Proceedings of the 8th Hungarian Congress on Plant Physiology and the 6th Hungarian Conference on Photosynthesis, 2005

Volume 49(1-2):107-109, 2005
Acta Biologica Szegediensis
http://www.sci.u-szeged.hu/ABS

Response of rice seedlings to aluminium stress with varying phosphate supplies

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ABSTRACT In this study the effects of Al and phosphate (P) treatments on the growth and potassium influx in roots and transport toward the shoots of rice (*Oryza sativa* L. cv. Oryzella) was examined. Seedlings were grown hydroponically at pH 4.1 with different levels of Al and P. Increased levels of Al in the growth solution reduced root growth in accordance of the Al-concentration. Shoot growth was only moderately influenced in 14 day experiment. P (as phosphate) supply in the growth solution enabled rice plants to overcome the Al toxicity symptoms, however, the available P concentrations must exceed the Al concentration in the external medium. In short-term (6h) uptake experiments, P reduced the Al-stimulated $K^+(^{86}Rb)$ influx anomaly in roots, which indicates a definite Al-P interaction at the plant level too. Stimulation of glutamine synthetase activity was confirmed by in vitro tests and western blot detection after native PAGE separation of enzyme isoforms.

Acta Biol Szeged 49(1-2):107-109 (2005)

KEY WORDS

aluminium
potassium transport
growth
phosphate
rice
glutamine synthetase

Aluminium (Al^{3+}) toxicity is regarded as one of the major causes of nutritional disorders of upland crops on acid soils. The Al concentration in the soil solution is high, when the soil has low pH. As the pH increases in the submerged soil the Al concentration in the soil solution decreases below the critical level for Al toxicity. For this reason Al toxicity is rare in ordinary lowland rice, but can occur under some conditions if reduction after flooding is slow or in upland rice, when the soil is not submerged. Recently we found that in acidic environment phosphate ions exert significant influence on the toxic effect of Al in different cereals (Zsoldos et al. 2004). However, the exact explanation for the mechanism of Al detoxification is not completely understood so far. Organic Al(III)-complexes added *in vitro* to the reaction mixture can enhance the glutamine synthetase (GS, EC 6.3.1.2) activity, depending on the organic ligand (Kertész et al. 2002). Our aim in the present work was to investigate the effect of Al and P, separately and in combination, on the growth and potassium uptake of rice seedlings at low pH and measurement of GS activity in crude extracts and detection of the amount of GS isoforms in these samples.

Materials and Methods

Lowland rice variety (*Oryza sativa* L. cv. Oryzella) provided the experimental material in this study. Seedlings were grown hydroponically in 0.5 mM $CaSO_4$ solution at pH 4.1 with different levels of Al and P in a Conviron growth chamber under controlled conditions.

^{86}Rb was used to monitor the K^+ transport in plants. The $K^+(^{86}Rb)$ influx experiments lasted for 6 hours. Roots and shoots were then separated and the radioactivity of ^{86}Rb in the plant material was measured by a liquid scintillation counter (Zsoldos et al. 2000).

The dry weights of the roots and shoots of all plants were determined upon harvesting. Shoots and roots were harvested separately and subsequently dried at 70°C to constant weight. Organs (leaves, roots) of 14-day-old rice were used as source of glutamine synthetase (GS, EC 6.3.1.2). GS was measured *in vitro* ("synthetase" reaction), according to the colorimetric assay of Rhodes et al. (1975), with slight modification. Native PAGE was performed according Laemmli (1970).

Results

The effect of 0.1 mM Al at various (0.0, 0.1, 1.0 mM) phosphate supplies on the growth (DW) of the rice seedlings can be seen in Figure 1. The results clearly indicate that Al-treatment in P-starved (P_0) condition cause considerable decrease in root growth. Shoot growth was only moderately influenced in 14 day experiments, the presence of 0.1 and 1.0 mM P in the growth solution definitely ceased the toxic effect of Al.

The effects of Al- and P-treatments on the short-term (6h) $K^+(^{86}Rb)$ influx in roots are presented in Fig. 2. The data clearly show that the P reduces the toxic effect of Al on $K^+(^{86}Rb)$ transport in seedlings at pH 4.1. That is, P-treatment abolish the Al-stimulated $K^+(^{86}Rb)$ influx anomaly in roots. It is noteworthy, that $K^+(^{86}Rb)$ transport toward the shoot in the rice seedlings was always higher than in other species e.g. in wheat and other cereals, as experienced earlier.

