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Differences in the metabolic responses of root tips of wheat and rye to aluminium stress

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Abstract

The effects of aluminium (Al) ions on the metabolism of root apical meristems were examined in 4-day-old seedlings of two cereals which differed in their tolerance to Al: wheat cv. Grana (Al-sensitive) and rye cv. Dańkowskie Nowe (Al tolerant). During a 24 h incubation period in nutrient solutions containing 0.15 m*M* and 1.0 m*M* of Al for wheat and rye, respectively, the activity of first two enzymes in the pentose phosphate pathway (G-6-PDH and 6-PGDH) decreased in the sensitive cultivar. In the tolerant cultivar activities of these enzymes increased initially, then decreased slightly, and were at control levels after 24 h. In the Al-sensitive wheat cultivar a 50% reduction in the activity of 6-phosphogluconate dehydrogenase was observed in the presence of Al. Changes in enzyme activity were accompanied by changes in levels of G-6-P - the initial substrate in the pentose phosphate pathway. When wheat was exposed for 16 h to a nutrient solution containing aluminium, a 90% reduction in G-6-P concentration was observed. In the Al-tolerant rye cultivar, an increase and subsequently a slight decrease in G-6-P concentration was detected, and after 16 h of Al-stress the concentration of this substrate was still higher than in control plants. This dramatic Al-induced decrease in G-6-P concentration in the Al- sensitive wheat cultivar was associated with a decrease in both the concentration of glucose in the root tips as well as the activity of hexokinase, an enzyme which is responsible for phosphorylation of glucose to G-6-P. However, in the Al-tolerant rye cultivar, the activity of this enzyme remained at the level of control plants during Al-treatment, and the decrease in the concentration of glucose occurred at a much slower rate than in wheat. These results suggest that aluminium ions change cellular metabolism of both wheat and rye root tips. In the Al-sensitive wheat cultivar, irreversible disturbances induced by low doses of Al in the nutrient solution appear very quickly, whereas in the Al-tolerant rye cultivar, cellular metabolism, even under severe stress conditions, is maintained for a long time at a level which allows for root elongation to continue.

Abbreviations: G-6-PDH – glucose-6-phosphate dehydrogenase, 6-PGDH – 6-phosphogluconate dehydrogenase, G-6-P – glucose-6-phosphate, TEA – triethanolamine.

Introduction

Aluminium (Al) toxicity is a major factor limiting the growth of plants in mineral acid soils with a pH below 5.0 (Foy, 1988). The toxic effect of this ion is primarily expressed as a drastic inhibition in root elongation, possibly a result of Al-induced disturbances of cell division in root apical meristems (Bennet and Breen, 1991; Eleftheriou et al., 1993; Taylor, 1988). Foliar symptoms of aluminium toxicity resemble those of calcium and phosphorus deficiency as aluminium acts as an antagonist of these two elements (Foy, 1992).

Cultivated plants differ significantly in their response to Al-toxicity. Among cereals, rye is considered to be the most Al-tolerant, whereas wheat is regarded as very Al-sensitive (Foy, 1988; Ponnampe-

ruma, 1982; Ślaski, 1990). Although many mechanisms of Al-tolerance have been postulated, none of these can satisfactorily explain the observed differences among genotypes.

Our previous results (Ślaski, 1989, 1990) show that NAD^+ kinase, an enzyme which catalyses the only known biochemical reaction of $NADP^+$ synthesis, is involved in the response of cultivated plants to Al-stress. By regulating production of $NADP^+$, which is a rate-limiting factor in many enzymatic processes, NAD^+ kinase may control several biosynthetic pathways including pentose phosphate pathway, shikimic acid pathway, as well as pathways involved in the synthesis of fatty acids, amino acids and nucleic acids (Allan and Trewavas, 1987). Among these pathways, the pentose phosphate pathway may be of great importance in Al-tolerance mechanism, since this pathway provides a number of intermediates (pentoses, erythrose-4-phosphate, NADPH) which are involved in the synthesis of substances which are considered to play a role in root response to Al-stress. In addition, the first two enzymes of this pathway viz. glucose-6-phosphate dehydrogenase and 6- phosphogluconate dehydrogenase are $NADP^+$-dependent. Under Al-stress, NAD^+ kinase activity in an Al-tolerant rye cultivar was 4-times higher than in an Al-sensitive wheat cultivar (Ślaski, 1990), thus it appears that Al-tolerance may be connected with availability of $NADP^+$.

