Screening maize for adaptation to acid aluminium-toxic soils of Colombia*

Lutz Collet[1], Carlos de Leon[2], Walter J. Horst[1,3]

[1]Institute of Plant Nutrition, University of Hannover
[2]CIMMYT, Colombia
[3]Corresponding author: Institute of Plant Nutrition; Herrenhäuserstr. 2 30419 Hannover
Tel.: 0511 762 2626 Fax: 0511 762 3611 Email: Horst@mbox.pflern.uni-hannover.de
*Financial support of BMZ is highly acknowledged

Abstract

Yield of maize is considerably reduced on acid Al-toxic soils. A system allowing the non-destructive screening of individual maize seedlings (*Zea mays* L.) for Al resistance in nutrient solution and subsequent transfer to the field for yield assessment was applied. Seedlings with roots exposed to 25 µM Al in nutrient solution were transplanted to an Al-toxic soil and those not treated with Al to an Al non-toxic soil in Colombia. Plant growth as well as grain yield was significantly reduced on the acid Al-toxic site. Al-induced callose formation in nutrient solution was significantly negatively related to relative grain yield (r = - 0.79*) and above-ground dry matter (r = - 0.84*) at harvest.

Keywords: aluminium, callose, maize, resistance, screening

Introduction

In most acid soils throughout the world, covering up to 30% of the ice-free land surface potentially usable for food and biomass production, Al is the most growth and yield limiting factor (Uexküll von and Mutert, 1995). An economically and environmentally acceptable alternative to overcome subsoil acidity by deep liming is to develop plants tolerant to subsoil acidity (Foy, 1976). Inhibition of root elongation is the primary effect and has been used as parameter for screening of Al-resistance in many studies (Foy, 1976; Foy *et al*., 1993; Hanson and Kamprath, 1979; Sapra *et al*., 1982). Aluminium-induced callose formation has been proposed as an even more sensitive marker of Al sensitivity (Horst *et al*., 1997). The aim of the project was to evaluate the prospects of Al-induced callose formation as a tool for screening maize cultivars on acid Al-toxic soils in Colombia.

The experiments were aimed at answering the following questions:

- Can Al sensitivity be determined non-destructively on a single plant level using short-term Al-induced callose formation and the individual plant being transferred thereafter to the field to assess grain yielding capacity on an acid Al-toxic soil?
- Is Al-induced callose formation of cultivars related to plant growth and yield on an acid Al-toxic soil on a single plant level?

Material and methods

Experiments were conducted at CIMMYT/CIAT, Cali, Colombia. Nine maize cultivars differing in Al resistance or adaptation to acid Al-toxic soils were used in the study. Experiments in nutrient solution were conducted in an air-conditioned glasshouse with a maximum air temperature of 35°C during the day. One seed each was sown into a paper beaker sealed with a wax layer at the bottom and filled with peat moistened with nutrient solution. Twelve plants per cultivar were sown and split into three replicates of 4 plants. Paper beakers were placed in a rag on top of plastic boxes containing 20 l of nutrient solution of low ionic strength used by Horst *et al*. (1997). The sealing wax layer prevented the contact of substrate and nutrient solution. Nutrient solution was renewed every second day. Seedlings germinated after 4 days. When the roots of all plants roots had penetrated through the wax layer into the nutrient solution its pH was lowered incrementally to 4.3. Eight days after sowing concentrations of 0 µM or 25 µM Al were added to the nutrient solution. During the experiment the pH was controlled and adjusted using 0.1 M HCl and 0.1 M KOH when necessary. Monomeric Al was determined using the PCV method (Kerven *et al*., 1989; Menzies *et al*., 1992). After 12 h of Al treatment root tips of 5 mm length from the longest main axis, defined as primary root, were cut and stored in Eppendorf cups containing ethanol (96 %) until analysis for callose. Callose formation was determined as described by Köhle *et al*. (1985) and modified by Horst *et al*. (1997) and is expressed as pachyman equivalents (PE). Following the evaluation in nutrient solution seedlings were transplanted: Seedlings without Al supply were transplanted into non-toxic soil at Palmira, those treated with Al to an acid Al-toxic soil in S. Quilichao. Seedlings were planted in a distance of 16 cm within and 75 cm between rows. Solid fertiliser was applied twice, directly after transplanting and four weeks later. In total, 110 kg ha^{-1} N as ammoniumsulfate and 30 kg ha^{-1} K_2O as KCl were applied at both sites. 50 kg ha^{-1} P_2O_5 as Triple superphosphate were applied only in Palmira. Plants were irrigated regularly. Lorsban (0.25%, Chlorpyrifos, DowElanco) was applied twice as arial spray, 10 and 30 days after planting. A third application was sprayed directly to the cobs 10 days after the end of female flowering, when the stigma had dried. Hostathion (Triazophos, AgrEvo) was applied in granules repeatedly after first appearance of spodoptera larvae (*Heliothis zea*). In S. Quilichao plants were shaded for the first 10 days after transplanting. Plants were harvested 130 days after sowing at Palmira and at 120 days at S. Quilichao.

