and 0.50 was much lower compared to the other variants and the most intensive band was a band

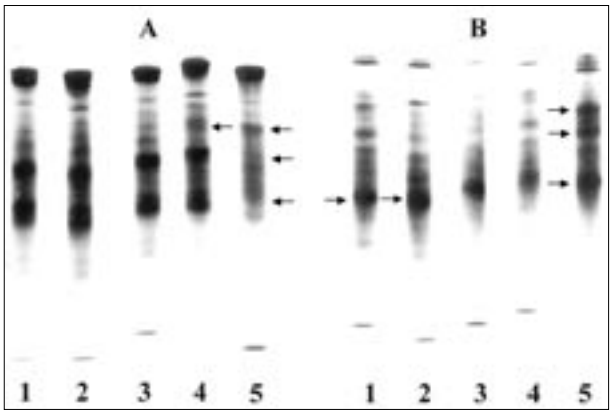

Figure 1. Total soluble proteins spectra (native PAGE) of maize colepotiles (A) and endosperm (B); lane 1 = control, lane 2 = ABA, lane 3 = BAP, lane 4 = 4PU-30, lane 5 = CHF; 100 μg of total protein was loaded in each tube; total protein profile of samples was visualized on gels by staining with Coomassie Blue

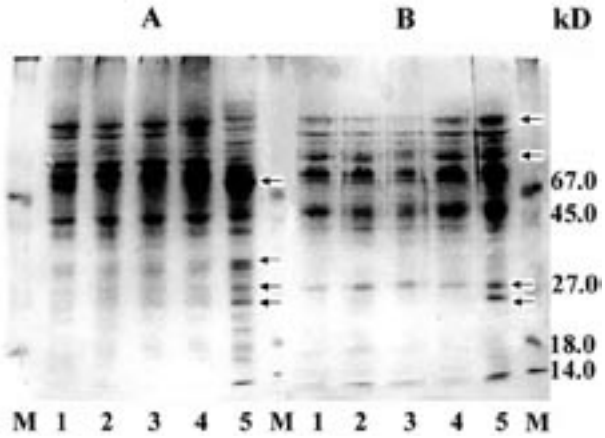

Figure 2. Polypeptide spectra (SDS-PAGE) of total proteins from maize colepotiles (A) and endosperm (B); lane 1 = control, lane 2 = ABA, lane 3 = BAP, lane 4 = 4PU-30, lane 5 = CHF, M = molecular mass, markers = BSA, egg albumin, tripsinogen, lactoglobulin, lyzozyme; 50 μg of total protein was loaded in each lane; polypeptide profile of samples was visualized on gels by staining with Coomassie Blue

with Rm value 0.22. The increase of intensity of this band was valid for 4PU-30 treated coleoptiles too. In the endosperm of maize seedlings 10–11 protein bands appeared (Figure 1B). The most intensive band for all variants was that with Rm value 0.50. The intensity of bands with Rm values 0.23 and 0.31 in the endosperms of CHF variant was higher than the same bands of the other variants.

Polypeptide spectra after SDS electrophoresis (Figure 2A) of coleoptiles from the control, ABA, BAP and 4PU-30 variants were very similar except in that the intensity of polypeptide with MW about 85 kDa in ABA and 4PU-30 treated plants was higher. The most intensively stained bands were those with MW about 120 kDa, 85 kDa, and 53 kDa. The greatest number of polypeptides (23) exhibited in the coleoptiles of CHF treated plants. A very intensive polypeptide band (MW 67 kDa) and the bands of many low molecular polypeptides in this variant were absent in the other variants however the quantity of polypeptide with MW 85 kDa was very low. The highest number of polypeptide bands was registered in the endosperm of CHF treated seedlings, which showed a higher intensity of staining in comparison to the other variants (Figure 2B). The most intensive were polypeptides with MW about 69 kDa and 53 kDa.

Thermostable proteins in the coleoptiles appeared only as traces (Figure 3A). In the endosperm well-manifested bands were revealed only in BAP and 4PU-30 treated plants (Figure 3B).

In the coleoptiles 12–14 anionic peroxidase isoenzymes were observed (Figure 4A). The most active was the fast moving group of peroxidase isoenzymes. A slight decrease in peroxidase activity for all isoenzymes from CHF treated coleoptiles was observed. A high peroxidase activity was established in the endosperm too (Figure 4B). Slight quantitative differences among the studied variants could be seen for moderate migrating isoenzymes.