The aim of this work was to determine the effect of aluminium on the cellular metabolism of root tips from two cereals that differ in their tolerance to Al.

Material and methods

Plant material

Four-day-old seedlings of an Al-tolerant rye cultivar (*Secale cereale* cv. Dańkowskie Nowe) and an Al-sensitive wheat cultivar (*Triticum aestivum* L. cv. Grana) were used for experiments. Seeds were sterilized with a 0.1% solution (w/v) of $HgCl_2$ for 10 min., rinsed thoroughly with tap water and germinated overnight at room temperature on filter paper in Petri dishes. Plants were subsequently grown for 4 days under conditions previously described (Ślaski, 1992) with the following modifications. The nutrient solution (pH 4.50) contained: 0.4 m*M* $CaCl_2$, 0.65 m*M* KNO_3, 0.25 m*M* $MgCl_2$, 0.01 m*M* $(NH_4)_2SO_4$ and 0.04 m*M* NH_4NO_3. Phosphorus was omitted in the nutrient solution to avoid precipitation with aluminium. After 4 days, seedlings were transferred to fresh nutrient solution with aluminium added in the form of $AlCl_3 \times 6H_2O$, at a concentration of 0.15 m*M* and 1.0 m*M* for wheat and rye, respectively. Control plants were transferred to fresh nutrient solution without aluminium. Immediately after Al-treatment the activity of enzymes and concentration of substrates in root tips were assessed as described below. Besides about 30–40 seedlings from each combination were transferred to nutrient solution without aluminium for 48 h to determine the ability of roots to grow after removal of Al-stress. The root regrowth was determined by staining roots with a 0.1% solution (w/v) of Eriochrome Cyanine R for 10 min. (Aniol, 1984).

Extraction of the enzymes

Root tips 3–4 mm long from 50–60 seedlings (200–300 mg fresh weight) were cut off from the root, pulverized in liquid nitrogen and extracted with 50 m*M* Tris buffer (1:10, w/v) (pH 7.4) containing 1 *M* KCl, 1 m*M* EDTA, 2 m*M* $MgCl_2$ and 2.5% (w/v) polyvinylpyrrolidone (MW 40 000). The mixture was shaken for 15 min. at 4°C and centrifuged at 22,000 *g* for 30 min. The pellet was discarded and the supernatant was used for assays of enzymes and metabolites.

Enzyme assays

The activities of G-6-PDH, 6-GPDH and hexokinase were measured at 340 nm with a Beckman spectrophotometer, model 26 according to Deutch (1983), Bergmayer (1974a) and Bergmayer (1974b), respectively. The incubation mixture for G-6-PGDH assay contained: 150 μL 0.5 *M* Tris-HCl (pH 7.5), 100 μL 3.8 m*M* $NADP^+$, 100 μL 0.63 m*M* $MgCl_2$, 200 μL 33 m*M* G-6-P, 0.5 mg maleimide and 100 μL extract. The volume was adjusted to 100 μL with H_2O. The incubation mixture for 6-GPDH assay contained: 250 μL 0.5 *M* Tris-HCl (pH 7.5), 200 μL 31 m*M* gluconate-6-phosphate, 100 μL 0.63 *M* $MgCl_2$, 100 μL 3.8 m*M* $NADP^+$ and 100 μL extract. The volume was adjusted to 1000 μL with H_2O. For the hexokinase assay the incubation mixture contained: 500 μL 50 m*M* TEA buffer (pH 7.6), 500 μL 0.6 *M* glucose, 100 μL 0.1 *M* $MgCl_2$, 100 μL 4 m*M* $NADP^+$, 50 μL 1.6 m*M* ATP, 0.6 unit G-6-PDH and 100 μL extract. The final volume of the mixture was 1360 μL.

Assay of metabolites

Glucose and G-6-P were assayed in deproteinized samples at 340 nm with a Beckman spectrophotometer, model 26 according to Kunst et al. (1984) and Michal (1984), respectively. The concentration of reagents in 2 mL of the incubation mixture for glucose assay were as follow: 66 m*M* NaH_2PO_4, 3.8 m*M* $MgSO_4$, 1.5 m*M* ATP, 1.5 m*M* $NADP^+$. Subsequently 100 μL of extract (glucose) and finally enzyme solution consisted of hexokinase and G-6-PDH were added. The incubation mixture for G-6-P assay contained: 500 μL 0.4 *M* TEA buffer (pH 7.6), 100 μL 24 m*M* $NADP^+$, 100 μL 0.5 *M* $MgCl_2$, 500 μL extract and 150 units G-6-PDH.