Figure 3 shows the effect of 3 days long Al- and P-treatment on glutamine synthetase (GS) activities. A set of plants were pretreated with Al and P in 0.5 mM $CaSO_4$ solution,

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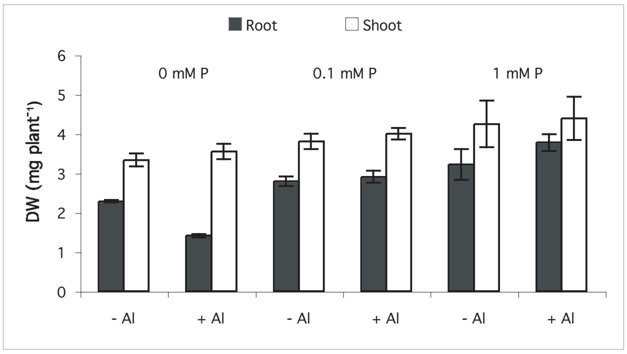

Figure 1. Effects of Al- and P-treatments on the growth of rice seedlings. Plants were grown for 14 days in 0.5 mM $CaSO_4$ solution at pH 4.1 without/with 0.1 mM Al ($AlCl_3$) and P (Na_2HPO_4) as indicated on the graph, pH was 4.1. All data show the means ±SD (n=8).

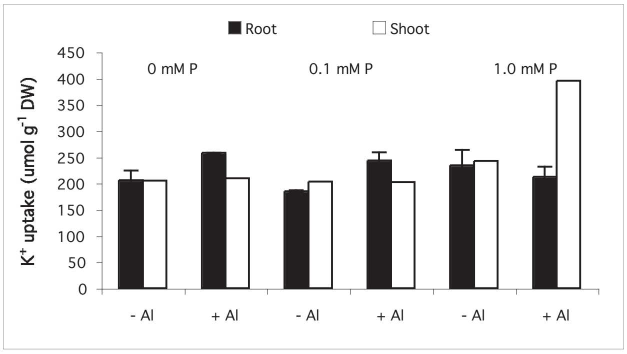

Figure 2. Effects of Al- and P-treatments on the K^+(^{86}Rb) uptake of the roots and the translocation toward the shoots of rice seedlings. Plants were grown for 14 days in 0.5 mM $CaSO_4$ solution at pH 6.5. After the 14th day the seedlings were treated for 6h with 1 mM K(^{86}Rb)Cl + 0.5 mM $CaCl_2$ + 0.1 mM $AlCl_3$ and Na_2HPO_4 solution as indicated on the graph, pH value was 4.1. All data show the means ±SD (n=8).

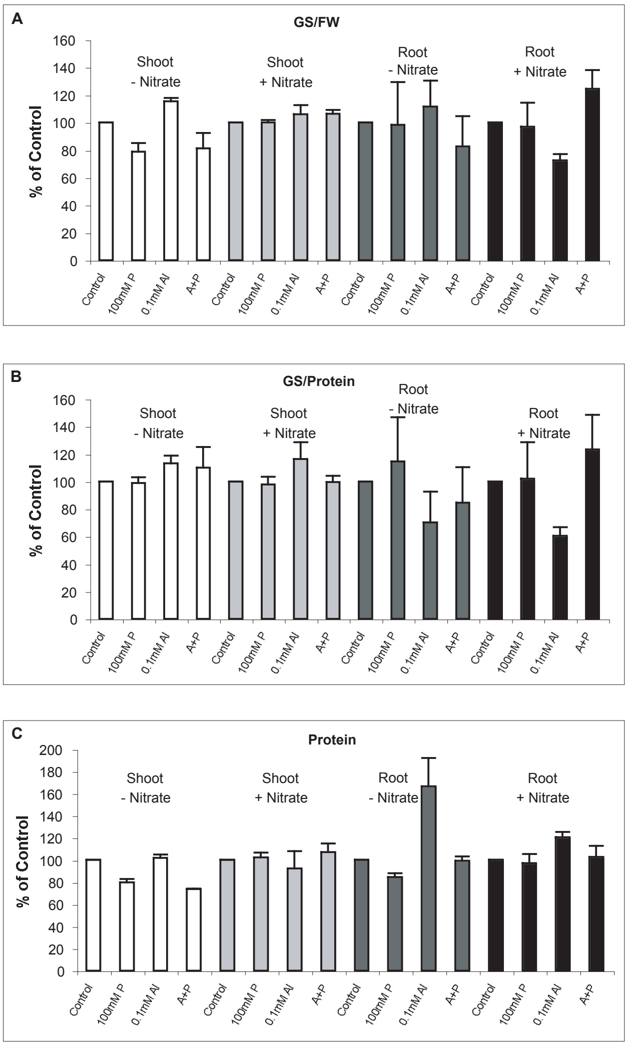

Figure 3. In vitro glutamine synthetase (GS) activity (A, B) and the protein content (C) in the organs of Al- and P-treated rice seedlings. Plants were grown for 14 days in 0.5 mM $CaSO_4$ and 0.5 mM $CaSO_4$ + 4 mM KNO_3 containing solution (pH 6.5), Al and P was added in the last 3 days (pH 4.1) as indicated on the graphs.

the other set had 4 mM KNO_3 in the medium, at pH 4.1. The protein contents were lower in P- containing shoot and root samples grown without nitrate, but the Al-treated seedlings show identical or increased protein level compared to the control. In the nitrate added samples the effects of Al/P were less characteristic (Fig. 3C). The tendency is visible in the results of *in vitro* GS activity tests. The GS activity was significantly enhanced in shoots of Al-treated plants in nitrate-free condition calculated on fresh weight basis (Fig. 3A), but these increases are still clear in the other set of data calculated for protein amount (Fig. 3B). In case of roots the fresh weight based activities were similar to the results in shoots, but the protein based activities proved lower. The nitrate containing root and shoot samples gave less characteristic or opposite results, because of the pH shift in the apoplast due to the $NO_3^-/2H^+$ co-transport.

