RESEARCH PAPER

Spatial aluminium sensitivity of root apices of two common bean (Phaseolus vulgaris L.) genotypes with contrasting aluminium resistance

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Abstract

The initial response of plants to aluminium (Al) is an inhibition of root elongation. In the present study, short and medium-term effects of Al treatment (20 μM) on root growth and Al accumulation of two common bean (Phaseolus vulgaris L.) genotypes, VAX-1 (Al-sensitive) and Quimbaya (Al-resistant), were studied. Root elongation of both genotypes was severely inhibited during the first 3–4 h of Al treatment. Thereafter, both genotypes showed gradual recovery. However, this recovery continued in genotype Quimbaya until the root elongation rate reached the level of the control (without Al) while the genotype VAX-1 was increasingly damaged by Al after 12 h of Al treatment. Short-term Al treatment (90 μM Al) to different zones of the root apex using agarose blocks corroborated the importance of the transition zone (TZ, 1–2 mm) as a main target of Al. However, Al applied to the elongation zone (EZ) also contributed to the overall inhibition of root elongation. Enhanced inhibition of root elongation during the initial 4 h of Al treatment was related to high Al accumulation in root apices in both genotypes (Quimbaya>VAX-1). Recovery from Al stress was reflected by decreasing Al contents especially in the TZ, but also in the EZ. After 24 h of Al treatment the high Al resistance of Quimbaya was reflected by much lower Al contents in the entire root apex. The results confirmed that genotypic differences in Al resistance in common bean are built up during medium-term exposure of the roots to Al. For this acquisition of Al resistance, the activation and maintenance of an Al exclusion mechanism, especially in the TZ but also in the EZ, appears to be decisive.

Key words: Abiotic stress, aluminium toxicity, apical root zones, root acclimation, root growth pattern.

Introduction

Toxicity of aluminium (Al) in acid soils in the tropics is a serious problem, and correcting soil acidity by amending the soils with lime is difficult and prohibitively expensive for most small farmers (Rao et al., 1993). Common bean (Phaseolus vulgaris L.) needs significant improvement in Al resistance to reduce farmer’s dependence on lime and fertilizers (Rao, 2001). Genotypic differences in Al resistance in common bean were also reported based on Al-inhibited root elongation in nutrient solution (Massot et al., 1999; Rangel et al., 2005; Manrique et al., 2006).

Reduction of root growth is the most widely recognized symptom of Al toxicity (Foy, 1976). It can be measured from 30 min to 90 min after Al exposure in maize (Zea mays L.) (Llugany et al., 1995; Sivaguru and Horst, 1998). However, more frequently, genotypic differences in Al resistance entailed measurements of root growth between 24 h and 72 h (Furlani and Clark, 1981; Horst et al., 1983; Bennet and Breen, 1991a). Detailed studies on the kinetics of the response of root growth rates to exposure to Al offer the possibility to differentiate between constitutive or inducible Al resistance mechanisms and to verify whether this is consistent across genotypes (Parker, 1995). Evidence of root acclimation to...
Al stress has been observed in maize (Llugany et al., 1995; Barceló and Poschenrieder, 2002), cowpea (Vigna unguiculata) (Hrst et al., 1983), and yellow lupin (Lupinus luteus L.) (Grauer and Hrst, 1990). Cumming et al. (1992) proposed that Al resistance is an inducible trait in common bean requiring a period of stress before a resistance mechanism is ‘switched on’. In fact, these authors observed an initial decline in the root elongation of an Al-resistant cultivar followed by a substantial increase after 24 h of Al exposure, while the Al-sensitive cultivar showed a steady decline in the elongation rate over the experimental time. Resistance to Al might be achieved by chelation or detoxification of Al by organic acids, either within the plant (Al tolerance) or in the rhizosphere by root exudation (Al exclusion) (Foy, 1988; Taylor, 1988). The Al-stimulated exudation of citrate was first reported in common bean (Miyasaka et al., 1991). Since then, Al-induced secretion of organic anions has been reported in several plant species or cultivars. [For more detailed information, see Ma et al. (2001); Ryan et al. (2001); and Kochian et al. (2004).] Lower Al contents were observed in root tips of Al-resistant compared with Al-sensitive common bean cultivars after 1 d or 3 d of Al treatment, respectively (Mugai et al., 2000; Shen et al., 2002). The lower Al contents were related to a higher capacity to exude citrate in response to Al treatment.