The concentration of pentoses in root tips was determined according to Douglas (1981) as the difference in absorption at 552 and 510 nm of the reaction product of phosphoglycilol and xylose.

Results

The first visual symptom of aluminium toxicity is inhibition of root elongation. A 24-h incubation of seedlings in nutrient solution containing Al (0.15 m*M*) caused irreversible damage to the root apical meristems in the Al-sensitive wheat cultivar. This damage was reflected by a lack of root regrowth, when seedlings were transferred to the medium without aluminium for 2 days (Fig. 1). In rye a 7-times higher concentration of Al in the nutrient solution (1.0 m*M*) had little effect on the ability of roots to continue the elongation growth. One would expect that Al-induced inhibition of root growth is preceded by changes in root cellular metabolism. Among other pathways, the pentose phosphate pathway was supposed to play an important role in the response of Al-tolerant genotypes to Al-stress, possibly by providing intermediates and NADPH for the synthesis of different compounds which may be involved in protecting the root against Al-induced injury (Ślaski,1990).

Results of these studies indicate that in the Al-sensitive wheat cultivar, the activity of the first two enzymes of the pentose phosphate pathway i.e. glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase decreased under conditions of severe Al-stress (24h of Al treatment (Fig. 2A, B). The latter enzyme appeared to be more sensitive to aluminium than G-6-PDH as a 50% reduction of its activity was observed after 24-h stress (Fig. 2B). When the Al-tolerant rye cultivar was incubated

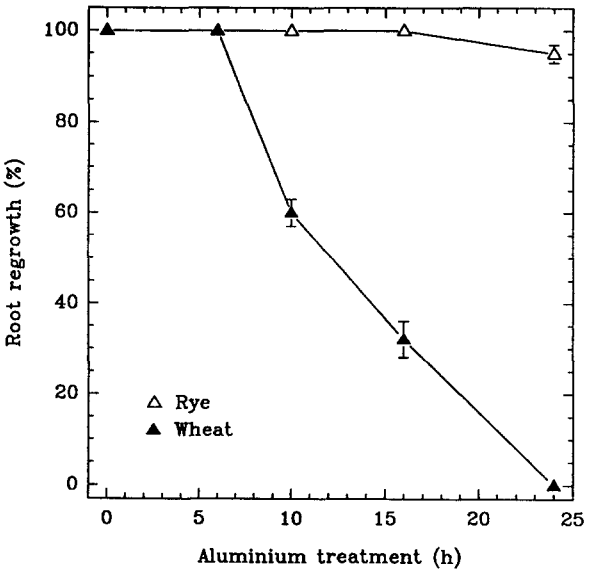

Fig. 1. Effect of aluminium treatment on regrowth of roots in cultivars of rye and wheat. About 40 four-day-old seedlings were incubated in a nutrient solution containing 0.15 m*M* and 1.0 m*M* $AlCl_3$ for wheat and rye, respectively. Data represents percent of seedlings which were able to continue to grow during the 48 h following treatment with aluminium. Values represent the mean of at least 3 replicates ± SE.

for 24 h in the presence of 1.0 m*M* Al, the activity of these enzymes increased initially, then decreased slightly, and after the Al-stress period the activity still remained at a level comparable to that of control plants (Fig. 2A, B).

The ability to maintain the activities of pentose phosphate pathway enzymes at relatively high levels in rye root tips under severe Al-stress may contribute to an increase of production of pentoses, which are one of the intermediates of this pathway (Fig. 3).

Changes in enzyme activities were also accompanied by changes the concentration of glucose-6-phosphate - a substrate which initiates the pentose phosphate pathway (Fig. 4).