Eight-nine cationic peroxidases are presented in the coleoptiles (Figure 5A). The most active were

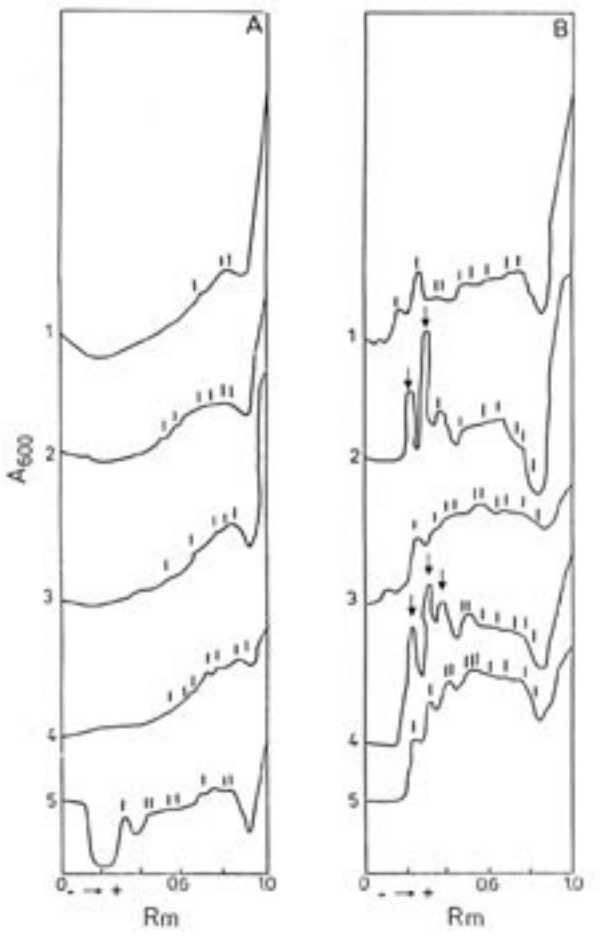

Figure 3. Densitometric scans of termostable soluble proteins spectra (native PAGE) from maize coleoptiles (A) and endosperm (B); 1. control, 2. ABA, 3. BAP, 4. 4PU-30, 5. CHF; 200 μg of total protein was loaded in each tube

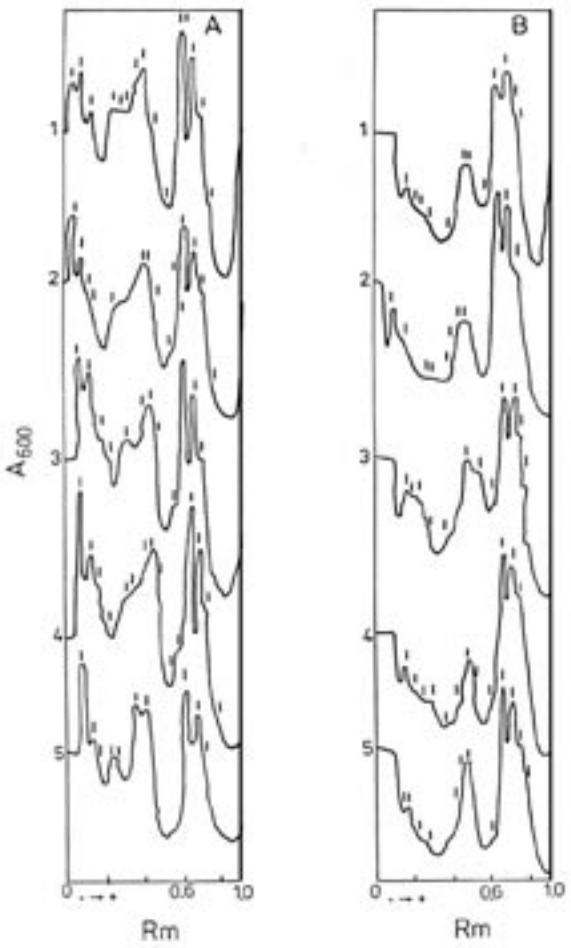

Figure 4. Densitometric scans of anionic peroxidase isoenzymes from maize coleoptiles (A) and endosperm (B); 1. control, 2. ABA, 3. BAP, 4. 4PU-30, 5. CHF; 50 μg of total protein was loaded in each tube

the peroxidases from 4PU-30 treated coleoptiles and especially the isoenzymes with Rm values 0.67 and 0.87. The same bands were very intensive in the ABA-treated coleoptiles. CHF treated coleoptiles showed the lowest basic peroxidase activity. The activity of basic peroxidases in the endosperm was significantly lower than that of coleoptiles (Figure 5B). The lowest peroxidase activity was established for CHF. An increase of enzyme activity was detected in the isoenzyme band with Rm value 0.87 for 4PU-30 as it was in coleoptiles samples too.

