Proton toxicity interferes with the screening of common bean (*Phaseolus vulgaris* L.) genotypes for aluminium resistance in nutrient solution

Andrés F. Rangel[1], Mohammad Mobin[1], Idupulapati M. Rao[2], and **Walter J. Horst[1]***

[1] Institute of Plant Nutrition, University of Hannover, Herrenhäuser Str. 2, D-30419 Hannover, Germany
[2] Tropical Soil Biology and Fertility Institute, International Center for Tropical Agriculture (CIAT), AA 6713, Cali, Colombia

Summary—Zusammenfassung

Common bean (*Phaseolus vulgaris* L.) proved to be very sensitive of low pH (4.3), with large genotypic differences in proton sensitivity. Therefore, proton toxicity did not allow the screening of common bean genotypes for aluminium (Al) resistance using the established protocol for maize (0.5 mM $CaCl_2$, 8 μM H_3BO_3, pH 4.3). Increasing the pH to 4.5, the Ca^{2+} concentration to 5 mM, and addition of 0.5 mM KCl fully prevented proton toxicity in 28 tested genotypes and allowed to identify differences in Al resistance using the inhibition of root elongation by 20 μM Al supply for 36 h as parameter of Al injury. As in maize, Al treatment induced callose formation in root apices of common bean. Aluminium-induced callose formation well reflected the effect of Ca supply on Al sensitivity as revealed by root-growth inhibition. Aluminum-induced callose formation in root apices of 28 bean genotypes differing in Al resistance after 36 h Al treatment was positively correlated to Al-induced inhibition of root elongation and Al contents in the root apices. However, the relationship was less close than previously reported for maize. Also, after 12 h Al treatment, callose formation and Al contents in root apices did not reflect differences in Al resistance between two contrasting genotypes, indicating a different mode of the expression of Al toxicity and regulation of Al resistance in common bean than in maize.

Key words: aluminium / toxicity / resistance / genotypic differences / proton toxicity / callose formation / common bean

Protonen-Toxizität beeinflusst das Screening von Buschbohnen (*Phaseolus vulgaris* L.)-Genotypen auf Aluminium-Resistenz in Hydrokultur

Buschbohnen (*Phaseolus vulgaris* L.) sind sehr empfindlich gegenüber niedrigen pH-Werten (4,3), wobei jedoch große genotypische Unterschiede bestehen. Daher war ein Screening von Buschbohne unter den für Mais etablierten Versuchsbedingungen (0,5 mM $CaCl_2$, 8 μM H_3BO_3, pH 4,3) bislang nicht möglich. Eine Erhöhung des pH-Wertes auf 4,5, der Ca^{2+} Konzentration auf 5 mM sowie die Zugabe von 0,5 mM KCl verhinderte Protonentoxizität bei allen 28 untersuchten Genotypen und erlaubte die Identifikation von großen Unterschieden in der Al-Resistenz, wenn die durch 20 μM Al induzierte Hemmung des Wurzellängenwachstums als Maß für die Al-Schädigung herangezogen wurde. Wie bei Mais induzierte eine Al-Behandlung die Bildung von Kallose in den Wurzelspitzen auch bei Buschbohne. Diese Induktion der Kallose-Synthese spiegelte die Melioration der durch Al induzierten Hemmung des Wurzellängenwachstum durch erhöhtes Ca-Angebot gut wider. Der Vergleich von 28 Buschbohnen-Genotypen hinsichtlich ihrer Al-Resistenz ergab positive Korrelationen zwischen der Al-induzierten Kallose-Bildung, der Hemmung des Wurzelwachstums und den Al-Gehalten in den Wurzelspitzen. Aber die Beziehungen waren deutlich weniger eng als für Mais berichtet. Bei kurzfristigem Al-Angebot (12 h) spiegelten die Kallose- und Al-Gehalte von Wurzelspitzen die Unterschiede in zwei sehr unterschiedlich Al-resistenten Genotypen nicht wider. Wir schließen daraus, dass Al-Toxizität und Al-Resistenz bei Buschbohne anders als bei Mais reguliert sind.

1 Introduction

Common bean (*Phaseolus vulgaris* L.) is the most important food legume for more than 300 million people, most of them in the developing world. It is the second source of protein in Eastern and Southern Africa and fourth in Tropical America, where it is also the third-most important caloric source after cassava and maize (*CIAT*, 1999; *Rao*, 2001). Common bean is mainly produced on small-scale farms (80% of dry-bean production) in developing countries in Latin America and Africa, where about 40% of the bean-growing area is affected by Al toxicity, resulting in yield reductions from 30% to 60% (*Thung* and *Rao*, 1999; *Wortman* et al., 1998).

Acid soils comprise up to 40% of the world's arable land (*von Uexküll* and *Mutert*, 1995), and soil acidity represents a major growth-limiting factor for plants (*Foy*, 1984). Although poor crop growth on acid soils is mainly correlated with Al^{3+} or H^+ activities, other factors like manganese toxicity, low nitrogen supply, and deficiencies of phosphorus, calcium, magnesium, and molybdenum may also play a role (*Foy*, 1984; *Rao* et al., 1993).

Developing genotypes tolerant of acid soils is an ecologically friendly, energy-conserving, and economical solution for resource-poor farmers in the tropics. This genetic approach and the application of adapted agronomic practices in addition to maintenance lime applications are key factors for sustainable cropping systems on acid soils (*Rao* et al., 1993).

The relative impacts of Al and proton toxicities on plant performance in acid soils differ among plant species. The relative sensitivity to protons of three legume species increased in the order *Pisum sativum* < *Glycine max* < *Phaseolus vulgaris* (16%–65% inhibition of root elongation, respectively) when the pH of the nutrient solution was reduced from 6.0 to 4.05 (*Lazof* and *Holland*, 1999).

* Correspondence: Prof. Dr. W. Horst;
e-mail: horst@pflern.uni-hannover.de

Preliminary evaluation indicated significant genotypic variation in grain yield among bean genotypes grown on Al-toxic soils. These genotypic differences could be related to differences in Al resistance (*Thung* and *Rao*, 1999; *CIAT*, 1999). However, a systematic screening for Al resistance independently from soil factors other than Al toxicity requires a suitable screening procedure, which is not yet available for common bean. The development of such a technique would be facilitated by a better understanding of the physiological mechanisms responsible for Al resistance in common bean.

Solution-culture techniques allow to study the effects of one factor of the soil-acidity complex without affecting others, provided the adaptation of plant roots to low pH are considered (*Edmeades* et al., 1995). The present work aimed at developing the basic nutrient solution required to assess genotypic differences in Al resistance of common bean, independently of interactions with proton toxicity, and at evaluating the possible use of Al-induced callose formation to assess genotypic differences in Al resistance.

2 Materials and methods

2.1 Plant materials and growing conditions

The experiments were conducted with a set of 28 common bean (*Phaseolus vulgaris* L.) genotypes (Tab. 1), including land races and bred lines differing in adaptation to acid, Al-toxic soils. Seeds were provided by the International Center for Tropical Agriculture (CIAT), Cali, Colombia.

Common bean seeds were germinated for 3 d in plastic trays filled with peat limed to pH 5.5. Seedlings were carefully removed from the peat and the roots gently rinsed with distilled water to remove the attached peat. The seedlings were then transferred to a constantly aerated 22 L nutrient solution in pots equipped with an automatic pH titration device. The seedlings were grown for 24 h at pH 6.0 ± 0.2, followed by lowering the pH in steps of 0.3 pH units until the target pH was reached after 18 h. Thereafter, the pH was kept constant by adding 0.1 M HCl or 0.1 M KOH. Plants were grown under controlled environmental conditions in a growth chamber with a 16 h/8 h light/dark regime, 27°C/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 plant height.