Two patterns of organic anion secretion have been identified. In wheat, buckwheat (Fagopyrum esculentum Moench), tobacco (Nicotiana spp., L.), and maize, the activation of the organic acid anion efflux is rapid and occurs without any discernible delay after exposure to Al (pattern I). In chakod (Cassia tora L.), soybean (Glycine max L.), triticale (× triticosecale), and rye (Secale cereale), a lag phase was observed between the addition of Al and the start of citrate release (pattern II). Subsequently, the exudation is enhanced with time (Ma et al., 2001; Ryan et al., 2001).

In plants, growth is confined to distinct zones along which diverse spatial patterns of growth intensity exist (Erickson and Sax, 1956). Hence, a physiological study about regulation of growth and its modification under stress conditions requires a detailed quantitative description of spatial growth profiles (Peters and Bernstein, 1997). In maize, specialized zones of the root apex have been classified on a millimetre scale based on physiological (Ishikawa and Evans, 1990), morphological (Ishikawa, 1993), and cytological (Baluska et al., 1990, 1996) approaches. These studies allowed an improvement of our understanding of the important role of the root apex in the Al perception and response mechanism. Bennet and Breen (1991a, b) hypothesized a major role for the root cap in Al-triggered signal perception and transduction. Later, Ryan et al. (1993) showed that not the root cap but the root meristem was the most Al-sensitive zone in maize. Further research work by Sivaguru and Horst (1998) and Kollmeier et al. (2000) presented evidence that the distal transition zone (DTZ) is the most Al-sensitive zone of the root apex in maize. In this zone, Al accumulation was the greatest (Sivaguru and Horst, 1998) and caused severe changes in the organization of microtubules and actin microfilaments, leading to root growth inhibition that could possibly be mediated by the interaction of Al with the apoplastic side of the cell wall–plasma membrane–cytoskeleton continuum (Hrst et al., 1999; Sivaguru et al., 1999). Application of Al to the DTZ and not to the EZ significantly reduced the root elongation, indicating the presence of Al-induced signal transduction between the DTZ and the EZ which could be mediated by auxin (Kollmeier et al., 2000).

In previous studies, significant genotypic differences were found in Al resistance of common bean in nutrient solution based on inhibition of root elongation after 36 h at 20 μM Al supply as a parameter for Al injury (Rangel et al., 2005). Callose formation was found to be a sensitive marker of Al stress (Wissenmeier et al., 1992) and a reliable parameter for the classification of maize genotypes for Al resistance (Eticha et al., 2005). In common bean, short-term Al supply (4 h) led to maximum accumulation of callose in the root apex. However, no relationship was observed between Al-induced callose contents and root-growth inhibition, hampering the use of this parameter as a screening tool for Al resistance in common bean (Rangel et al., 2004). It thus appears that the Al perception and response mechanism in common bean could differ from that in maize. Therefore, a better understanding of the temporal and spatial effect of Al on root growth and of the accumulation of Al is necessary to quantify genotypic differences in Al resistance and to develop quick screening techniques for Al resistance in common bean.

Materials and methods

Plant material and growth conditions

Seeds of the common bean genotypes Quimbaya (Al-resistant) and VAX-1 (Al-sensitive) were germinated between filter-paper styrofoam-sandwiches soaked with tap water, in an upright position. Three-day-old uniform seedlings were transferred to 18 litre pots with constantly aerated simplified nutrient solution containing 5 mM CaCl₂, 0.5 mM KCl, and 8 μM H₂BO₃ (Rangel et al., 2005). This solution allows optimum root elongation for 3 d at least. After 24 h of root growth, the pH of the solution was decreased from 5.6 to 4.5 within 24 h in steps of 0.3 units using an automatic pH titration device. The pH was controlled in each pot by adding 0.1 M HCl or 0.1 M KOH. All experiments were conducted under controlled environmental conditions in a growth chamber with a 16/8 h light/dark regime, 27/25 °C day/night temperatures, 70% relative air humidity, and a photon flux density of 230 μmol m⁻² s⁻¹ (photosynthetic active radiation) at the plant-canopy level (Sylvania Cool White, 195 W, Philips, Germany).
Partial elongation of 1 mm root zones

Assessment of spatial growth patterns of individual root sections was based on experiments (Erickson and Sax, 1956; Peters and Bernstein, 1997) where the first 10 mm of the root apex was marked with consecutive black and red dots at 1 mm intervals using a fine brush and Indian ink (Peltkan, Hanover, Germany). The process of marking did not affect root growth during the experimental period. Subsequently, plants were transferred to simplified nutrient solution containing 0 μM or 20 μM AlCl₃. The distances between the dots were measured after 4 h, and then the elongation rates of each specific root zone were calculated and plotted as a continuous curve against the distance from the root apex. Measurements were made with a precision of 25 μm at a 40-fold magnification against a 1 mm scale using a stereo microscope (Askania GZS 2T, Ratheonow, Germany).

