

## **Aluminium toxicity in roots: correlation between root elongation and potassium fluxes in aluminium-sensitive and aluminium-tolerant cereal species**

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**Abstract.** The objective of this study was to examine the effects of aluminium (Al) and phosphate (P)-treatments on the growth and potassium uptake of roots and transport toward the shoots in Al-sensitive and Al-tolerant cereal species. Seedlings were grown hydroponically at pH 4.1 in the presence and absence of Al and P. Al in the growth solution reduced root growth in the order of Al-tolerance (rye>triticale>common wheat>durum wheat). Shoot growth was only moderately influenced at 50  $\mu$ M Al concentration. PO<sub>4</sub>-P in the growth solution enabled plants to overcome Al toxicity symptoms, however, different species respond differently to P-application. In the short term (6h) K<sup>+</sup>(<sup>86</sup>Rb) uptake experiments P reduced Al-P interaction at the plant level.

**Keywords:** aluminium, phosphate, phytotoxicity, growth, potassium uptake, durum wheat, common wheat, triticale, rye, pH

### **Introduction**

Al toxicity is the major factor limiting crop productivity on acid soils, which comprise up to 40% of the world's arable soils (Bóna et al. 1995; Foy 1992; von Uexkuell and Mutert 1995). Much of the damage to plant production is due to excess Al, the most common metal in soils. Al in soils

with  $\text{pH} > 5$  mostly forms insoluble oxides and alumino-silicates. At lower pHs there is a release of bioactive forms of Al, particularly  $\text{Al}^{3+}$  and „ $\text{Al}_{13}$ ”, which is toxic to plants (Marschner 1995). In this text, we denote Al as Al without implying a specific Al species.

Aluminium toxicity is primarily expressed by drastic inhibition of root growth in Al-sensitive genotypes (Taylor 1988; Bóna et al. 1998). Typically, the main axis of the roots is inhibited, and the root become stubby, thickened, brown, brittle and occasionally necrotic (Archambault et al. 1997; Hecht-Buchholz and Foy 1981). The toxic effect of Al on plants are well documented, however, the physiological reasons for inhibition of root elongation by Al is not completely understood so far.

Differential sensitivity of species and genotypes has been extensively studied and several mechanisms of Al tolerance have been suggested including chelation of Al via formation of Al complexes with organic acids, acidic polypeptides and/or proteins (Kochian 1995; Kochian and Jones 1997; Libaga et al. 2004; Lipton et al. 1987; Ma et al. 2001; Miyasaka et al. 1991; Ogawa and Matsumoto 2001). Al-phosphate complexes may also occur on the surface of the roots, furthermore, within cell wall or on the surface of the plasma lemma influencing the Al toxicity of plants (Clarkson 1967; Lüttge and Clarkson 1992; McCormick and Borden 1973; Miyasaka et al. 1991, Naidoo et al. 1978; Wagatsuma et al. 1983).

The objective of our study was to determine the effects of Al stress on the growth and potassium transport of roots of different cereal species with varying phosphate (P)-supplies hoping that our results could be valuable tools for studying the physiological basis of mechanisms of Al tolerance in plants.

## Materials and Methods

### *Plant Materials*

Common wheat (*Triticum aestivum* L. cv. Jubilejnaja 50), durum wheat (*T. durum* Desf. cv. GK Betadur), triticale (*xTriticosecale* Wittmack cv. GK Marco) and rye (*Secale cereale* L. cv. GK Wibro) provided the experimental material in this study. Earlier tests in acid soil and culture solutions showed that GK Wibro is an Al-tolerant, Jubilejnaja 50 and GK Marco are moderately tolerant type and GK Betadur is a moderately sensitive to Al toxicity (Bóna et al. 1992, 1995; Vashegyi et al. 2002).

Seeds were washed and germinated in Petri dishes in darkness at 25°C, then seedlings were placed on stainless screens over glass beakers. Each beaker contained 300 ml growth solution and 8 seedlings. Seedlings were

grown hydroponically for 7 days in different growth solutions under controlled conditions in growth chamber.

