Phosphorus ameliorates aluminium toxicity of Al-sensitive wheat seedlings

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Abstract
The role of phosphorus (P) in the amelioration of aluminium (Al) toxicity to plants is still unclear. The aim of this study was to examine the amelioration of Al toxicity by P supply. The study involved growing Al-sensitive wheat seedlings for 13 days in an acidic soil (pH 4.5 in $CaCl_2$) with increasing added rates of P (0, 20, 40 and 80 mg P/kg soil) and Al (0, 50 and 150 mg $AlCl_3$/kg soil). The results indicated that the effects of Al toxicity in this soil could be fully alleviated by the application of P at 50 mg $AlCl_3$/kg. The highest 150 mg/kg $AlCl_3$ treatment significantly reduced root growth, but this was partially overcome by the 80 mg/kg P treatment. High P significantly reduced the concentration of Al in the apoplast, and in the root and shoot. It is possible that an insoluble Al-P complex forms in the soil and this decreases Al bound in apoplast as well as uptake into the roots. High P decreased the translocation of Al from root to shoot.

Key Words
Al tolerance, Al translocation, Apoplast Al, P-Al interactions

Introduction

Aluminium toxicity and P deficiency are two common constraints limiting crop production in acid soils (Liao *et al.,* 2006). Aluminium toxicity is considered to be closely associated with the phosphorus nutrition of plants and P may be an effective agent for detoxifying excess Al (Bollard, 1983). However, relatively few studies have been done to investigate how improved P nutrition alleviates Al toxicity in plants (Tan and Keltjens, 1990). Understanding the mechanisms underlying Al and P interactions will help to develop management options to sustain crop production in acid soils. The objectives of this study were i) to investigate the growth response of Al-sensitive wheat seedlings to increasing P and Al supply in an acid soil, and ii) to understand how P alleviates Al toxicity. We hypothesized that P will ameliorate Al toxicity in both the soil and within the plant.

Materials and methods

Soil, plants, experimental design and procedure

The subsurface layers of an acidic Podosol (Isbell 2002) were used in the experiment. The soil had extractable Al of 4.98 mg/kg, a field capacity of 13% (w/w), a pH buffer capacity of 0.24 cmol/kg/pH, a sand texture (95.2 % sand, 0.4 % silt and 4.4 % clay) and a total organic C of 0.69%. The experiment consisted of 3 levels of added Al (0, 50 and 150 mg $AlCl_3$/kg soil) × 4 levels of P (0, 20, 40 and 80 mg P/kg soil) with 5 replications. Basal nutrients were not applied to minimize the interactions between nutrients and Al in the soil, so plant growth over the 13 day study relied on the seed reserve. $AlCl_3$ was added as a stock solution to soil and the soil was pre-incubated at 30^0C for 7 days. Before sowing, KH_2PO_4 was applied directly to the soil after pre-incubation and thoroughly mixed. The Al-sensitive wheat genotype (ES8) was used as a testing plant. Eight pre-germinated uniform seeds were sown in each replication in plastic cups containing 200 g soil, and subsequently thinned to 6 plants/cup. Plants were grown in a growth cabinet with day/night temperatures 20/18 ^{0}C, 10 h dark and 14 h light conditions, and an average light intensity of 9660 lm-m^{-2}.

Measurements

The experiment was harvested 13 days after sowing. After roots were washed free of soil particles, plant height, and root and shoot biomass were recorded. Root morphological parameters were measured using the WinRHIZO image analysis system (WIN MAC, Regent Instruments Inc., Quebec, Canada). The soil-available Al in the same extract was measured by using a modified pyrocatechol violet (PCV) method (Kerven *et al.,* 1989). Apoplastic Al was extracted in 50 mM $BaCl_2$ solution, chilled to 0^0C for 45 minutes and determined spectrophotometrically at 585 nm using the PCV method with standards prepared in the extacting solution (Wang *et al.,* 2004). The dried root and shoot samples were digested in HNO_3/$HClO_4$ mixture (4:1,v/v) (Zheng *et al.,* 2005). After digestion, root and shoot Al concentrations were determined using the colorimetric method described by Nursyamsi *et al.* (2002). Concentrations of P in the roots and shoots were determined colorimetrically using malachite green (Motomizu *et al.,* 1983)

Statistical analysis
Results were analysed by a two-way analysis of variance (ANOVA) using Genstat 5^{th} ed^{n} for Windows (Lawes Agricultural Trust, UK).

Results and Discussion

Increasing P supply substantially decreased extractable Al in bulk soil (Figure 1). This decrease in Al extractability in the soil is likely to have resulted from the chemical precipitation of Al with the added P thereby lowering the activity of Al^{3+} in the soil solution (Nakagawa *et al.,* 2003; Sanzonowicz *et al.,* 1998; Silva *et al.,* 2001).