In addition, relative elemental growth rates (REGRs) were calculated as the derivative of the fifth-order sigmoidal function of growth rate profiles (Erickson and Sax, 1956; Silk, 1984; Peters and Bernstein, 1997) and the spatial organization of the root apex determined following the methodology used by Ishikawa and Evans (1995). Briefly, the EZ was divided arbitrarily into six subzones: meristematic zone (MZ), transition zone (TZ), apical elongation zone (AEZ), central elongation zone (CEZ), basal elongation zone (BEZ), and proximal elongation zone (PEZ) based on rates of elongation relative to the peak rate in the CEZ.

Localized effect of Al in 1 mm PVC blocks or 20 mm plastic cylinder blocks

Local Al treatments were performed in low gelling temperature agarose [1% (w/v), Fluka, Deisenhofen, Germany] dissolved in simplified nutrient solution containing 0 μM or 20 μM AlCl₃. Nominal 200 μM Al added to cooled agarose yielded 90±6 μM Al₃⁺ following the procedure described by Sivaguru and Horst (1998) and the aluminon method according to Kerven et al. (1989).

Al was applied to specific 1 mm apical root zones using a polyvinylchloride (PVC) block system previously described by Sivaguru and Horst (1998). Briefly, five uniform seedlings were placed on the PVC blocks with different apical root positions 1.5 cm behind the root tip, agarose was poured into the horizontal slit, which was vertically sealed with vaseline. Thereafter, agarose was poured into the horizontal slit using a fine-tipped Pasteur pipette just before solidification. Subsequently, the PVC blocks were placed into the horizontal 1 mm slit, which was vertically sealed with vaseline. Thereafter, agarose was poured into the horizontal slit using a fine-tipped Pasteur pipette just before solidification. The PVC blocks were placed in a growth chamber in an upright position, under the conditions described above. The whole root system was kept moist during all manipulations by soaking the root system with nutrient solution, covering the roots with moistened filter paper, and repeatedly spraying nutrient solution with a hand sprayer. The root elongation rate was calculated from the measurements of root lengths every hour for up to 6 h as described above.

Alternatively, Al was applied to 20 mm apical root zones using a plastic cylinder block system. Each block consisted of five vertical cylinders (20 mm length×0.5 mm width) mounted on a plant-supporting filter-paper styrofoam sandwich previously soaked with simplified nutrient solution. Five uniform seedlings were placed at different apical root positions into the 20 mm chambers, and sealed at the base with vaseline. In contrast to the 1 mm PVC block system, the apical root zone in consideration was located outside the Al-treated zone. Thereafter, the Al-containing agarose was poured into the cylinder using a fine-tipped Pasteur pipette, and the plants were covered with a second filter-paper styrofoam sandwich. Afterwards, each block system was placed in an upright position into plastic pots containing enough nutrient solution to cover the base of each block, and kept in a growth chamber under the conditions described above. Root elongation rates were calculated from measurements of root length after 4 h of Al treatment.

Effect of Al on root growth

Tap roots of 3 cm behind the root tip were marked at 2 h before the Al treatment using a fine point permanent marker (Sharpie blue, Stanford), and this did not affect root growth during the experimental period. Afterwards, the plants were transferred to simplified nutrient solution (see above) containing 0 μM or 20 μM AlCl₃ for up to 24 h. Root elongation was determined every hour during the first 10 h and then at 12, 16, 20, and 24 h of Al treatment using a 1 mm scale.