The western blot detection of GS isoforms in shoots (up) and in roots (down) of Al- and P-treated rice seedlings are presented in Figure 4. The same amount of tissue extracts were applied and separated on the gels. This is identical with the fresh weight calculated data of Figure 3A. Densities show the same tendency as the *in vitro* data of Figure 3A.

Discussion

Rice is a highly Al-resistant species (Fig. 1), but the mechanism responsible for this is still unknown. Earlier we demonstrated that P-nutrition has significant role in Al-detoxification (Zsoldos et al. 2004). In case of rice and other cereals as well, P-supply proved effective in decreasing Al-toxicity symptoms by binding Al as Al-phosphate complexes at the root surface

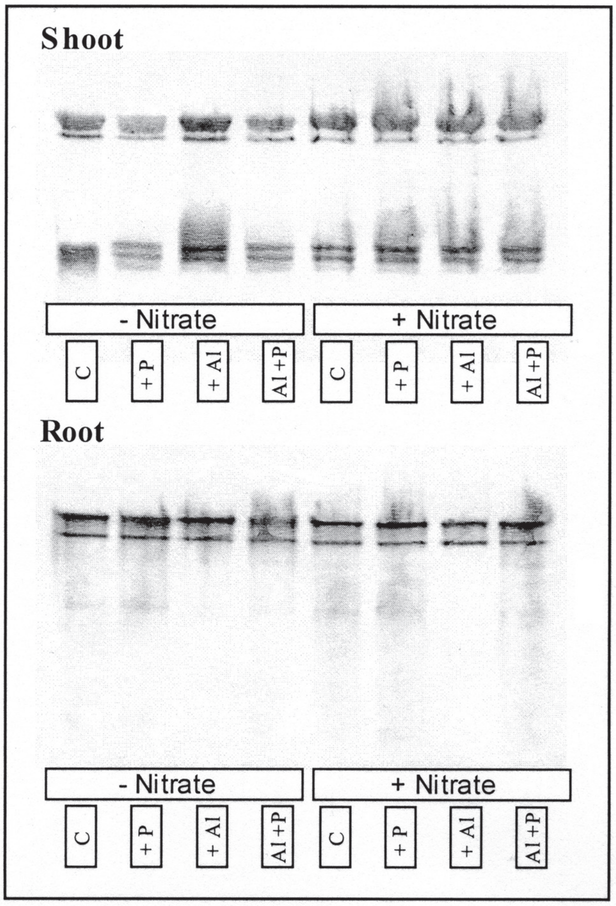

Figure 4. Western blot visualization of glutamine synthetase (GS) activity in the organs of Al- and P-treated rice seedlings. Treatments as in Fig. 3.

or in apoplast. However the availability of phosphate must exceed the Al^{3+} in the growth medium. The results with rice presented in Fig. 1 are in accordance with our earlier findings in different cereal species.

Al^{3+} and other metal ions like Cu^{2+} (Tari et al. 2002) is able to cause membrane perturbations, which appear in K^+ transport. Zsoldos et al. (2000) reported, that in short-term experiments the influx of K^+(^{86}Rb) and its transport toward the shoot was positively correlated with Al concentration of the outer medium. Present data clearly show P-treatments abolish this formerly experienced stimulatory effect of Al on K^+ uptake of roots (Fig. 2).

In higher plants, GS is responsible for the primary assimilation of ammonium originating from soil or generated in dinitrogen fixation and nitrate reduction. In addition, GS is also involved in the cellular detoxification of the ammonium released in many metabolic processes such as photorespiration or proteolytic degradation (Lea et al. 1990). It has two Mg-binding sites in its structure. The enhanced GS activity in the *in vivo* Al-stressed plants is similar to the effect of *in vitro* added Al, and it can be considered as a beneficial sideeffect, which can help to reassimilate the stress-born ammonium, preventing loss of nitrogen and ammonium toxicity.

Acknowledgements

This research was supported by the Hungarian Scientific Research Fund (OTKA T 046692). The supply of rice seeds from I. Simon-Kiss (Szarvas, Hungary) is gratefully acknowledged. The authors are grateful to J. Cullimore (Toulouse, France) for GS-AB. A.P. is a grantee of Bolyai J. scholarship.

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