At start of the growth period the low salt culture solutions contained 0.5 mM CaSO<sub>4</sub>. Phosphorus (as phosphate) was added to the solutions at concentration of 0.1 and 1.0 mM. In growth experiments AlCl<sub>3</sub> was present at concentrations of 0 (control) and 0.05 mM. The initial pH values of growth solutions were adjusted with 0.1 M HCl or 0.1 M NaOH as needed, and checked and renewed every day to maintain nutrient and Al concentration. Seedlings were illuminated for 16 h at about 65% relative humidity and 25/20°C day/night temperatures. The light intensity at plants level was 60 W m<sup>-2</sup> (120 μmol m<sup>-2</sup> s<sup>-1</sup>).

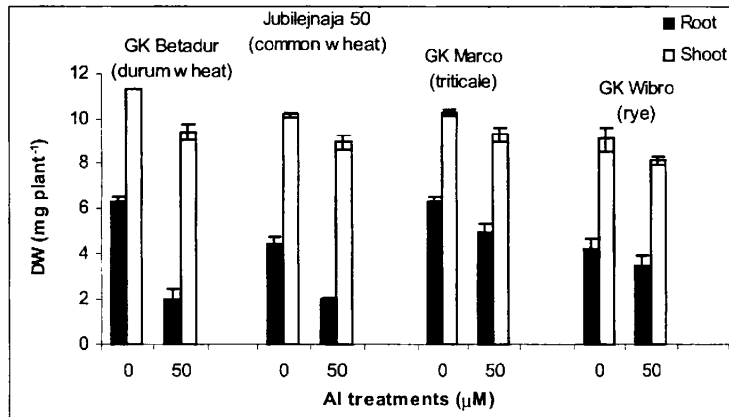
Shoots and roots were harvested separately and plant parts were dried at 70°C to constant weight. Dry weights of all plant were determined upon harvesting. All experiments were performed in triplicate with whole plants, the data given below are averages with SD (n=8). A typical series of results from three independent experiments are presented in the figures.

#### *Potassium uptake experiments*

<sup>86</sup>Rb was used to monitor the K<sup>+</sup> transport in plants (Erdei and Zsoldos 1977; Zsoldos et al. 2001). In the K<sup>+</sup>(<sup>86</sup>Rb) influx experiments plants were precultured in 0.5 mM CaSO<sub>4</sub> solution in the presence or absence of phosphate. After the 7th day plants were transferred to different uptake solutions containing 1 mM K(<sup>86</sup>Rb)Cl + 0.5 mM CaCl<sub>2</sub> + AlCl<sub>3</sub> and Na<sub>2</sub>HPO<sub>4</sub> as indicated in figure legends. The pH of the absorption solution was initially adjusted to the appropriate value with 0.1 M HCl or 0.1 M NaOH, and checked again at the end of the absorption period. The K<sup>+</sup>(<sup>86</sup>Rb) influx experiments lasted for 6 hours. Roots and shoots were then separated and radioactivity of <sup>86</sup>Rb in the plant material was measured by scintillation counter as described earlier (Zsoldos et al. 2001).

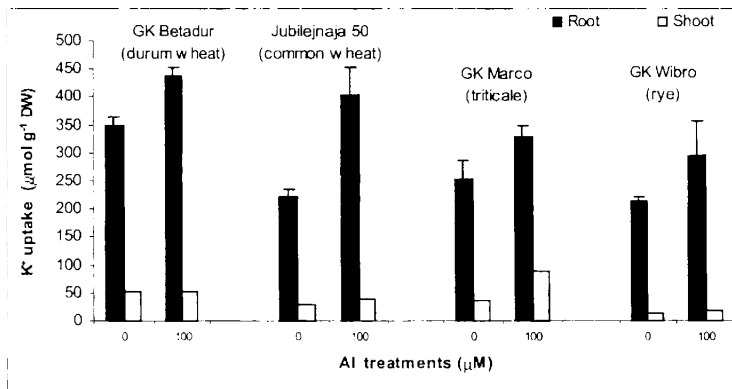
## **Results**

In Figure 1 growth data are presented, showing that 50 μM Al concentration causes a significant decrease in root dry weight in the order of Al-tolerance [rye (17%)>triticale (20%)>common wheat (56%)>durum wheat (63%)]. Shoot growth was only moderately influenced in 7d experiments.