Determination of Al in 5 mm and 2 mm root segments

For Al analysis, roots of four plants per replicate were rinsed with distilled water and 5 mm (primary root) or 2 mm segments along the first 10 mm of root tips (primary plus the two longest basal roots) were excised using a razor blade, placed in separate Eppendorf reaction vials (four 5 mm and twelve 2 mm root sections per vial), and digested in 500 μl of ultra-pure HNO₃ by overnight shaking on a rotary shaker (Heidelberg, Reax 20, Germany). Preliminary studies did not reveal any differences between primary and basal roots. The digestion was completed by heating the samples in a water bath at 80 °C for 20 min. Then 1.5 ml of ultra-pure deionized water (18.2 MΩ; E-pure, D4642; Barnstead, Dubuque, IA, USA) was added after cooling the samples in an ice-water bath. Samples were diluted and measured with a Unicam 939 QZ graphite furnace atomic absorption spectrometer (GFAAS; Analytical Technologies Inc., Cambridge, UK) at a wavelength of 308.2 nm with an injection volume of 20 μl.

At harvest, the culture solutions were filtered immediately through 0.025 μm nitrocellulose membranes (Schleicher & Schuell, Dassel, Germany). Mononuclear Al (Al₃⁺) concentrations were measured colorimetrically using the pyrocatechol violet method (PCV) according to Kerven et al. (1989). Nominal 20 μM Al treatments resulted in 16±2 μM Al₃⁺ after 24 h.

Statistical analysis

Each experiment had a completely randomized design with eight (root elongation), four (Al contents), six (partial elongation rate), and four (spatial sensitivity) independent replicates for each treatment. Analysis of variance was performed using the ANOVA procedure of the statistical program SAS 9.1 (SAS institute, Cary, NC, USA) and means were compared using the Tukey test.

Results

Effect of Al on partial elongation of specific 1 mm root zones

To obtain information on partial growth patterns along the 10 mm root apex, elongation growth of individual segments was measured by identifying the main zone of Al-induced growth inhibition. Previous experiments had shown that there was no observable root elongation beyond the 10 mm zone (data not shown). In the absence of Al, the growth patterns of both genotypes were similar (Fig. 1), however, the genotype×segment interaction was highly significant. The calculated REGRs suggest that the EZ extended from 1.7 or 1.5 mm to 10.5 or 8.9 mm behind the root tip in Quimbaya and VAX-1, respectively. The peaks of maximum relative elongation rate were located at 3.9 mm (31% h⁻¹) in Quimbaya and 3.3 mm
behind the root tip in VAX-1. Based on the position of maximum relative elongation, the EZ was divided arbitrarily on a millimetre scale in both genotypes. The pattern of root growth inhibition induced by 4 h of Al supply was similar in both genotypes (no significant genotype x treatment x segment interaction) and resulted from a general inhibition along the entire EZ without shortening the growth zone. The inhibition was greater in the CEZ where the maximal rate of relative elongation was decreased to 15% h⁻¹ in both genotypes. Although the genotype x treatment interaction was not significant, there was a trend showing that Quimbaya was less severely inhibited by Al in the EZ (40%) than VAX-1 (60%), which was particularly Al sensitive in the TZ (88%).

Effect of localized application of Al on root elongation
Application of Al for 2 h to individual 1 mm root zones of the 5 mm root apex significantly inhibited root elongation in both genotypes (Fig. 2). Al inhibited root elongation when applied to all segments except the apical segment. Calculated across the genotypes (not shown), the Al treatment x segment interaction was significant, suggesting that Al was particularly toxic when applied to the 1–2 mm zone.

This study was extended using a different experimental approach where increasing parts of the 10 mm root apex contributing to root elongation (compare Fig. 1) were not treated with Al (Fig. 3). Al significantly inhibited root elongation even if it was not applied to the 0–6 mm root zone (both genotypes) or even the 0–8 mm zone (VAX-1). Particularly in VAX-1 (significant difference between segments and Al treatment x segment interaction), the comparison of means indicates that Al applied to the 0–2 mm segment was most toxic, although basal root segments also contributed to the inhibition of the overall root elongation.

Effect of Al on overall root elongation
In the presence of Al, root elongation of both genotypes was inhibited as early as 1 h after the beginning of the Al treatment. The inhibition was enhanced up to a maximal level after 3 h and 4 h for VAX-1 and Quimbaya, respectively (Fig. 4). Thereafter, both genotypes gradually recovered, but the recovery with Quimbaya was much faster than with VAX-1. Whereas this recovery continued in Quimbaya until the root elongation rate reached the
level of the control (without Al), VAX-1 was increasingly damaged by Al after 12 h of Al treatment, which is reflected by the highly significant Al treatment × time interaction.