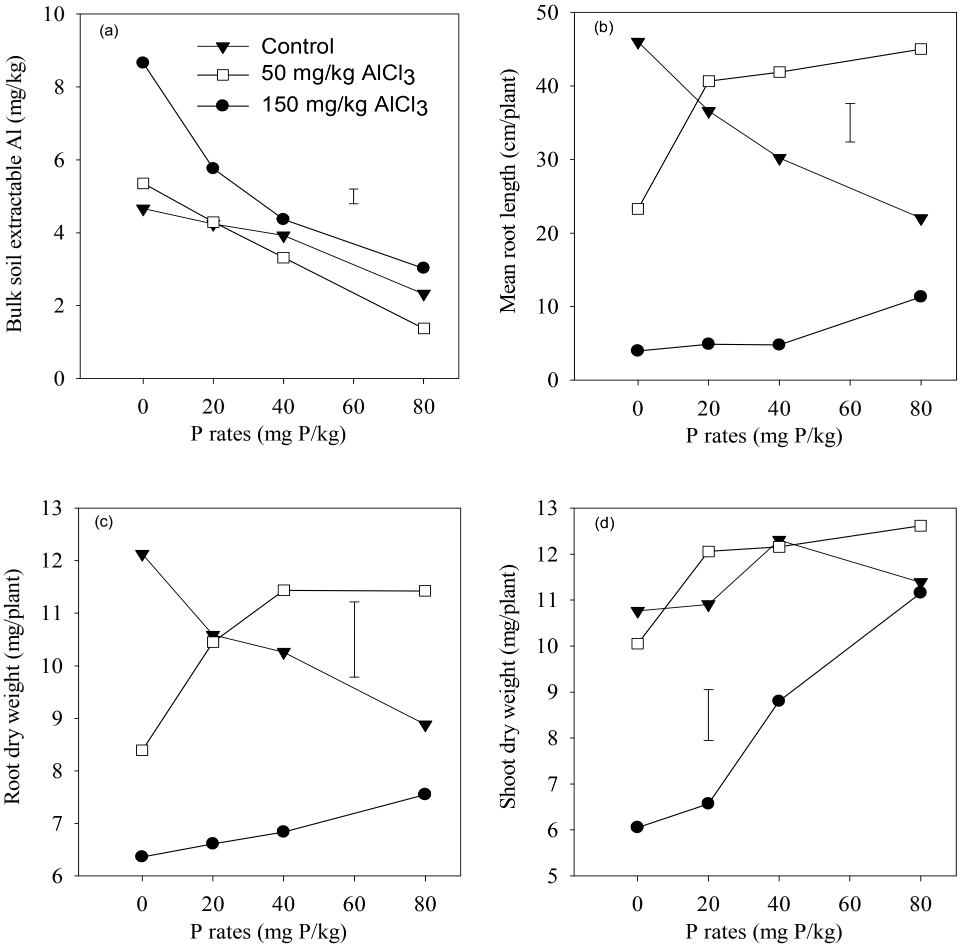

Figure 1. Effect of P supply on (a) extractable Al in bulk soil, (b) root length, (c) root dry weight and (d) shoot dry weight of Al-sensitive wheat seedlings after 13 days of growth. Bar represents LSD (*P*=0.05) for Al×P interaction.

A marked Al by P interaction occurred with the growth of the wheat roots. Increasing P supply resulted in a linear decline in root length and root dry weight in the absence of added Al (Figure 1b, c). With the50 mg/kg $AlCl_3$ treatment, both root length and root dry weight increased significantly (p<0.05) with the 20 mg/kg P treatment and then remained consistently high with further added P. However, the 150 mg/kg Al treatment markedly depressed root growth with nil P; root length and dry weight were then increased by 65% and 16%, respectively with the 80 mg/kg P supply. These results confirm that increasing P supply can ameliorate the toxic effects of Al on root growth root growth, but the extent of the amelioration is dependent on the severity of the toxicity.

High P supply was more effective at ameliorating the effect of Al toxicity on shoot growth than on the roots. Despite the 40% reduction in shoot dry weight with the 150 mg/kg $AlCl_3$ treatment with nil P, there was no difference in shoot dry weight when 80 mg/kg P had been added (Figure 1d). Shoot mass did not differ significantly between the control and 50 mg/kg $AlCl_3$ treatment. Thus there was a very positive effect of increasing P supply on shoot growth under severe Al toxicity. It has been suggested that this is related to indirect effects associated with nutrient uptake in the plant growth response (Tan and Keltjens 1990).

High P reduced Al in the apoplast and inside roots. With the 80 mg P/kg treatment, apoplast Al was reduced by 72%, 57% and 65% in 0, 50 and 150 mg/kg $AlCl_3$ treatments, respectively. However, total root Al was reduced by only 4%, 22% and 29% with these Al treatments (Figure 2a, b). These results indicate that the exclusion of Al by P was greater in the apoplast than in the roots. In addition, our result showed that apoplast Al was around 37% of the total root Al. Other research showed that apoplastic Al was consistently 30 to 40% of total root Al (Tice *et al.* 1992).