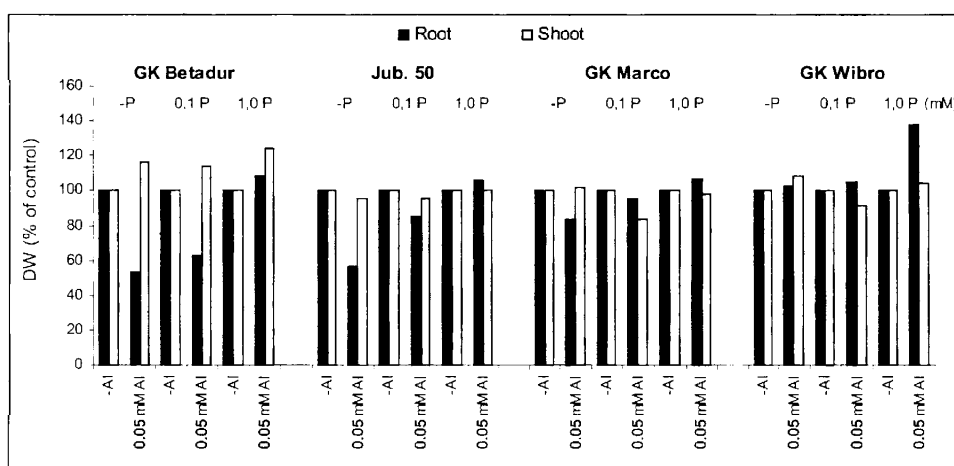
**Figure 1.** Effects of Al treatments on the growth of different cereal seedlings. Plants were grown for 7 days in 0.5 mM CaSO<sub>4</sub> + 50 μM AlCl<sub>3</sub> solution as indicated on the graph, pH was 4.1. All data show the means±SD (n=8).

Figure 2 shows the effect of short-term (6h) Al exposure on K<sup>+</sup>(<sup>86</sup>Rb) influx of the roots and transport toward the shoots in different cereal seedlings at pH 4.1. From the data it is visible that Al treatments increased the K<sup>+</sup>(<sup>86</sup>Rb) influx in roots in comparison with control plants. It is noteworthy that Al-induced K<sup>+</sup>(<sup>86</sup>Rb) transport toward the shoot in rye was more moderate than in the other species.

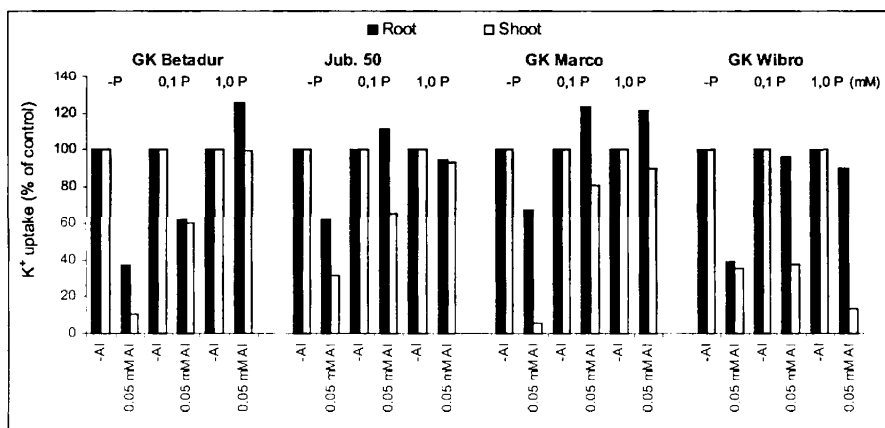


**Figure 2.** Effects of Al treatments on the K<sup>+</sup>(<sup>86</sup>Rb) uptake of the roots and translocation toward the shoots of different cereal seedlings. Plants were grown for 7 days in 0.5 mM CaSO<sub>4</sub> solution at pH 6.5. After the 7<sup>th</sup> day the seedlings were treated for 6h with 1 mM K(<sup>86</sup>Rb)Cl + 0.5 mM CaCl<sub>2</sub> + AlCl<sub>3</sub> solution as indicated on the graph, pH value was 4.1. All data show the means±SD (n=8).