The relative inhibition of root elongation more clearly visualizes the response of the genotypes to Al supply (Fig. 5). The maximum inhibition of root elongation reached ~80% in both genotypes after 3 h or 4 h and then recovered. Whereas Quimbaya completely recovered after 24 h, VAX-1 recovered only to 40% after 12 h and then became increasingly damaged up to 80% again after 24 h of Al treatment.

**Al contents in root apices**

The Al contents of the 5 mm root apex (Fig. 6) reflected the inhibition of root elongation induced by Al (Fig. 4). Enhanced inhibition of root elongation up to 4 h of Al treatment was related to increasing Al contents in the root tips in both genotypes. However, the Al contents reached higher levels in Quimbaya. This higher Al content did not lead to more severe inhibition of root elongation compared with VAX-1 (both absolutely and relatively, compare with Figs 4 and 5). Subsequently, Al contents decreased in both genotypes. However, in Quimbaya, the decrease continued up to 24 h while in VAX-1 the Al contents started to increase again after 10 h and reached a higher level after 24 h than after 4 h of Al treatment.

Apical root zones differ in their response to Al (see above). Therefore, the Al contents in 2 mm root zones along the root apex were determined. The distribution of Al along the 10 mm root apex clearly shows that enhanced Al injury in both genotypes during the first 4 h
of Al treatment were particularly due to increasing Al contents in the 0–2 mm zone (Fig. 7). The recovery in root elongation corresponded to a decrease of Al particularly in this zone. Complete recovery of Quimbaya after 12 h of Al exposure also resulted in lower Al contents in the more basal zones (Fig. 7, Quimbaya). In VAX-1, the enhanced Al injury after 12 h of Al treatment was accompanied by an increase in Al contents in all root segments (Fig. 7, VAX-1).

Discussion

Typically, the zones of development in roots include the cap, the apical meristem, the EZ, and the maturation zone (Ishikawa and Evans, 1995). However, it is widely acknowledged that root growth in plants is confined to the apical regions along which diverse spatial patterns of growth intensity exist as a result of different gradients of cellular activities (Erickson and Sax, 1956; Gandar, 1983; Pritchard, 1994). These regions of growth are described in terms of growth rate (R) and REGR profiles (Silk, 1984). Although both R and REGR are true rates (the physical dimension is time$^{-1}$), R describes the velocity of change of material entities, i.e. fresh weight or organ length in time, while REGR characterizes the distribution of growth intensities in the space (Erickson and Sax, 1956; Gandar, 1983; Peters and Bernstein, 1997). Identification and analysis of spatial growth profiles are a prerequisite for the physiological study about regulation of growth and its modification under stress conditions.

In the absence of Al, the determination of REGR in common bean revealed that the pattern of elongation in both genotypes was similar (Fig. 1). However, the extension of the EZ of both genotypes differed slightly. It was larger in Quimbaya than in VAX-1. Consequently, the point of maximum elongation occurs in a more basal part of the EZ (3.9 mm) in Quimbaya than in VAX-1 (3.3 mm). Elongation at the root axis is caused by the production and expansion of cells (Green, 1976). After cessation of cell division in the meristem, cells undergo a preparatory phase for rapid elongation resulting in an increase in width as well as in length (Baluska et al., 1990). Thereafter, cell elongation is characterized by extensive vacuolar expansion and an increase in the area of lateral cell walls (Pritchard, 1994). In common bean, differences in seed size among genotypes of different origin have been directly related to variation in cell volumes of different tissues (White and Gonzales, 1990). Normally, genotypes of Meso-American origin (e.g. interspecific genotype, VAX-1) are typically smaller seeded than those of Andean (i.e. Quimbaya) origin (Gepts et al., 1986; Singh, 1989). This is in agreement with microscopic observations of root cross-sections at the TZ (AF Rangel, unpublished results), where higher numbers and larger cortical cells in Quimbaya than in VAX-1 could explain the greater size of the EZ in Quimbaya (8.8 mm) compared with VAX-1 (7.4 mm) (Fig. 1).