To verify the suitability of the nutrient solution medium used for maize (*Horst* et al., 1997; *Collet* et al. 2002; *Wang* et al., 2004; *Eticha* et al., 2005) for common bean, seedlings of the genotypes SEA-5 and VAX-1 were grown at 0.5 mM CaCl$_2$ and 8 μM H$_3$BO$_3$ at pH 6.0, 4.5, and 4.3. Six hours after the target pH was reached, the plants were treated with nominal Al concentrations of 0, 10, and 15 μM as AlCl$_3$ for up to 36 h.

Seedlings of the genotypes SEA-5 and VAX-1 were further grown at pH 6.0 and 4.5 in nutrient solutions containing 8 μM H$_3$BO$_3$ and different KCl (0 and 0.5 mM) and CaCl$_2$ concen-

Table 1: List of the common bean genotypes used in the Al-screening experiments.
Tabelle 1: Liste der Buschbohnen-Genotypen, die in das Al-Screening einbezogen wurden.

Cultivar	Classification		
A-774	Breed	Mesoamerican	Advanced line for America, drought-tolerant
A-785	Breed	Mesoamerican	Advanced line for America, drought-tolerant
BAT-477	Breed	Mesoamerican	Bean adapted to the tropics, drought-tolerant
BAT-881	Breed	Mesoamerican	Bean adapted to the tropics
BRB-191	Breed	Andean	Bush bean resistant to black root
BRB-198	Breed	Andean	Bush bean resistant to black root
Carioca	Land race	Mesoamerican	Commercial variety
Dicta-17	Breed	Mesoamerican	Drought-tolerant
DOR-364	Breed	Mesoamerican	Bean resistant to golden mosaic virus
DOR-390	Breed	Mesoamerican	Bean resistant to golden mosaic virus
DOR-714	Breed	Mesoamerican	Bean resistant to golden mosaic virus
FEB-190	Breed	Mesoamerican	Experimental bush bean
G-685			
G-855			
G-3513	Land race	Mesoamerican	
G-5273	Land race	Andean	
G-19227A	Land race	Mesoamerican	
G-19833	Land race	Andean	Nueva
G-21212	Land race	Mesoamerican	Monoculture on the Mexican high plateau
MAM-38	Breed	Mesoamerican	Monoculture on the Mexican high plateau
MAM-49			
1-Mar	Breed	Mesoamerican	Resistance to angular leaf spot
MD-23-24			
Quimbaya	Variety	Andean	Commercial variety
SEA-5	Breed	Mesoamerican	Advanced drought-resistant line
Tiocanela-75	Variety	Mesoamerican	
VAX-1	Breed	Mesoamerican	Interspecific hybrid
VAX-6	Breed	Mesoamerican	Interspecific hybrid

J. Plant Nutr. Soil Sci. 2005, *168*, 607–616

Screening of common bean for aluminum resistance   609

trations (0.5, 1, and 5 mM). Six hours after the target pH was reached, the plants were treated with nominal Al concentrations of 0, 10, 20, and 50 μM as $AlCl_3$ for 12 and 36 h.

To prove if modifications of the basal nutrient solution alleviated proton toxicity, a set of 28 common bean genotypes was grown at 5 mM $CaCl_2$, 0.5 mM KCl, and 8 μM H_3BO_3 at pH 6.0 and at 4.5 for 36 h. Half of the pots were treated with a nominal Al concentration of 20 μM as $AlCl_3$ for up to 36 h.

2.2 Determination of the growth of roots and their callose and Al contents

Root elongation was determined by measuring the primary root length at the beginning and 36 h after Al treatment using a 1 mm scale keeping the root submerged in nutrient solution. The difference between the initial and the final length during the treatment period was defined as root-elongation rate (RER). Aluminum-induced inhibition of root elongation was calculated as:

$$Al\text{-inhibited root elongation }[\%] = \frac{RFR_{control} - RFR_{Al}}{RFR_{control}} \times 100$$

$RER_{control}$: root-elongation rate at 0 μM Al

RER_{Al}: root-elongation rate at 20 μM Al

After treatment, roots were rinsed with distilled water, and 10 mm root tips were excised using a razor blade, stored in Eppendorf tubes (Eppendorf AG, Hamburg, Germany), and fixed immediately in liquid nitrogen. The root tips were homogenized in 500 μL of 1 M NaOH with a mixer mill (MM 200, Retsch GmbH & Co. KG, Haan, Germany) at a speed of 20 cycles s^{-1} for 2 min. After homogenization, another 500 μL of 1 M NaOH were added, and callose was solubilized by heating in a water bath at 80°C for 20 min. Thereafter, samples were centrifuged at 21000 g for 5 min. Callose was measured according to *Kauss* (1989) after addition of aniline blue reagent, using a Microplate Fluorescence Reader (Flx 800, Bio-Tek Instruments Inc, Winooski, Vermont, USA), at excitation and emission wavelengths of λ = 400/30 and λ = 485/40 nm, respectively, sensitivity 50. Pachyman (1,3-ß-D-glucan, Calbiochem, Deisenhofen, Germany) was used as calibration standard. Hence, callose contents were expressed as pachyman equivalents (PE) per root tip.

After treatment, roots were rinsed with distilled water and 10 mm root tips were excised using a razor blade, stored in Eppendorf cups and kept at 4°C. Samples were placed in teflon centrifuge tubes and digested in 500 μL ultrapure HNO_3 (65% v/v) in a Microwave-Laboratory-System (MLS-ETHOS Plus, MLS GmbH, Leutkirch, Germany) for 3 h. Thereafter, the volumes of the samples were adjusted to 2 mL with ultrapure water. Aluminium in the samples was analyzed by ICP-OES (Spectro Analytical Instruments GmbH, Kleve, Germany) at a wavelength of λ = 308.21 nm.

2.3 Experimental design and statistical analysis

Completely randomized designs were used in all experiments with four or eight replicates, depending on the experiment. After analysis of variance (Proc GLM), the means were compared using the Tukey test. Data were statistically analyzed using SAS 8 (SAS Institute Inc., Cary, NC, USA). *, **, *** denote significant differences at $p < 0.05$, 0.01, 0.001, respectively; ns, nonsignificant.

3 Results

3.1 Adaptation of the basal incubation medium used for maize to common beans

When the solution pH decreased from pH 6.0 to 4.3, the root-elongation rate was significantly inhibited in both genotypes tested (Fig. 1a). In general, growth inhibition was more
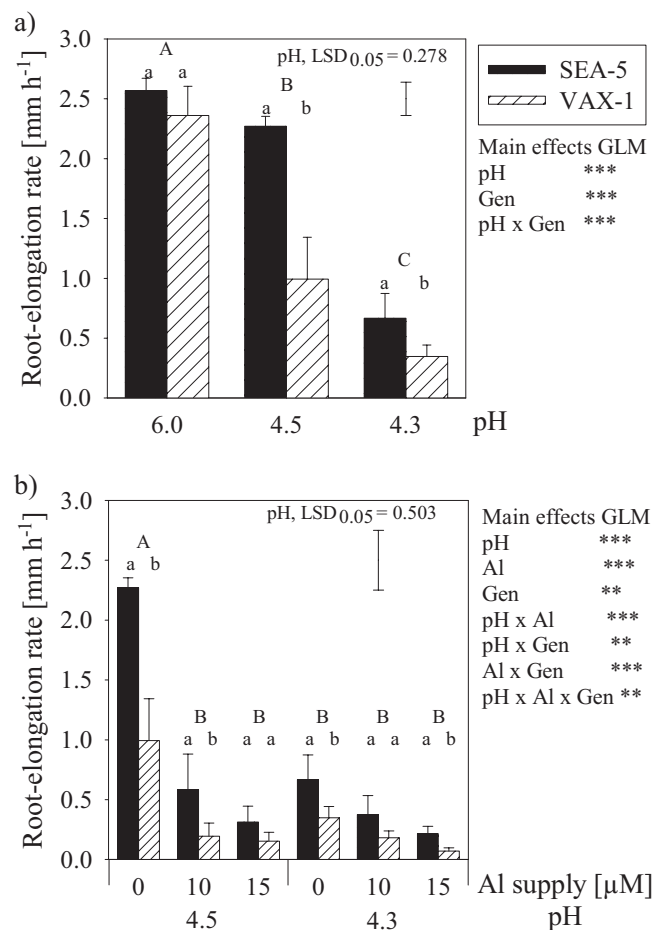