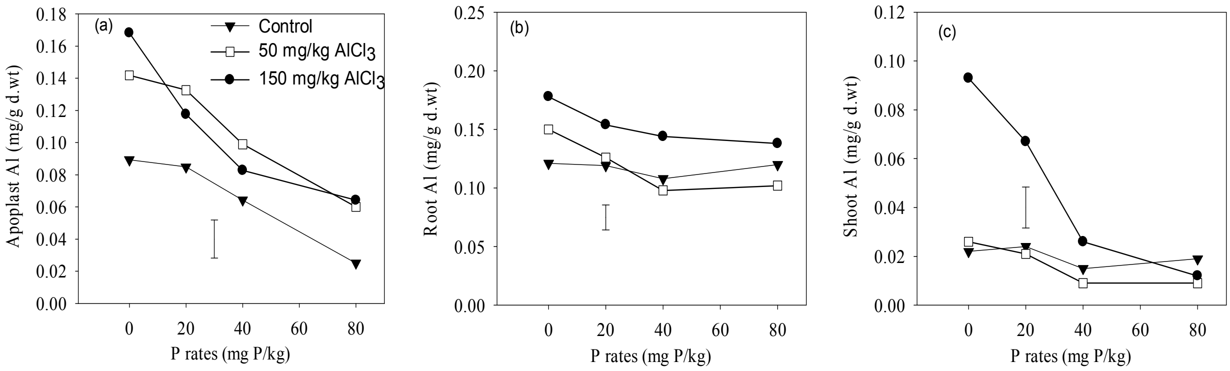

Figure 2. Effect of P supply on Al concentrations in (a) apoplast, (b) root and (c) shoot of Al-sensitive wheat seedlings after 13 days of growth. Bar represents LSD (*P*=0.05) for Al×P interaction.

A key finding was that Al translocation from the root to the shoot with the 150 mg $AlCl_3$/kg treatment, which was 5 times that of the 0 and 50 mg $AlCl_3$/kg treatments with no added P, was markedly reduced with 80 mg P/kg (Figure 2c). This was associated with a significant reduction ($P<0.05$) in shoot P concentration, suggesting a possible causal relationship. Irrespective of the level of added $AlCl_3$, the wheat seedlings were able to take up more P from the soil, translocated more P to the shoots and utilize P more efficiently for shoot growth and development, with increasing P supply, although total P uptake was reduced with the high Al treatment.

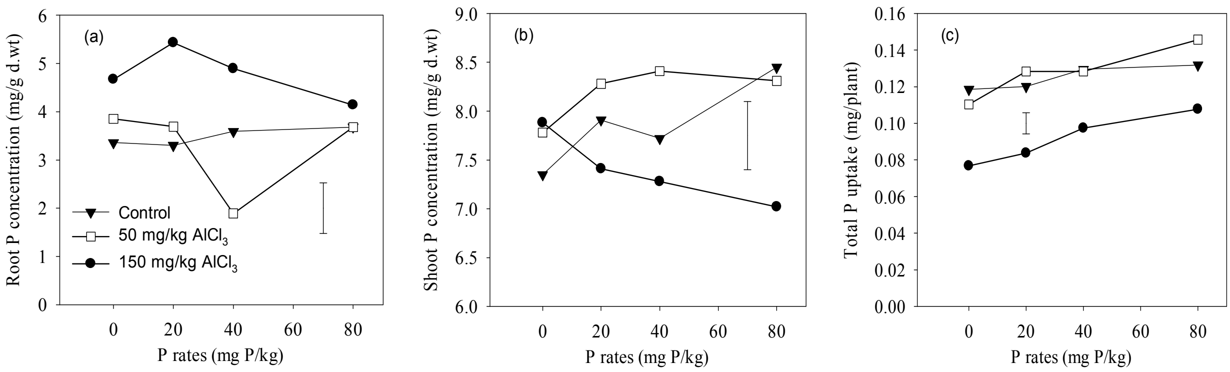

Figure 3. Effect of P supply on P concentrations in (a) root and (b) shoot, and (c) total P uptake of Al-sensitive wheat seedlings after 13 days of growth. Bar represents LSD (*P*=0.05) for Al×P interaction.

The reductions in apoplast root and shoot Al concentrations with increasing P supply shows that high concentrations of soil P can ameliorate the toxic effects of Al on these seedlings (Figure 2). These findings have only previously been found in solution culture studies (Gaume *et al.,* 2001; Nakagawa *et al.,* 2003).

Conclusions

This study demonstrated that increasing P supply improves the tolerance of these Al-sensitive wheat seedlings to Al toxicity. There are at least four ways in which P alleviates Al toxicity. First, P directly reacted with Al in soil presumably to form Al-P precipitates, and thus lower Al^{3+} activity in soil solution. Second, P decreased the amount of apoplastic Al that was bound to the root cell walls and this binding was around 37% of the total Al uptake by the root. Third, high P supply decreased total uptake of Al into the plants (50%) with the reduction in Al concentration in the roots (12%) being less than that in the shoots (88%) with the high Al treatment. Finally, P decreased the translocation of Al from roots to shoot by up to 90% in high P and high Al supply.

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