The division of the root apex into growth zones in common bean (Fig. 1) is arbitrary following the proposal by Ishikawa and Evans (1993), and is in agreement with previous results obtained in mungbean [Vigna radiata L. (Blamey et al., 2004)] and maize (Baluška et al., 1990,
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1996; Ishikawa and Evans, 1993, 1995). Using a computerized video system, Blamey et al. (2004) showed that in mungbean the zone of greatest expansion (CEZ) was located at 1.9–4.6 mm from the root tip. In maize, the EZ extended from 1.5 mm to 9.2 mm behind the tip, and the maximum relative elongation rate was 26% h⁻¹ at 4 mm in the CEZ extending from 3.2 mm to 6.5 mm behind the tip (Ishikawa and Evans, 1993). The TZ was located between 1.7 mm and 3.4 mm from the root tip in nutrient solution experiments (Ishikawa and Evans, 1993) or between 1 mm and 2 mm from the root tip in moist air experiments (Kollmeier et al., 2000). The localization of the DTZ in maize corresponded with the cytological studies conducted by Baluška et al. (1990).

The overall dynamics of root growth and Al accumulation in the present study confirmed previous results on the characterization of the genotypes Quimbaya and VAX-1 as Al-resistant and Al-sensitive, respectively (Rangel et al., 2004, 2005; Manrique et al., 2006). However, as observed previously by Cumming et al. (1992), the dynamics of the Al stress perception and response varies on the spatial (Figs 1–3) and temporal (Figs 4–7) scale.

In both genotypes, accumulation of Al in the 5 mm root tips (Fig. 6) and even more in the 2 mm apical root zone (Fig. 7) during the first 4 h of Al treatment led to enhanced inhibition of root elongation (Fig. 4). This initial inhibition of root elongation resulted from a generalized effect along the entire EZ (reduced maximal rate of relative elongation) without changing the shape or the length of the EZ. This observation is in agreement with the findings of Blamey et al. (2004) in mungbean and Kollmeier et al. (2000) in maize who did not find any changes in the shape and length of the EZ due to Al treatment. In Arabidopsis, phosphorus deficiency shortened the EZ by reducing the production rate of epidermal cells but not cortical cells, and moderately decreased the maximal rate of relative elongation, while ethylene regulated the maximal rate of relative elongation rather than the length of the growth zone independently of the phosphorus status (Ma et al., 2003). Greater inhibition of root elongation by Al in the Al-sensitive VAX-1 cannot be related to a higher Al accumulation but appears to be related to its higher Al sensitivity of the TZ (Fig. 1). Localized application of Al to different zones of the root apex (1 mm or 20 mm agarose blocks) confirmed the previous results with maize obtained by Sivaguru and Horst (1998) and Sivaguru et al. (1999), and confirmed that the TZ is the most Al-sensitive apical root zone not only in maize but also in common bean.