A high catalase activity was observed in coleoptiles as well as in the endosperm of maize seedlings (Figure 6A). Two slow migrating isoenzymes could be seen. There were no significant differences among the tested variants with exception for BAP and ABA treated coleoptiles where the catalase activity was slightly lower (Figure 6B).

Five to six bands with SOD activity differing in intensity of staining were visible in the coleoptiles (Figure 7A). The most intensive were slow migrating isoenzymes with Rm values 0.18 and 0.28 respectively. The activity of both isoenzymes was significantly inhibited by BAP and 4PU-30 and a slight activation could be seen for ABA-treated coleoptiles. There were no significant differences among SOD-isoenzymes in the endosperm (Figure 7B).

DISCUSSION

A common feature of plant response towards the substances at these concentrations was the reduction of coleoptile and root growth. ABA, BAP and 4PU-30 applied in concentrations of 500μM inhibited similarly the growth of seedlings of about 25%. CHF applied at the same concentration reduced seedling growth approximately with 75% (Petkova et al. 2003). The results from the present investigation indicated that the protein quantity of bands with Rm values 0.35 and 0.50 in CHF treated coleoptiles was significantly lower compared to

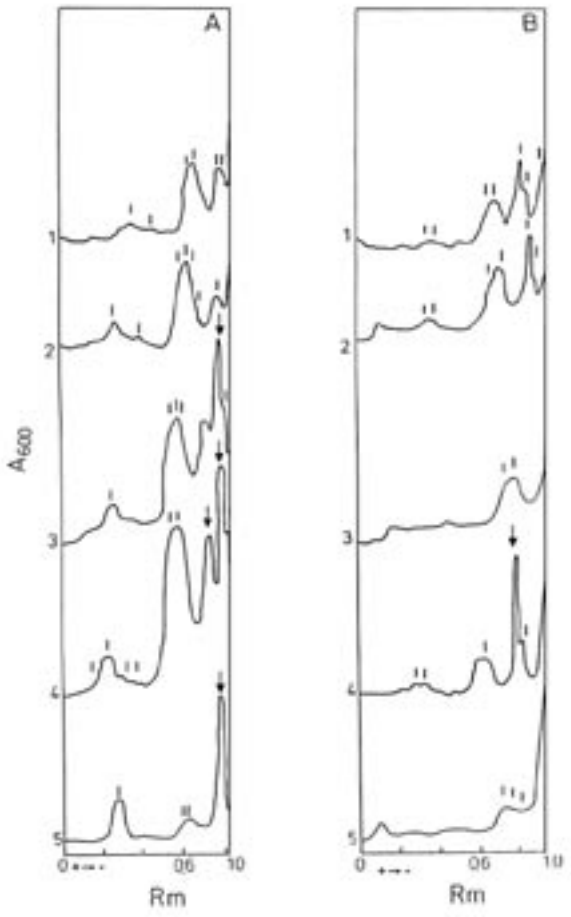

Figure 5. Densitometric scans of cationic peroxidase isoenzymes from maize coleoptiles (A) and endosperm (B); 1. control, 2. ABA, 3. BAP, 4. 4PU-30, 5. CHF; 50 μg of total protein was loaded in each tube

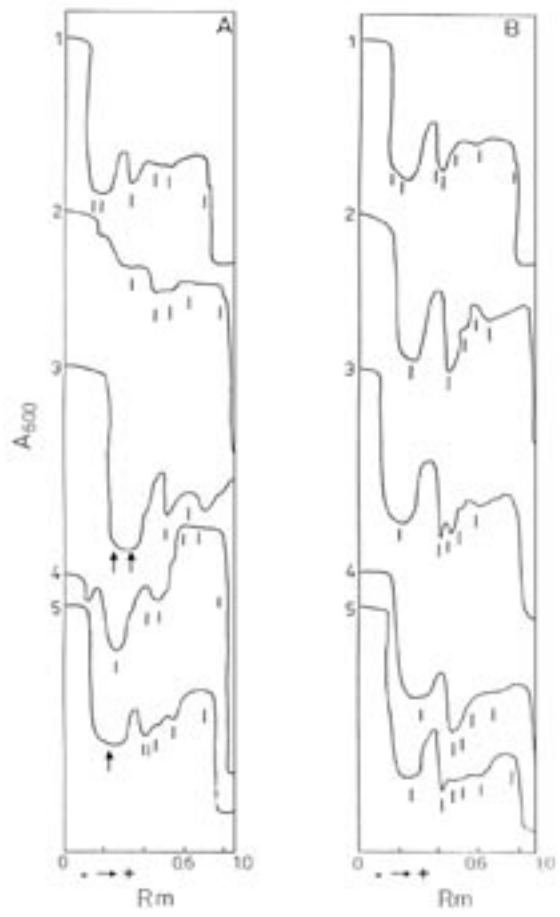

Figure 6. Densitometric scans of catalase isoenzymes from maize coleoptiles (A) and endosperm (B); 1. control, 2. ABA, 3. BAP, 4. 4PU-30, 5. CHF; 50 μg of total protein was loaded in each tube