Figure 1: Root-elongation rate of two common bean genotypes grown for or 36 h in a solution containing 0.5 mM $CaCl_2$ and 8 μM H_3BO_3 at different pH (a) or different pH and Al supplies (b). Bars represent means ± SD (n = 4). Significant differences between means are indicated by different letters at $p < 0.05$ (Tukey test). Capital letters: differences between treatments; small letters: differences between genotypes within treatments.
Abbildung 1: Wurzellängenwachstum von zwei Buschbohnen-Genotypen nach 36 h Kultur in einer Nährlösung, die 0,5 mM $CaCl_2$ und 8 μM H_3BO_3 enthielt, in Abhängigkeit vom pH-Wert (a) oder vom Al-Angebot (b). Die Balken zeigen Mittelwerte ± SD (n = 4). Unterschiedliche Buchstaben kennzeichnen signifikante Unterschiede zwischen Mittelwerten bei $p < 0,05$ (Tukey-Test). Großbuchstaben zeigen Unterschiede zwischen, Kleinbuchstaben Unterschiede innerhalb von Behandlungen an.

severe in VAX-1 than in SEA-5. At pH 4.5, root growth of SEA-5 was less affected (12% reduction compared to pH 6.0) than of VAX-1 (58% reduction compared to pH 6.0). At pH 4.3, root-elongation rates of both genotypes were greatly reduced (74% and 85% for SEA-5 and VAX-1 compared to pH 6.0, respectively). Al supply additionally inhibited root-elongation rate (Fig. 1b). However, significant differences were only observed at pH 4.5. Treatment with 10 μM Al reduced root growth of genotypes SEA-5 and VAX-1 by more than 74% and 80%, respectively. At pH 4.3, the control roots particularly of VAX-1 were too much damaged by H$^+$ toxicity to show a clear Al effect.

3.2 Modifications of the basal incubation medium

Addition of K (0.5 mM) to the basal nutrient solution (0.5 mM CaCl$_2$ and 8 μM H$_3$BO$_3$) fully prevented the observed reduction in the root growth of SEA-5 grown at pH 4.5 (Fig. 2). Likewise, K supply enhanced the root growth of VAX-1 (*cf.,* Fig. 1a). However, complete recovering of the root growth (relative to pH 6.0) was only achieved at 5 mM CaCl$_2$ (Fig. 3a). Genotype SEA-5 showed higher root-elongation rates than VAX-1, independent of pH and Ca concentration.

Aluminum application (10 μM Al) strongly inhibited root elongation of both genotypes similarly at low Ca supply (Fig. 3b) and K supply (Fig. 2). Increasing the Ca supply improved root growth of the controls (Al 0) only in VAX-1. However, in the presence of Al, increasing the Ca supply greatly improved root growth, suggesting a reduction of Al toxicity by Ca in
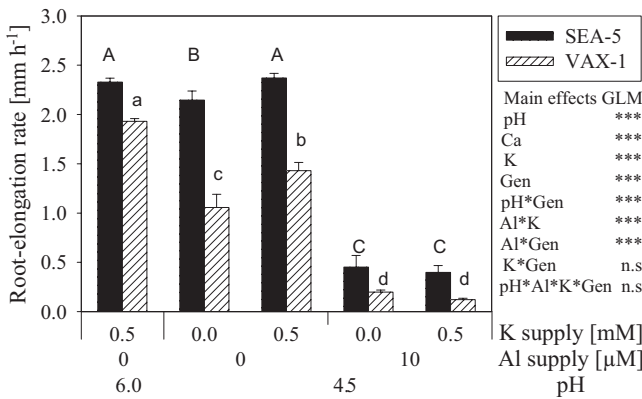
Figure 3: Root-elongation rate of two common bean genotypes grown for 36 h in a solution containing 0.5 mM KCl and 8 μM H$_3$BO$_3$ at different pH and Ca supplies (a) or different Ca and Al supplies at pH 4.5 (b). Bars represent means $\pm$ SD (n = 4). Significant differences between means are indicated by different letters at *p* < 0.05 (Tukey test). Capital letters: differences between treatments for SEA-5; small letters: differences between treatments for VAX-1.
Abbildung 3: Wurzellängenwachstum von zwei Buschbohnen-Genotypen nach 36 h Kultur in einer Nährlösung, die 0,5 mM KCl und 8 μM H$_3$BO$_3$ enthielt, in Abhängigkeit vom pH-Wert (a) oder vom Ca- und Al-Angebot bei pH 4,5 (b). Die Balken zeigen die Mittelwerte $\pm$ SD (n = 4). Unterschiedliche Buchstaben kennzeichnen signifikante Unterschiede zwischen Mittelwerten bei *p* < 0,05 (Tukey Test). Großbuchstaben zeigen Unterschiede zwischen Behandlungen für SEA 5, Kleinbuchstaben für VAX-1 an.
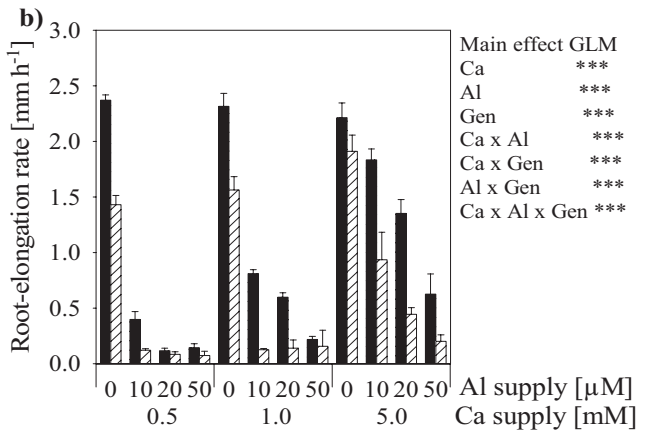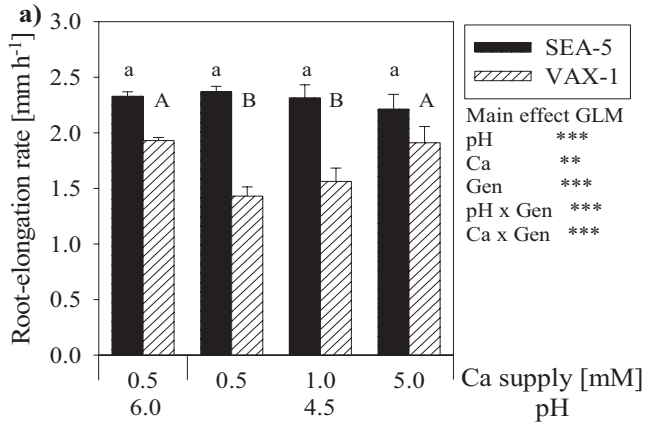
Figure 2: Effect of potassium on root-elongation rate of two common bean genotypes grown for 36 h in a solution containing 0.5 mM CaCl$_2$ and 8 μM H$_3$BO$_3$ at different pH and Al supplies. Bars represent means $\pm$ SD (n = 4). Significant differences between means are indicated by different letters at *p* < 0.05 (Tukey test). Capital letters: differences between treatments for SEA-5; small letters: differences between treatments for VAX-1.
Abbildung 2: Einfluss von Kalium auf das Wurzelwachstum von zwei Buschbohnen-Genotypen nach 36 h Kultur in einer Nährlösung, die 0,5 mM CaCl$_2$ und 8 μM H$_3$BO$_3$ enthielt, bei unterschiedlichen pH-Werten und Al-Angeboten. Die Balken zeigen die Mittelwerte $\pm$ SD (n = 4). Unterschiedliche Buchstaben kennzeichnen signifikante Unterschiede zwischen Mittelwerten bei *p* < 0,05 (Tukey-Test). Großbuchstaben zeigen Unterschiede zwischen Behandlungen für SEA 5, Kleinbuchstaben für VAX-1 an.