The post-effect (3d) of 0.05 mM Al at various (0.1 and 1.0 mM) phosphate (P) supplies on the growth (DW) of different cereal seedlings can be seen in Figure 3. The results clearly indicate that Al treatment in low salt condition (0.5 mM CaSO<sub>4</sub>) influence significantly the growth of roots of different cereal species in the absence of P. The presence of 0.1 mM P (low P-roots) in the growth solution, however, cause a significant decrease in the toxic effect of Al and at 1.0 mM P-concentration (high P-roots) and Al-treatments do not show any inhibitory effect on the growth (DW) of cereal seedlings.



**Figure 3.** Effects of Al- and P-treatments on the growth of different cereal seedlings. Plants were grown for 4 days in 0.5 mM CaSO<sub>4</sub> solution at pH 6.5. After the 4th day the seedlings were treated for 3 days with 0.5 mM CaSO<sub>4</sub> solution in presence and absence of Al and P as indicated on the graph, pH value was 4.1.



**Figure 4.** Post-effects of Al- and P-treatments on the K<sup>+</sup> (<sup>86</sup>Rb) uptake of the roots and translocation toward the shoots of different cereal seedlings. Plants were grown for 4 days in 0.5 mM CaSO<sub>4</sub> solution at pH 6.5. After the 4th day the seedlings were treated for 3 days with 0.5 mM CaSO<sub>4</sub> solution in presence and absence of Al and P as indicated on the graph, pH value was 4.1. After the Al- and P-pretreatments the seedlings were tested for 6h with 1 mM K (<sup>86</sup>Rb)Cl + 0.5 mM CaCl<sub>2</sub>, pH value was 6.5.

The effects of Al and P (phosphate) pretreatments on the short-term (6h) K<sup>+</sup> (<sup>86</sup>Rb) uptake (influx) of roots of different cereal seedlings are presented in Figure 4. The data clearly indicate that the P-treatments significantly influence (decrease) the toxic effect of Al on K<sup>+</sup> (<sup>86</sup>Rb) transport in seedlings.

## Discussion

Formerly we reported that in short-term (6-24h) experiments the influx of K<sup>+</sup> (<sup>86</sup>Rb) in roots of wheat was positively correlated with Al concentration of the outer medium (Zsoldos et al. 2000). The mechanism(s) whereby the Al enhances the influx of K<sup>+</sup> (<sup>86</sup>Rb) is not known.

In our experiments with different cereal seedlings (Fig. 2) no phosphate was used in the growth solution supplied simultaneously with Al, therefore in low salt condition (0.5 mM CaSO<sub>4</sub>) precipitation of P-complexes in culture solution or on the surface of roots can be excluded.

Considering the dry weight data and visual observations, Al inhibits root growth in accordance with Al tolerance of cereal species (Fig. 1). Inhibition of root elongation at pH values below 5 is a common feature in many plant species and is caused by various factors such as impairment of H<sup>+</sup> efflux and related processes (Marschner 1995). In soil-grown plants inhibition of root

elongation at these low pH values is often closely and causally related to high activities of monomeric Al, and thus, Al-toxicity. The positive correlation of P and Al in roots of sorghum was reported earlier (Bergmann 1992; Okki 1987).

In summary, our results indicate that there is an interaction between Al and P (Figs. 3 and 4). In Clarkson's work (1967) the P associated with Al-treated roots was inorganic and almost entirely exchangeable. Increasing P supply in the growth medium (Fig. 3) can precipitate and thus detoxify Al. When plants are growing in a soil where Al and P are both present it is possible to envisage this adsorption precipitation reaction as a continuous process which would effectively reduce the amounts of P and toxic Al available in soil solution for plants.

## Conclusion

Four cereal species were tested in this study. The durum wheat (GK Betadur) proved to be much more sensitive to the stress conditions examined than the common wheat (Jubilejnaja 50) and the triticale (GK Marco). As it was expected rye (GK Wibro) turned out the most tolerant type in our experiments. The results confirmed that in acidic environment phosphate ion cause a significant decrease in the toxic effect of Al.

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