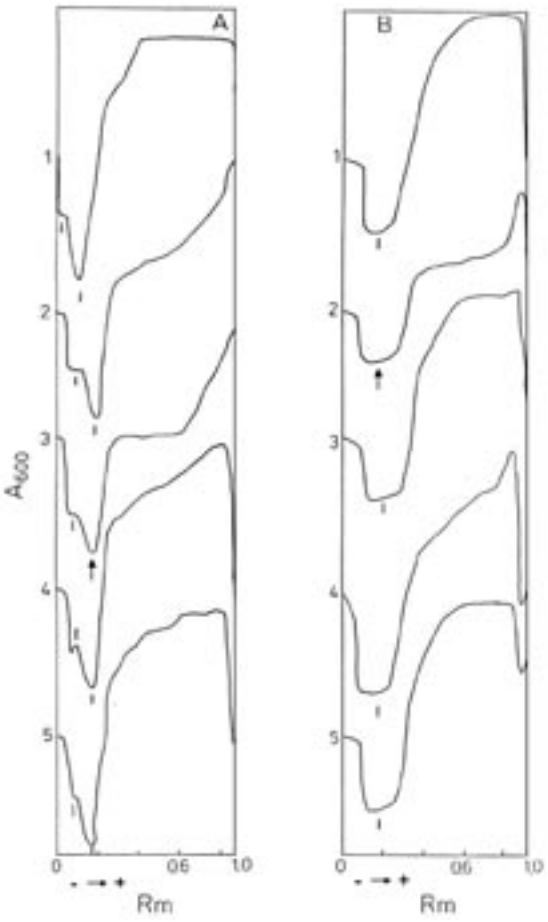

Figure 7. Densitometric scans of superoxidismutase isoenzymes from maize coleoptiles (A) and endosperm (B); 1. control, 2. ABA, 3. BAP, 4. 4PU-30, 5. CHF; 100 μg of total protein was loaded in each tube

the control and the rest of the variants. This was in agreement with the reduction of protein biosynthesis resulting from the decrease of synthesis of branched chain amino acids (LaRossa and Falko 1984, Ray 1984). It was noteworthy that the band with Rm value 0.22 was very intensive in CHF and 4PU-30 treated coleoptiles. This effect may be due to the similarity in the structure of both compounds (Brown and Catterman 1994). The more pronounced stimulating effect of CHF was observed in the protein quantity of bands with Rm 0.23 and 0.31 in the endosperm. Contrary to Ray's data (1984), where protein synthesis was unaffected under the influence of CHF and cell division in maize roots was reduced 80–90%, we found that the reduction of growth was accompanied by a significant decrease in protein quantity and specific qualitative changes in electrophoretic patterns for coleoptiles. More convenient evidence that CHF influences protein synthesis and pattern was in data obtained for the polypeptide composition of CHF treated coleoptiles. *De novo* synthesized amounts of polypeptide (MW 67 kDa) and low molecular polypeptides appeared in the coleoptiles. The enhanced intensity or increased quantity of polypeptides with MW 69 kDa and 53 kDa in the endosperm was found. There was a good correlation between data from native and SDS-PAGE electrophoresis. They both indicated the appearance of protein with Rm 0.22 respectively and polypeptide with MW 67 kDa under the influence of CHF. What is its role in the metabolism of CHF treated plants remains unclear. On the other hand the decrease in the protein quantity of native protein bands with Rm values 0.35 and 0.50 correlated with the availability of great number of low molecular polypeptides established in the coleoptiles and endosperm of CHF treated plants. This group of polypeptides remained free probably because they cannot be involved in the composition of heteromeric proteins due to the lack of polypeptides containing branched amino acids. Another possible explanation was that CHF inhibited the degradation of proteins in the endosperm and as a result of this inhibition; the flux of free amino acids to the growing coleoptiles was reduced.