both genotypes. At 1 mM and particularly at 5 mM Ca supply, clear genotypic differences in Al resistance appeared: SEA-5 proved to be more Al-resistant than VAX-1. This differentiation was best at 20 μM Al supply.

The callose and Al contents of the controls (Al 0) were low and independent of the pH of the nutrient solution (not shown). Thus, proton toxicity did not induce callose formation in bean. At 0.5 mM Ca supply, even the lowest Al supply of 10 μM strongly increased callose contents in the root apices (Fig. 4a). A higher Al supply only slightly further enhanced callose formation. Increasing the Ca supply particularly
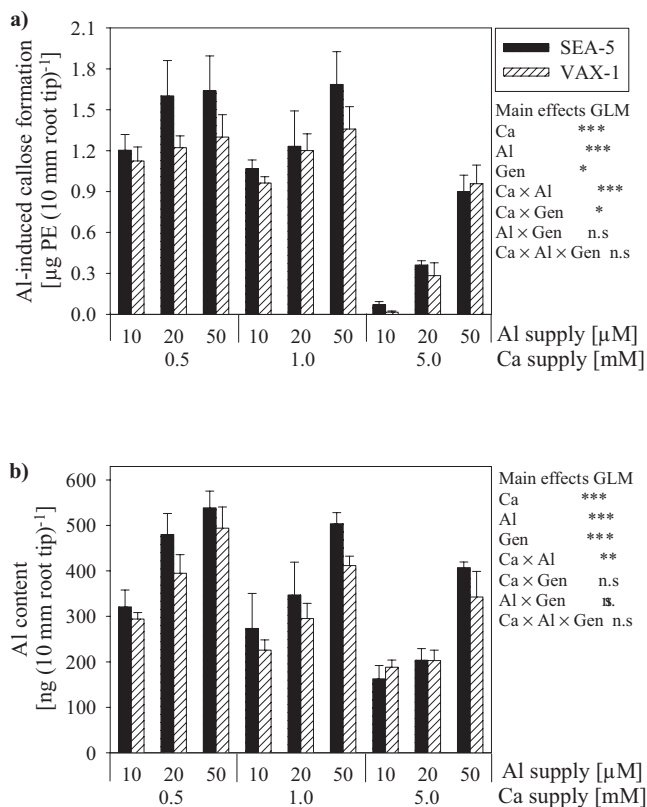
Figure 4: Effect of different Al and Ca supplies on Al-induced callose formation (a) and Al contents (b) in 10 mm root tips of two bean genotypes grown for 12 h in a solution containing 0.5 mM KCl and 8 μM H_3BO_3, pH 4.5. Bars represent means ± SD (n = 4).
Abbildung 4: Einfluss des Al- und Ca-Angebotes auf die durch Al induzierten Kallose- (a) und Al-Gehalte (b) in 10-mm-Wurzelspitzen von zwei Buschbohnen-Genotypen nach 12 h Kultur in einer Nährlösung, die 0,5 mM KCl und 8 μM H_3BO_3 enthielt, pH 4,5. Die Balken zeigen Mittelwerte ± SD (n = 4).

decreased the callose formation induced by the lower Al supplies. However, at 50 μM Al supply, callose contents were high, independent of the Ca supply. Al-induced callose contents well reflected Al-induced inhibition of root elongation (Fig. 5a) in this experiment, where the Ca supply was the main factor determining the Al response of both genotypes. However, the greater Al resistance of SEA-5 (as indicated by less Al-inhibited root elongation) was not reflected in lower callose contents. Particularly at 5 mM Ca supply, which best differentiated the genotypes regarding their level of Al resistance, Al-resistant SEA-5 produced significantly more callose than Al-sensitive VAX-1.

The Al content of the root apices increased with Al supply and decreased with Ca supply (Fig. 4b), reflecting competition between Al^{n+} and Ca^{2+} for root uptake/binding. Significant differences between genotypes and Al treatments were observed at all Ca levels. In general, the Al content reflected Al-induced inhibition of root elongation ($R^2 = 0.45$; Fig. 5b). However, the greater Al resistance of SEA-5 (see above) was not reflected in a lower Al content, especially at 5 mM Ca supply. The Al contents in the root tips were positively correlated with callose formation ($R^2 = 0.74$).

3.3 Confirming the lack of proton (H^+) toxicity at pH 4.5

As shown above, the two genotypes differed substantially in their sensitivity of low pH. The modification of the nutrient solution by increasing the pH to 4.5 and the Ca supply to 5 mM and the addition of 0.5 mM K ameliorated the proton toxicity in the Al-sensitive genotype VAX-1. To confirm that this treatment generally prevents proton toxicity in all bean genotypes to be screened for Al resistance, we compared the root growth of 28 genotypes at pH 6 and pH 4.5 using the modified nutrient solution (Fig. 6). The genotypes differed significantly in root-elongation rate, independent of the solution
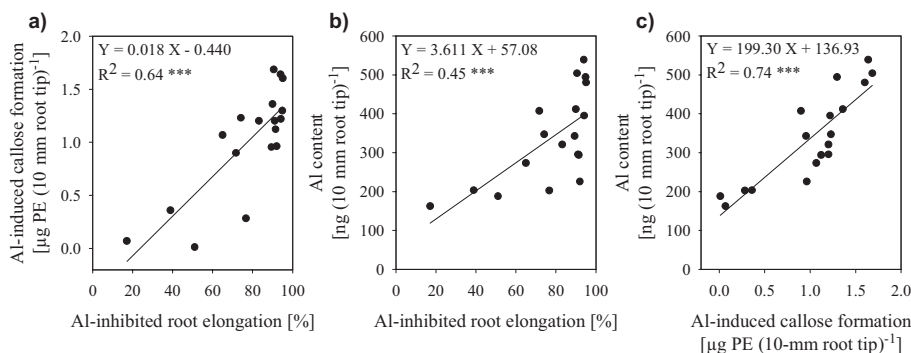