When the Al-sensitive wheat cultivar was incubated for 16 h in nutrient solution containing 0.15 m*M* Al, a 90% reduction in G-6-P concentration was observed. However, in the Al-tolerant rye cultivar, an increase and subsequently a slow decrease in concentration of the G-6-P was detected, and after 16 h of Al-stress the concentration of this substrate was still higher than in control plants. This dramatic decrease in G-6-P concentration in the Al-sensitive wheat cultivar under Al-stress was connected with a decrease in both, the glucose concentration in the root tips and in hexokinase

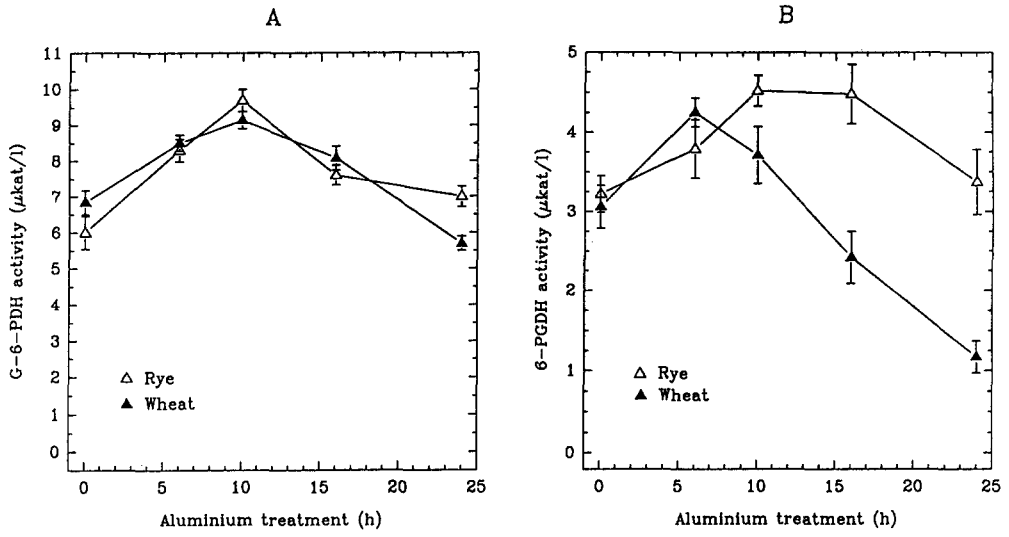

Fig. 2. Effect of aluminium treatment on activity of glucose-6-phosphate dehydrogenase (G-6-PDH) (**A**) and 6-phosphagluconate dehydrogenase (6-PGDH) (**B**) in root tips of wheat and rye. Plants were grown in a nutrient solution supplied with 0.15 m*M* and 1.0 m*M* of $AlCl_3$ for wheat and rye, respectively. The values indicated at time '0' represents levels of activity found in control plants which were not exposed to Al. Values represent the mean for at least 4 independent experiments ± SE.

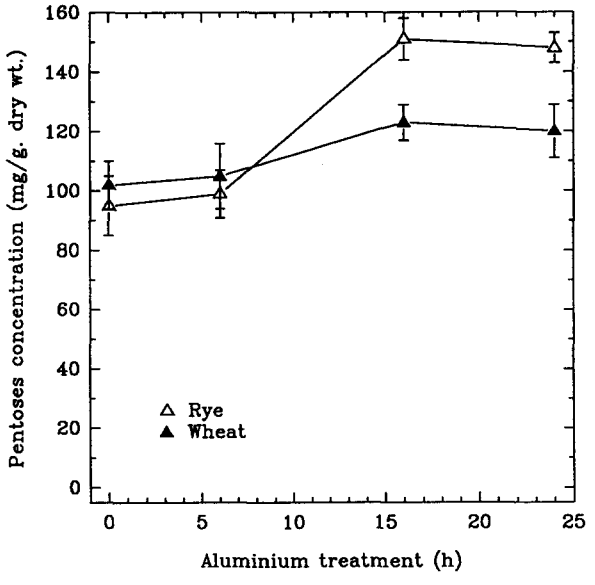

Fig. 3. Changes in the concentration of pentoses in root tips of wheat and rye in the presence of aluminium. Four-day-old plants were grown for 24 h in the presence of 0.15 m*M* and 1.0 m*M* $AlCl_3$ for wheat and rye, respectively. Values represent the mean of at least 3 replicates ± SE.