As well as in maize (Sivaguru and Horst, 1998; Kollmeier et al., 2000), localized application of Al to the MZ (0–1 mm zone) was significantly less inhibitory than when applied to more basal parts of the EZ in both common bean genotypes (Fig. 2). This effect normally has been explained by the strong capacity of the root cap mucilage to bind Al, thus protecting the root tip from Al injury (Horst et al., 1982; Archambault et al., 1996; Li et al., 2000). Even more importantly, it is widely acknowledged that the DTZ is the root zone most responsive to a variety of hormonal and environmental stimuli (Ishikawa and Evans, 1993, 1995; Borch et al., 1999; Kollmeier et al., 2000; Ma et al., 2003). The importance of the DTZ in Al perception and response has been demonstrated using different indicators (see Introduction). In the present experiments, application of Al to the TZ in both common bean genotypes resulted in root growth inhibition to the same extent as if the whole root tip would have been treated (Fig. 2). Additionally, the pattern of Al accumulation along the 10 mm root tip of both genotypes suggests that enhanced Al injury during the first 4 h of Al treatment was particularly due to increasing Al contents in the MZ and TZ (Fig 7, 0–2 mm zone). This assumption is supported by a closer relationship (R²=0.61 and 0.45 for Quimbaya and VAX-1, respectively) between root elongation rate and Al content of the 0–2 mm zone than with any other root zone. However, in contrast to maize (Kollmeier et al., 2000) and wheat (Ryan et al., 1993), application of Al to the EZ even up to 8 mm from the root tip (Figs 2, 3) also reduced root growth in both common bean genotypes, though to a lesser extent than when applied to the TZ. Dickotyledons and grasses (Poales) are well known to differ widely in the composition of their cell walls, particularly in their pectin content which is higher in dicotyledons (Carpita and Gibeaut, 1993), thus enhancing the capacity to accumulate Al. The important role of the cell wall pectin content for Al accumulation and Al sensitivity has been demonstrated by modifying the pectin contents of the maize root apex (NaCl treatment) and maize cell cultures (Horst et al., 1999; Schmohl and Horst, 2000), and by the strong positive relationship between Al accumulation and the localization of pectin contents along the root apex (Schmohl and Horst, 2001; Eticha et al., 2005). Comparing faba bean and maize, Schmohl and Horst (2001) showed that the four times greater Al accumulation in the first 5 mm of the root apex in faba bean corresponded to higher pectin contents. This higher Al binding capacity of cell walls in faba bean could explain the differences in radial movement of Al in the DTZ. Marienfeld et al. (2000) showed that after short-term Al supply, Al was mostly restricted to the rhizoderms and outer cortical cells in faba bean, while in maize Al was detected even in the inner cortex. The greater binding of Al to the cell walls in the EZ thus affecting the extensibility of the cell wall directly or indirectly by creating mechanical stress which is transferred to the cytoskeleton, leading to a disturbance of the processes that are necessary for cell elongation (Carpita and Gibeaut, 1993; Horst et al., 1999), might be responsible for the inhibitory effect of Al when applied to the EZ in common bean (Figs 2, 3).
The two common bean genotypes did not differ in the initial response of root elongation to Al exposure (Fig. 5); both were severely inhibited up to 4 h of Al treatment duration. After 4 h of Al treatment, both genotypes gradually recovered from the initial inhibition of root elongation (Fig. 4). Whereas this recovery continued in Quimbaya almost to the level of the control (without Al) after 24 h, with VAX-1 it was again increasingly inhibited by Al after 12 h of Al treatment. Similar patterns of recovery have been detected after 8 h of Al treatment in common bean (Cumming et al., 1992) and after 6 h of Al treatment in soybean (Yang et al., 2000, 2001). This pattern of root elongation during medium-term Al exposure was reflected in decreasing Al contents of root apices (Fig. 6), particularly of the apical 2 mm (Fig. 7) in both genotypes and again increasing Al contents in the Al-sensitive VAX-1. However, only after 24 h of Al exposure were the clear genotypic differences observed in the recovery of root elongation. These differences were reflected in lower Al contents in the Al-resistant genotype Quimbaya which maintained the higher Al contents of the root tips for up to 16–20 h (Figs 6, 7). A similar or even smaller Al-induced inhibition of root elongation at higher Al contents in the root apices calls for an Al tolerance mechanism acting in the Al-resistant genotype Quimbaya in addition to an Al exclusion mechanism (see below).

The recovery of root growth after short-term (<6 h) Al treatment is typical for plant species characterized by a pattern II Al-induced release of organic acid anions (Ma et al., 2001; Ryan et al., 2001) such as soybean (Yang et al., 2000) and chakod (Ma et al., 1997). In both plant species, the recovery of Al-resistant cultivars was directly related to an Al-induced citrate exudation. Our own unpublished work clearly shows that, also in common bean, recovery from short-term Al stress and medium-term established genotypic differences in Al resistance are related to an Al-induced increase in release of citrate from root apices. This is in agreement with the studies of Shen et al. (2002) demonstrating that lower Al contents of root tips of the Al-resistant cultivar G 19842 compared with the Al-sensitive cultivar ZPV corresponded with its higher capacity to exude citrate that was assessed after 3 d of Al treatment (10 μM and 20 μM Al). Earlier, Miyasaka et al. (1991) had provided evidence that Al resistance in common bean involves the efflux of citrate.

In conclusion, the results presented confirm that the expression of Al toxicity and Al resistance in common bean differs from that in maize. Although in both species the TZ appears to be the most Al-sensitive root zone, in common bean Al inhibits root elongation also when applied exclusively to the EZ. Al resistance in common bean thus requires the protection of the entire EZ from Al injury. Genotypes differing in Al resistance in medium-and long-term studies do not differ in their short-term sensitive response to Al. Al resistance in common bean is building up during medium-term exposure to Al and is related to a reduced Al accumulation in the root tips, supporting the idea of a mechanism of Al resistance based on exclusion from the root apex mediated by citrate exudation. In addition to Al exclusion, it appears that the Al-resistant genotype Quimbaya possesses an Al tolerance mechanism detoxifying part of the Al accumulated in the root apices. Results on organic acid anion synthesis and exudation, and Al compartmentation and binding in root apices of common bean will be published elsewhere.

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