Figure 5: Relationships between Al-inhibited root elongation and Al-induced callose formation (a), Al-inhibited root elongation and Al content (b), and Al-induced callose formation and Al content (c) of two common bean genotypes grown in a solution containing 0.5 mM KCl and 8 μM H_3BO_3 at different Ca and Al supplies, pH 4.5. Callose and Al contents in 10 mm root tips were measured in plants treated for 12 h. Al-inhibited root elongation was measured in plants treated for 36 h (n = 4); *** denotes significance at $p < 0.001$.
Abbildung 5: Beziehungen zwischen Al-induzierter Hemmung des Wurzelwachstums und Al-induzierter Kallose-Bildung (a), Al-induzierter Hemmung des Wurzelwachstums und Al-Gehalten (b) sowie Al-induzierter Kallosebildung und Al-Gehalten (c) bei zwei Buschbohnen-Genotypen nach Kultur in einer Nährlösung, die 0,5 mM KCl und 8 μM H_3BO_3 enthielt, pH 4,5, bei variiertem Al- und Ca-Angebot. Die Kallose- und Al-Gehalte in 10-mm-Wurzelspitzen wurden nach 12 h, Al-induziertes Wurzelwachstum nach 36 h Behandlungsdauer bestimmt (n = 4); *** zeigt Signifikanz bei $p < 0,001$ an.
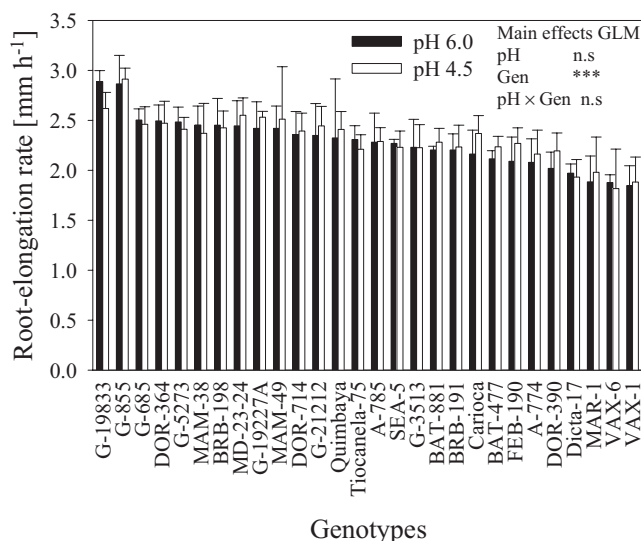
Figure 6: Effect of pH on root-elongation rate of 28 common bean genotypes grown for 36 h in a solution containing 5 mM CaCl$_2$, 0.5 mM KCl, and 8 µM H$_3$BO$_3$. Bars represent standard deviations from the means (n = 8).
Abbildung 6: Einfluss des pH-Wertes auf das Wurzelwachstum von 28 Buschbohnen-Genotypen nach Kultur für 36 h in einer Nährlösung, die 5 mM CaCl$_2$, 0,5 mM KCl und 8 µM H$_3$BO$_3$ enthielt. Die Balken zeigen die Mittelwerte ± SD (n = 8).

Figure 7: Al-induced inhibition of root elongation of 28 common bean genotypes grown in a solution containing 5 mM CaCl$_2$, 0.5 mM KCl, and 8 µM H$_3$BO$_3$ for 36 h at 20 µM Al, pH 4.5. Bars represent standard deviations from the means (n = 8).
Abbildung 7: Al-induzierte Hemmung des Wurzelwachstums von 28 Buschbohnen-Genotypen nach Kultur für 36 h in einer Nährlösung, die 5 mM CaCl$_2$, 0,5 mM KCl, 8 µM H$_3$BO$_3$ und 20 µM Al enthielt, pH 4,5. Die Balken zeigen die Mittelwerte ± SD (n = 8).

pH. However, there were no differences between the growth at pH 6.0 and pH 4.5, suggesting that screening for Al at pH 4.5 is possible without interference with proton toxicity using this solution.

3.4 Screening common bean genotypes for Al resistance based on root elongation and callose formation

Based on the previous results, 28 common bean genotypes were screened for Al resistance in a solution with 5 mM Ca, 0.5 mM K, 8 µM B, pH 4.5, without and with 20 µM Al. The effect of Al on root-elongation rate is best shown as Al-induced inhibition of root elongation (Fig. 7). The genotypes showed highly significant differences in response to Al supply. The genotypes were arbitrarily ranked for Al resistance in three categories, based on the percentage of Al-induced inhibition of root elongation. Accordingly, nine genotypes were classified as Al-sensitive (inhibition >50%) with VAX-1, MAR-1, and DOR-714 being the most Al-sensitive genotypes. Ten genotypes were classified as intermediate (inhibition between 50% and 30%), and seven genotypes were classified as Al-resistant (inhibition <30%). Among these latter seven, three Andean genotypes (G-5273, Quimbaya, and BRB-198) showed outstanding levels of Al resistance.

Callose contents of the root apices after 36 h of Al exposure (Fig. 8a) were much lower than after 12 h (compare Fig. 4a). However, the genotypes differed highly significantly in Al-induced callose formation. Genotypes classified as Al-sensitive generally had higher callose contents than those in the
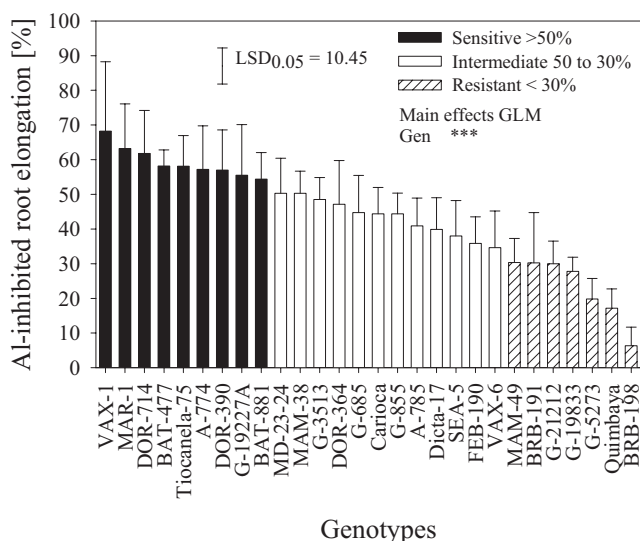
Al-resistant category. The genotypes also differed in Al contents of the root apices (Fig. 8b), which were again generally lower in resistant genotypes after 12 h exposure to Al (compare Fig. 4b). Genotypes in the Al-sensitive category were characterized by generally higher Al contents than those classified as Al-resistant.

The linear-regression analysis of all genotypes revealed highly significant correlations between Al-induced callose formation in root apices and Al-inhibited root elongation (Fig. 9a), Al contents in root apices and root-growth inhibition (Fig. 9b), and callose and Al contents in root apices treated with Al (Fig. 9c). However, the lower values of regression coefficients suggest that the existence of other sources of variation and the prediction of genotypic Al resistance based on callose formation and Al contents after 36 h Al treatment alone will be subjected to large errors.