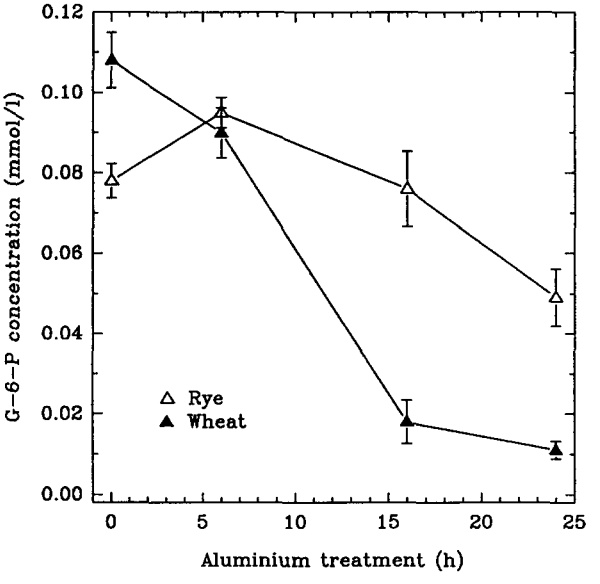

Fig. 4. Changes in the concentration of glucose-6-phosphate in root tips of wheat and rye exposed to aluminium. Plants were incubated in a nutrient solution containing 0.15 m*M* and 1.0 m*M* $AlCl_3$ for wheat and rye, respectively. Values represent the mean of at least 3 replicates ± SE.

activity, an enzyme which is responsible for phosphorylation of glucose to glucose-6-phosphate (Fig. 5A, B). However, in the Al-tolerant rye cultivar, activity of hexokinase remained at the control level during exposure to Al, and the decrease of glucose concentration occurred at a much slower rate than in wheat.

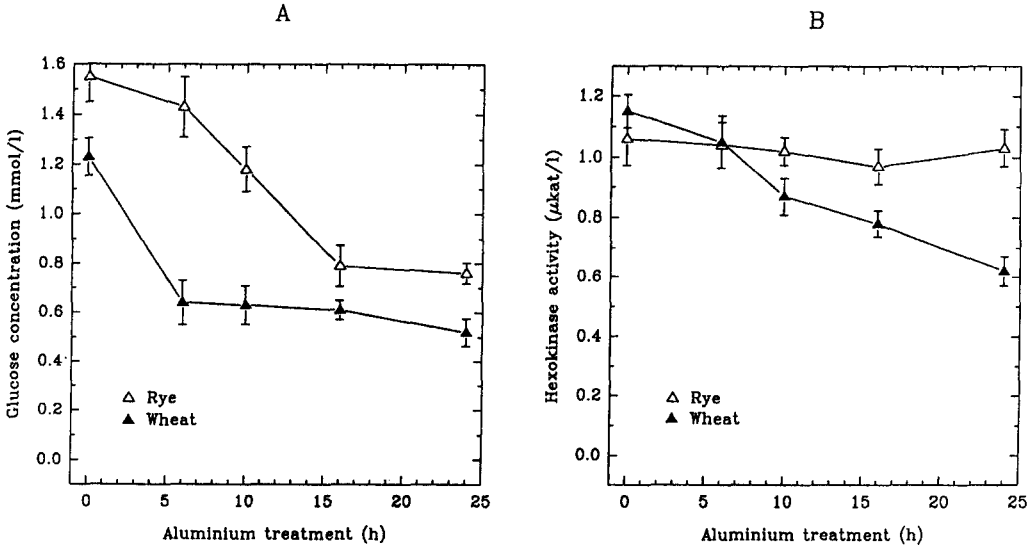

Fig. 5. Effect of aluminium treatment on glucose concentration (**A**) and hexokinase activity (**B**) in root tips of wheat and rye. Seedlings were grown for 24 h in the presence of 0.15 m*M* and 1.0 m*M* $AlCl_3$ for wheat and rye, respectively. Values represent the mean of at least 3 replicates ± SE.

Discussion

During the past few years the effect of aluminium on biochemical processes in roots has been widely discussed and contradictory data concerning Al influence on cellular metabolism has been presented.

One group of evidences shows that in spite of Al-induced inhibition of root elongation, during the first few hours of Al-treatment these ions do not directly disturb intracellular metabolism, presumably because Al does not cross the plasma membrane (Horst et al., 1992; Kinraide, 1988). These results suggest that inhibition of root growth must be due to some kind of impairment of processes occurring in the root apoplast.

The other group of data shows that in plants exposed to relatively low level of Al-stress this ion influences the metabolic activity inside the cell, suggesting that inhibition of root growth is a consequence of metabolic disorders (Copeland and de Lima, 1992; Karataglis, 1986; Pfeffer et al., 1986; Ślaski, 1990). Many authors have also found genotypical differences in metabolic responses to Al between Al-tolerant and Al-sensitive cultivars (Foy et al., 1987; Gakuru and Lefebvre, 1991; Galvez et al., 1991; Moustakas et al., 1992; Ślaski, 1989).