There was no specific effect of the tested substances studied on the activity of anionic peroxidases (Mader and Fussl 1982). Significant differences in the manner and degree of influence on the activity of basic peroxidases between growth regulators tested and CHF were observed. It is well known that these enzymes function as IAA-oxidases as well (Gordon and Henderson 1973) and participated in the cessation of plant growth. Recently Quiroga et al. (2001) have shown that basic peroxidases function in the lignification too. The high activity of basic peroxidases in ABA treated coleoptiles was in agreement with growth inhibiting role of ABA. It could be mention that both cytokinins studied influenced differentially the activity of basic peroxidases (Figure 5). BAP had no effect on the individual isoenzymes, while 4PU-30 drastically increased their activity. The low enzyme activity under the influence of CHF might be a result of the reduction of total protein biosynthesis (Figure 1). The data presented in this work show that sulfonilurea herbicides (CHF) may influence not only the activity of ALS (Ray 1984) but also the activity of other enzymes (basic peroxidase). Similar results were obtained by Zabalza et al. (2002) for enzymes of fermentative metabolism.

The presence of two active catalase isoenzymes was in agreement with the data of Scandalios et al. (1997). They reported that CAT-1 and CAT-3 are the only catalase isoenzymes presented in the coleoptile of the germinating maize seedling. In our case a very slight effect of ABA on catalase isoenzymes was established. This finding was in disagreement with the data for strong effect of ABA on CAT-1 activity in maize (Guan et al. 2000).

The strong inhibiting effect of BAP and 4PU-30 on the activity of both slow migrating SOD isoenzymes were of interest, and should be mentioned. Only a slight activation could be seen for ABA-treated coleoptiles. Guan and Scandalios (1998) found that Sod 4A transcript was increased under the influence of ABA in young maize leaves. They stated that ABA mediated changes in oxygen free radical levels activated antioxidant enzymes. There was a difference between our and their results about the degree of ABA effect on the activity of catalase as well as SOD isoenzymes.

The significant effect of CHF on seedling growth (Petkova et al. 2003) correlated with the data established in the present investigations. The reduction of growth by CHF was accompanied by significant decrease in quantity of main native proteins and specific qualitative changes in electrophoretic patterns of coleoptile samples. The appearance of a native protein with Rm 0.22, respectively and polypeptide with MW 67 kDa was shown. A similar effect on protein band with Rm value 0.22 was observed of 4PU-30 too. In the endosperm the effect of CHF was manifested as a delay of protein degradation. CHF and 4PU-30 influenced the activity of basic peroxidases in the opposite manner. The strong inhibitory effect of the two cytokinins BAP and 4PU-30 on the activity of both slow migrating SOD isoenzymes was observed. In conclusion, that was an object of our study, each substance exhibited differential effect on the proteins and antioxidant enzymes despite the fact that all of them were applied at concentrations, which inhibited the growth of seedlings.

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ABSTRAKT

Vliv růstových regulátorů a chlorsulfuronu na elektroforetické profily rozpustných bílkovin, polypeptidů a antioxidačních enzymů v klíčních rostlinách kukuřice

Výzkum proběhl na klíčních rostlinách kukuřice (*Zea mays* L.). Byl porovnáván vliv chlorsulfuronu (CHF) na elektroforetické profily všech bílkovin dohromady a termostabilních bílkovin jednotlivě, polypeptidů a některých enzymů s antioxidačními vlastnostmi s vlivem kyseliny abscisové (ABA), benzylaminopurinu (BAP) a N^1-(2-chloro-4-pyridyl)-N^2-fenylmočoviny (4PU-30). Sledované látky byly aplikovány v koncentracích, které inhibovaly růst klíčních rostlin. Bylo zjištěno, že CHF má nejprůkaznější efekt na růst klíčních rostlin stejně jako na studované biochemické charakteristiky. Snížení růstu pomocí CHF bylo doprovázeno průkazným snížením množství hlavních proteinů a specifickými kvantitativními změnami elektroforetických profilů v koleoptilech. Byl prokázán výskyt nového nativního proteinu s Rm 0,22 a polypeptidu s MW 67 kDa. Podobný účinek na proteinový proužek s hodnotou Rm 0,22 byl také pozorován u 4PU-30. V endospermu se vliv CHF projevoval opožděnou degradací a 4PU-30 působil opačně na aktivitu bazických peroxidáz. Byl pozorován silný inhibiční efekt obou cytokininů (BAP a 4PU-30) na aktivitu pomalu postupujících izoenzymů superoxidismutázy (SOD). Každá z testovaných látek působí rozdílně na bílkoviny i antioxidační enzymy, i když všechny byly aplikovány v koncentracích, které inhibují růst klíčních rostlin.

Klíčová slova: kyselina abscisová; benzylaminopurin; kataláza; chlorsulfuron; kukuřice; peroxidáza; proteiny; N^1-(2-chloro-4-pyridyl)-N^2-fenylmočovina; superoxiddismutáza

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