4 Discussion

4.1 Adaptation of the basal incubation medium used for maize to common bean

In the present study, the basal incubation medium commonly used for maize was tested for common bean genotypes. The reduction of the pH from 6.0 to 4.3, which is the standard pH used for the Al-screening in maize (*Horst* et al., 1997), greatly inhibited the root elongation of bean genotypes in the absence of Al. This makes it difficult to select for Al resistance, because of the lack of a proper control (Fig. 1a). This
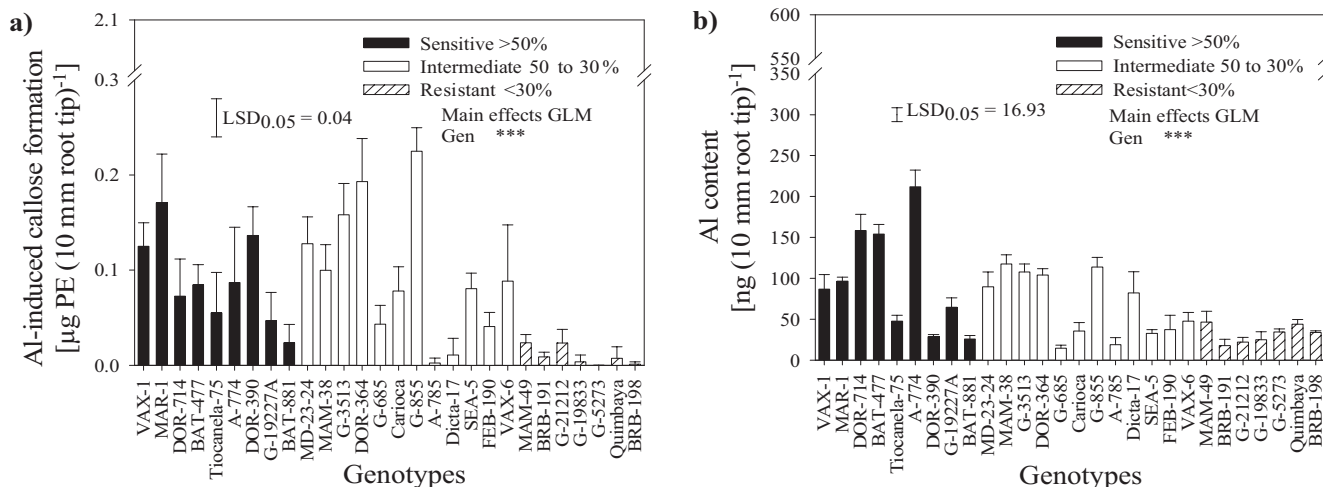
Figure 8: Al-induced callose formation (a) and Al contents (b) in 10 mm root tips of 28 common bean genotypes grown in a solution containing 5 mM $CaCl_2$, 0.5 mM KCl, and 8 μM H_3BO_3 for 36 h at 20 μM Al, pH 4.5. Genotypes are arranged from left to right according to increasing Al resistance based on Al-inhibited root elongation (Fig. 7). Bars represent standard deviations from the means (n = 4).

Abbildung 8: Al-induzierte Kallose- (a) und Al-Gehalte (b) in 10-mm-Wurzelspitzen von 28 Buschbohnen-Genotypen nach Kultur für 36 h in einer Nährlösung, die 5 mM $CaCl_2$, 0,5 mM KCl, 8 μM H_3BO_3 und 20 μM Al enthielt, pH 4,5. Die Genotypen sind von links nach rechts angeordnet, entsprechend ihrer auf der Al-induzierten Hemmung des Wurzelwachstums basierenden Al-Resistenz (Abb. 7). Die Balken zeigen Mittelwerte ± SD (n = 4).
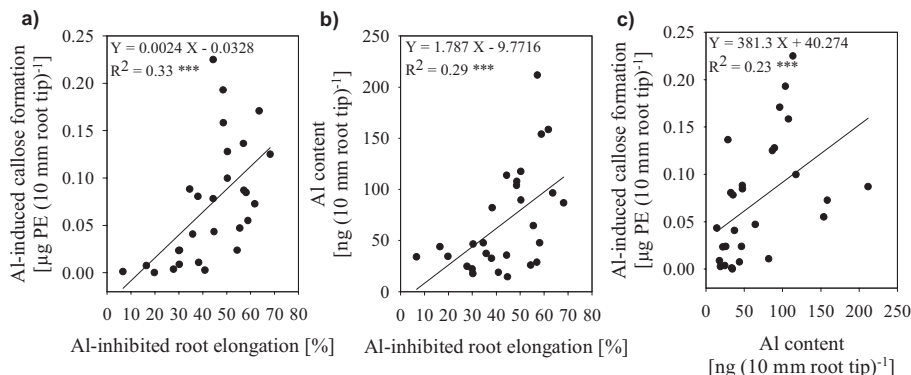

Figure 9: Relationships between Al-inhibited root elongation and Al-induced callose formation (a), Al-inhibited root elongation and Al content (b), and Al-induced callose formation and Al content (c) of 28 common bean genotypes grown in a solution with 5 mM $CaCl_2$, 0.5 mM KCl, and 8 μM H_3BO_3 for 36 h at 20 μM Al, pH 4.5 (n = 4); *** denotes significance at $p < 0.001$.

Abbildung 9: Beziehungen zwischen Al-induzierter Hemmung des Wurzelwachstums und Al-induzierter Kallose-Bildung (a), Al-induzierter Hemmung des Wurzelwachstums und Al-Gehalten (b) sowie Al-induzierter Kallose-Bildung und Al-Gehalten (c) bei 28 Buschbohnen-Genotypen nach Kultur für 36 h in einer Nährlösung, die 5 mM $CaCl_2$, 0,5 mM KCl, 8 μM H_3BO_3 und 20 μM Al enthielt, pH 4,5 (n = 4); *** zeigt Signifikanz bei $p < 0,001$ an.

is in agreement with *Lazof* and *Holland* (1999), who clearly showed the high proton sensitivity of two common bean genotypes. They concluded that screening bean for Al resistance is not possible at pH 4.05 and proposed to evaluate recovery from Al stress as indicator of Al resistance. Because of the high proton sensitivity of common bean, *Massot* et al. (1990) classified six genotypes for Al resistance using a solution pH of 4.8, but without control of pH and also without monitoring Al activity in solution. The high proton sensitivity of common bean may be related to a low efficiency of the plasma-membrane proton ATPase (*Yan* et al., 1992, 1998), and thus the inability to maintain the cytosolic pH leading to cell injury (*Schubert* and *Yan*, 1997).

Calcium and other polyvalent cations play a crucial role in maintaining the integrity of the ion-absorption process, especially in the acid pH range (*Moore*, 1971). Calcium concentrations in the nutrient solution (0.5 mM) have been proven to be sufficient to avoid proton toxicity in maize, but not in common bean (Fig. 1a). From their experiments conducted with maize and broad bean, *Yan* et al. (1992) concluded that the Ca^{2+} requirement to recover root growth at a given low pH was smaller for maize than for broad bean. In our experiments with common bean, a similar root growth at pH 4.5 as at pH 6.0 was observed with addition of 5 mM Ca^{2+} (Fig. 3a). Since we did not use Ca concentrations between 1 and 5 mM, we cannot exclude that Ca concentrations of less than 5 mM

may have been sufficient. The alleviation of H^+ toxicity by Ca^{2+} can be attributed to the displacement of H^+ from the cell wall and outer face of the plasma membrane, thus maintaining cell-wall (*Koyama* et al., 2001) and plasma-membrane (*Hanson*, 1984) properties, and the H^+ release from the cytosol through the H^+-ATPase, which is a prerequisite for root growth (*Kinraide*, 1998; *Yan* et al., 1992). Potassium (Fig. 2) has been shown to increase the activity of the H^+-ATPase (K^+ antiport) by increasing the affinity of the ATPase for ATP, therefore enhancing root growth (*Lindberg* and *Yahya*, 1994). The modified nutrient solution (5 mM Ca^{2+} and 0.5 mM K^+) resulted in equal root-elongation rates of bean genotypes at pH 6.0 and 4.5 (Fig. 6), thus obtaining a proper control for the Al treatments.