Data presented in this paper seem to support the second opinion since after only 6 h incubation of roots in nutrient solution containing Al a significant change, in both enzyme activity and metabolite concentration were observed. A different response to Al-stress was observed between rye and wheat. In the Al-tolerant rye cultivar the activity of G-6-PDH and 6-PGDH did not decrease below control level even after 24 h incubation in nutrient solution containing 1.0 m*M* Al (Fig. 2A, B). The ability to maintain undiminished flux through the pentose phosphate pathway in the face of severe Al-stress seems to play an important role in the mechanism of Al-tolerance in rye. This pathway provides intermediates such as pentoses, erythrose-4-phosphate, and NADPH. These intermediates are involved in the synthesis of several compounds (for example, amino acids, nucleic acids, coenzymes) which may be involved in the response of plants to Al-stress. This suggestion is supported by the observed increase in concentration of pentoses in rye root tips treated with Al, whereas in wheat no significant changes in the level of pentoses was observed (Fig. 3). In the Al-sensitive wheat cultivar, severe Al-stress led to decrease in the activity of G-6-PDH and a 50% reduction of 6-PGDH activity (Fig. 2A, B). These decreases in activity could be explained by a direct interaction of Al with enzymes in wheat, which may have a higher affinity for aluminium than similar enzymes in rye. To verify this supposition in vitro experiments examining the influence of Al on enzymes from both cultivars are needed. Contradictory data was recently presented by Copeland and de Lima (1992) which showed that after 24 h and 48 h of treatment with 0.075 m*M* Al the activity of G-6-PDH

in whole roots of wheat cv. Vulcan was not reduced. These results can be explained by the fact that this long-term Al-stress did not make irreversible changes in the root metabolism in this cultivar since even after 48 h of exposure to Al, a 25% of roots were still able to grow.

The activities of both enzymes are controlled by the ratio of NADPH to $NADP^+$ (Copeland and Turner, 1987; Hrazdina and Jensen, 1992). In some wheat cultivars the availability of $NADP^+$ is strongly reduced due to depression of NAD^+ kinase activity by Al ions (Ślaski, 1989). By disturbing other metabolic processes in which NADPH is used, Al could cause a reduction of the demand for this coenzyme and subsequent increase in concentration in root cells. Both these reasons may increase the NADPH/$NADP^+$ ratio and this in turn could lead to inhibition of enzymes. Another reason for the decrease in activity of pentose phosphate pathway enzymes may be a low level of glucose-6-phosphate – a substrate which is known to initiate this pathway (Lendzian, 1978; Turner and Turner, 1980). The 90% reduction in G-6-P concentration in wheat root tips during first the 16 h of Al-treatment (Fig. 4) suggests that besides the above mentioned reasons, the reduction of G-6-P availability may cause an addition reduction the activity of the pentose phosphate pathway. This hypothesis is supported by Hanson's (1991) work, which showed that in soybean Al-tolerant genotypes had higher concentrations of G-6-P than Al-sensitive selections.

Looking for a possible source of such a rapid decrease in G-6-P concentration in wheat, the substrate (glucose) and enzyme responsible for its phosphorylation (hexokinase) were examined (Fig. 5A, B). In the Al-sensitive wheat cultivar both factors were influenced by Al ions. The glucose level was drastically reduced by a 50% during the first 6 h of Al-stress. Since aluminium is known to be a strong inhibitor of hexokinase in pea seeds (Turner and Copeland, 1981), inhibition of hexokinase activity which can additionally disturb of G-6-P biosynthesis was expected. In fact, in the Al-sensitive wheat cultivar 50% reduction of hexokinase activity was observed, whereas this enzyme from rye root tips appeared to be completely insensitive to Al-toxicity, similarly to Copeland and de Lima (1992) results obtained for semi-tolerant wheat.

Taking into account the above presented results it may be concluded that Al changes the cellular metabolism in root tips of both wheat and rye. In the Al-sensitive wheat cultivar, irreversible disturbances induced by low doses of Al in the nutrient solution appeared very quickly, whereas in the Al-tolerant rye cultivar, cellular metabolism, even under severe stress conditions, was maintained for a long time at a level which allowed for root elongation to continue. In the future, studies are required to elucidate if the changes in metabolic activity observed in the presence of Al are connected with the concentration of aluminium at the site of enzyme action in the root symplast.

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