Al treatment caused a significant increase of callose formation in root tips of bean genotypes (Fig. 4a). Within the range of 6.0 to 4.3, no effect of pH on callose formation was observed. The best differentiation in callose formation between genotypes was obtained at the highest Ca^{2+} supply. Callose formation is one of the earliest physiological reactions of roots to Al stress and a sensitive parameter of Al injury in soybean (*Wissemeier* et al., 1992; *Staß* and *Horst*, 1995), wheat (*Zhang* et al., 1994), maize (*Collet*, 2001), and bean (*Massot* et al., 1999). According to current knowledge, callose formation is initiated through changes in plasma-membrane fluidity and permeability (*Jones* and *Kochian*, 1995) and increased cytosolic Ca^{2+} activities (*Rengel* and *Zhang*, 2003; *Sivaguru* et al., 2005). *Staß* and *Horst* (1995) also observed no effect of pH on callose induction when soybean cells were grown in the pH range between 4.3 and 7.0. *Wissemeier* and *Horst* (1995) found a reduction in the callose formation in soybean plants with increasing Ca^{2+} levels in the nutrient solution. They also demonstrated the necessity of sufficient Ca^{2+} in the external solution to promote Al-induced callose formation.

Calcium amelioration of Al toxicity as reflected by lower root-growth inhibition (Fig. 3b) and lower callose formation (Fig. 4a) can be related to decreased Al concentrations in the root apices (Fig. 4b, Fig 5). However, even at 50 μM Al supply, Al toxicity could not be prevented, confirming that Al toxicity cannot be explained by Al-induced Ca deficiency (*Ryan* et al., 1994).

4.2 Screening for Al resistance of common bean genotypes based on root elongation and callose formation

Using the modified Al solution allowed for the identification of significant differences in the Al resistance among the 28 genotypes on the basis of Al-inhibited root elongation (Fig. 7). This shows the existence of a genetic variability in response of common bean to Al stress and underlines the possibility of using the methodology for the screening of a larger set of genotypes. Aluminium-resistant genotypes like G-5273 (Andean) and G-21212 (Mesoamerican) have been found to be outstanding in their performance under acid soil conditions. However, genotypes VAX-1 and MAR-1, which were classified as highly Al-sensitive based on relative root elongation in nutrient solution, performed very well on acid soils

(*CIAT*, 1999, 2000). The superior performance of VAX-1 under acid soil conditions was associated with abundant basal and adventitious-root development (*Rao* et al., 2004).

However, Al toxicity may not have been the only growth-limiting factor on the acid soils used for the field tests. P-acquisition efficiency (*Baligar* et al., 1997; *Shen* et al., 2002a), adaptation to low levels of Ca and Mg, and establishment and maintenance of the N_2-fixing rhizobia symbiosis (*Rao*, 2001) may well have been equally or even more important. Also, *Horst* and *Klotz* (1990) reported a poor correlation between Al resistance of 31 soybean genotypes in solution and sand cultures. This indicates that in substrate genotypic Al resistance may be modified by root exudation as suggested by *Horst* et al. (1990).

Callose synthesis has been demonstrated to be a sensitive short- (1–8 h) and short- and medium-term (8–24 h) marker for Al injury in soybean (*Wissemeier* et al., 1992; *Staß* and *Horst*, 1995) and maize (*Horst* et al., 1997; *Kollmeier* et al., 2000; *Collet* et al., 2002), respectively. A decrease in Al-induced callose contents in root apices after 22 h Al treatment has been reported for soybean (*Wissemeier* and *Horst*, 1995) and after 24 h for maize (*Collet*, 2001). A decrease in the callose content through depolymerization of callose by (1,3)-β-glucanase has been suggested by *Wissemeier* and *Horst* (1995) to be the mechanism explaining the low callose contents in bean root apices found after 36 h Al treatment (Fig. 8a).

Differences in Al contents and thus callose contents of root tips between genotypes after medium- and longer-term (36 h) Al supply may be the result rather than the cause of differences in root-growth rate: Al-resistant genotypes maintained root growth and thus "diluted" Al and callose contents. Also, lower Al contents in Al-resistant genotypes may result from Al exclusion through the release of organic-acid anions (*Shen* et al., 2002b) after an Al-induction period typical for Pattern II-response plants (*Ma* et al., 2001). This may explain why after 36 h of Al treatment, significant correlations were found between callose and Al contents and Al-induced inhibition of root elongation (Fig. 9). However, much closer correlations were found with Ca supply and solution pH as the main sources of variation after a medium-term (12 h) exposure to Al (Fig. 5), which drastically varied the severity of the Al stress. Nevertheless, genotypic differences in Al resistance were not reflected in corresponding differences in Al-induced callose formation and Al contents in root apices (Fig. 4). This is in contrast to results obtained with maize, where short-term Al supply induced callose contents in root apices as a most suitable indicator of Al sensitivity, which may be used for the characterization of inheritance of Al resistance and as a screening tool for adaptation to acid, Al-toxic soils (*Eticha* et al., 2005). It thus appears that the expression of Al toxicity and the resistance in common bean differ from that of maize. Therefore, more detailed studies on the kinetics of Al-induced inhibition of root elongation, Al-induced callose formation in relation to the release of organic acid anions, and the accumulation of organic acids and Al are necessary to better understand genotypic differences in Al resistance and to develop quick screening techniques for Al resistance.

Acknowledgments

References

Baligar, V. C., Pitta, G. V. E., Gama, E. E., Schaffert, R. E., Bahia-Filho, A. F., De, C., Clark, R. B. (1997): Soil acidity effects on nutrient use efficiency in exotic maize genotypes. *Plant Soil* 192, 9–13.

CIAT (1999): Bean improvement for sustainable productivity, input use efficiency, and poverty alleviation. Annual Report of the project IP-1. CIAT, Cali, Colombia, pp. 14–23.

CIAT (2000): Bean improvement for sustainable productivity, input use efficiency, and poverty alleviation. Annual Report of the project IP-1. CIAT, Cali, Colombia, pp. 23–29.

Collet, L. (2001): Screening maize (*Zea mays* L.) for aluminium resistance – Contribution to the breeding for adaptation to acid soils. Ph.D. Thesis, University of Hanover, Germany.

Collet, L., de Leon, C., Kollmeier, M., Schmohl, N., Horst, W. J. (2002): Assessment of aluminium sensitivity of maize cultivars using roots of intact plants and excised root tips. *J. Plant Nutr. Soil Sci.* 165, 357–365.

Edmeades, D. C., Blamey, F. P. C., Farina, M. P. W. (1995): Techniques for assessing plant responses to acid soils, in Date, R. A., et al. (eds.): Plant-Soil Interactions at Low pH: Principles and Management. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 221–233.

Eticha, D., Thé, C., Welcker, C., Narro, L., Staß, A., Horst, W. J. (2005): Aluminium-induced callose formation in root apices: inheritance and selection trait for adaptation of tropical maize to acid soils. *Field Crops Res.* 93, 252–263.

Foy, C. D. (1984): Physiological effects of hydrogen, aluminium and manganese toxicities in acid soils, in Pearson, R. W., Adams, F. (eds.): Soil acidity and liming. 2nd edn., American Society of Agronomy, Wisconsin, pp. 57–97.

Hanson, J. B. (1984): The functions of calcium in plant nutrition, in Tinker, P. B., Läuchli, A. (eds.): Advances in plant nutrition, Vol. 1. Praeger Publ., New York, pp. 149–208.

Horst, W. J. (1995): The role of the apoplast in aluminium toxicity and resistance of higher plants: a review. *Z. Pflanzenernähr. Bodenkd.* 158, 419–428.

Horst, W. J., Klotz, F. (1990): Screening soybean for aluminium tolerance and adaptation to acid soils, in El Bassam, N., et al. (eds.): Genetic aspects of plant mineral nutrition. Kluwer Acad. Publ., Dordrecht, The Netherlands, pp. 355–360.

Horst, W. J., Klotz, F., Szulkiewicz, P. (1990): Mechanical impedance increases aluminium tolerance of soybean (*Glycine max* L.) roots. *Plant Soil* 124, 227–231.

Horst, W. J., Püschel, A.-K., Schmohl, N. (1997): Induction of callose formation is a sensitive marker for genotypic aluminium sensitivity in maize. *Plant Soil* 192, 23–30.

Jones, D. L., Kochian, L. V. (1995): Aluminum inhibition of the inisitol 1,4,5-trisphosphate signal transduction pathway in wheat roots: a role in aluminium toxicity? *Plant Cell* 7, 1913–1922.

Kauss, H. (1989): Fluorometric measurements of callose and other 1,3-ß glucans, in Linkens, H. F., Jackson, J. F. (eds.): Modern methods of plant analysis 10. Springer Verlag, Berlin, Germany, pp. 127–137.

Kinraide, T. B. (1998): Three mechanisms for the calcium alleviation of mineral toxicities. *Plant Physiol.* 118, 513–520.

Kollmeier, M., Felle, H. H., Horst, W. J. (2000): Genotypical differences in Al resistance of *Zea mays* (L.) are expressed in the distal part of the transition zone. Is reduced basipetal auxin flow involved in inhibition of root elongation by Al? *Plant Physiol.* 122, 945–956.

Koyama, H., Toda, T., Hara, T. (2001): Brief exposure to low-pH stress causes irreversible damage to the growing root in *Arabidopsis thaliana*: pectin-Ca interaction may play an important role in proton rhizotoxicity. *J. Exp. Bot.* 52, 361–368.

Lazof, D. B., Holland, J. M. (1999): Evaluation of the aluminium-induced root growth inhibition in isolation from low pH effects in *Glycine max, Pisum sativum* and *Phaseolus vulgaris. Aust. J. Plant Physiol.* 26, 147–157.

Lindberg, S., Yahya, A. (1994): Effects of pH and mineral nutrition supply on K^+ ($^{86}Rb^+$) influx and plasma membrane ATPase activity in roots of sugar beets. *J. Plant Physiol.* 144, 150–155.

Ma, J. F., Ryan, P. R., Delhaize, E. (2001): Aluminium tolerance in plants and the complexing role of organic acids. *Trends Plant Sci.* 6, 273–278.

Massot, N., Poschenrieder, Ch., Barcelo, J. (1990): Aluminum tolerance assessment in bush bean cultivars by root growth analysis and hematoxylin staining. *Suelo y Planta* 5, 25–32.

Massot, N., Llugany, M., Poschenrieder, Ch., Barcelo, J. (1999): Callose production as indicator of aluminium toxicity in bean cultivars. *J. Plant Nutr.* 22, 1–10.

Moore, D. P. (1971): Physiological effects of pH on roots, in Carson, E. W. (ed.): The plant root and its environment. University Press of Virginia, Charlottesville, pp. 135–151.

Rao, I. M. (2001): Role of physiology in improving crop adaptation to abiotic stresses in the tropics: The case of common bean and tropical forages, in Pessarakli, M. (ed.): Handbook of Plant and Crop Physiology. Marcel Dekker, Inc., New York, USA, pp. 583–613.

Rao, I. M., Zeigler, R. S., Vera, R., Sarkarung, S. (1993): Selection and breeding for acid-soil tolerance in crops: Upland rice and tropical forages as case studies. *BioScience* 43, 454–465.

Rao, I. M., Beebe, S., Ricaurte, J., Teran, H., Singh, S. (2004): Common bean (*Phaseolus vulgaris* L.) genotypes tolerant to aluminum-toxic soils in the tropics, in: Proceedings of the 6th Intern. Symp. on Plant-Soil Interactions at Low pH, Sendai, Japan 07-31 – 08-05-2004. Jap. Soc. Soil Sci. Plant Nutr., Sendai, Japan, pp. 272–273.

Rengel, Z., Zhang, W. H. (2003): Tansley Review. Role of dynamics of intracellular calcium in aluminium toxicity syndrome. *New Phytol.* 159, 295–314.

Ryan, P. R., Kinraide, T. B., Kochian, L. V. (1994): Al^{3+}-Ca^{2+} interaction in rhizo-toxicity. I. Inhibition of root growth is not caused by reduction in calcium uptake. *Planta* 192, 98–103.

Schubert, S., Yan, F. (1997): Nitrate and ammonium nutrition of plants: effects on acid/base balance and adaptation of root cell plasmalemma H^+-ATPase. *Z. Pflanzenernähr. Bodenkd.* 160, 275–281.

Sivaguru, M., Yamamoto, Y., Rengel, Z., Ahn, S. J., Matsumoto, H. (2005): Early events responsible for aluminium toxicity symptoms in suspension-cultured tobacco cells. *New Phytol.* 165, 99–109.

616   Rangel, Mobin, Rao, Horst

J. Plant Nutr. Soil Sci. 2005, *168,* 607–616

Shen, H., Yan, X., Zhao, M., Zheng, S., Wang, X. (2002a): Exudation of organic acids in common bean as related to mobilization of aluminium- and iron-bound phosphates. *Env. Exp. Bot.* 48, 1–9.

Shen, H., Yan, X., Wang, X., Zheng, S. (2002b): Exudation of citrate in common bean in response to aluminum stress. *J. Plant Nutr.* 25, 1921–1932.

Staß, A., Horst, W. J. (1995): Effect of aluminium on membrane properties of soybean (*Glycine max*) cells in suspension culture. *Plant Soil* 171, 113–118.

Thung, M., Rao, I. M. (1999): Integrated management of abiotic stresses, in Singh, S. P. (ed): Common Bean Improvement in the Twenty-first Century. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 331–370.

von Uexküll, H. R., Mutert, E. (1995): Global extent, development and economic impact of acid soils, in Date, R. A., et al. (eds): Plant-Soil Interactions at Low pH: Principles and Management. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 5–19.

Wang, Y., Stass, A., Horst, W. J. (2004): Apoplastic binding of aluminum is involved in silicon-induced amelioration of aluminum toxicity in maize. *Plant Physiol.* 136, 3762–3770.

Wissemeier, A. H., Diening, A., Hergenröder, A., Horst, W. J., Mix-Wagner, G. (1992): Callose formation as parameter for assessing genotypical plant tolerance of aluminium and manganese. *Plant Soil* 146, 67–75.

Wissemeier, A. H., Horst, W. J. (1995): Effect of calcium supply on aluminium-induced callose formation, its distribution and persistence in roots of soybean (Glycine max L. Merr.). *J. Plant Physiol.* 145, 470–476.

Wortman, C. S., Kirkby, R. A., Eledu, C. A., Allen, D. J. (1998): Atlas of Common Bean (*Phaseolus vulgaris* L.) Production in Africa. CIAT, Cali, Colombia.

Yan, F., Schubert, S., Mengel, K. (1992): Effect of low root medium pH on net proton release, root respiration, and root growth of corn (*Zea mays* L) and broad bean (*Vicia faba* L). *Plant Physiol.* 99, 415–421.

Yan, F., Feuerle, R., Schäffer, S., Fortmeier, H., Schubert, S. (1998): Adaptation of active proton pumping and plasmalemma ATPase activity of corn roots to low root medium pH. *Plant Physiol.* 117, 311–319.

Zhang, G., Hoddinott, J., Taylor, G. J. (1994): Characterization of 1,3-β-glucan (callose) synthesis in roots of *Triticum aestivum* in response to aluminium toxicity. *J. Plant Physiol.* 144, 229–234.