Tab.1: Site characteristics of Palmira and S. Quilichao.

Location	Org. matter	P	pH	Al	Ca	Mg	K	Al-satur.
	[%]	[ppm]		[meq (100 g soil)$^{-1}$]				[%]
Palmira	2.7	62.1	7.4	-	22.0	9.8	0.8	-
S.Quilichao	3.9	9.8	4.7	2.3	1.5	0.6	0.2	50

Plant height, the distance between ground level and last fully developed leaf node of each plant, was determined at 4, 6, 8, 10 and 16 weeks after sowing. Female as well as male flowering was recorded at 50 % silking and 50 % anthesis for each plant. 50 % silking was defined as the stage when stigma had a length of approx. 2-3 cm, 50 % anthesis was recorded when 50% of pollen containers were open. During that stage plants were checked every second day. Ear height of each plant was determined as the distance between ground level and the insertion of the cob. At grain maturity, shoots were cut at the soil surface and dried at 60°C for 5 days for above-ground dry matter determination. Grain yield was determined on single cobs when at least one kernel was developed. Grain yield loss due to spodoptera infestation was visually estimated in percentage of the whole cob and its grain yield adjusted by this factor.

Results

At 25 µM Al supply, the concentration of monomeric Al was 20±0.76 µM. Al-induced callose formation in root tips ranged from 0.55 to 1.25 µg PE (0.5 cm root segment)$^{-1}$. Roots of cultivar 3x4 in the nutrient solution were curled and thickened and only low callose formation was found. This cultivar was therefore excluded from the correlation analysis. Nearly all transplanted cultivars survived the transplanting. At harvest, from 94 % of transplanted plants above-ground dry matter could be determined.

Fig. 1: Steps of the experimental procedure.
A. Maize seedlings sown in paper beakers in nutrient solution.
B. Transverse section of a pot containing seedling.
C. Transplanting of individual seedlings.
D. Plant stand at flowering on the Al-toxic site, S. Quilichao.

On the acid Al-toxic soil plant height, ear height, above-ground dry matter and grain yield were significantly reduced. Time to 50 % silking was retarded (Tab. 2). Consequently, the interval between 50 % male and 50 % female flowering was extended as shown by anthesis silking interval (ASI). Grain yield on the Al-toxic site was not correlated with grain yield on the non-toxic site.

Tab. 2: Means of agronomic traits for 9 transplanted maize cultivars on an Al-toxic and non-toxic soil.

Variable	non-toxic soil Palmira	Al-toxic soil S. Quilichao
Plant height 4 WAS [cm]	13.5±4.9 a	8.7±2.7 b
Plant height 6 WAS [cm]	45.4±25.3 a	14.2±5.3 b
Plant height 8 WAS [cm]	126.8±27.5 a	45.8±25.7 b
Plant height 10 WAS [cm]	187.8±36.0 a	102.5±28.1 b
Plant height 16 WAS [cm]	188.4±38.2 a	110.7±30.1 b
50% silking [d]	61.8±4.8 a	72.2±8.7 b
50% anthesis [d]	61.8±4.4 a	70.2±8.6 b
ASI [d]	0±1.8 a	2.3±2.7 b
Ear height [cm]	100.3±30.4 a	40.2±20.4 b
Ears per plant	0.89±0.2 a	0.88±0.1 a
Above-ground dry matter [g]	301.5±141.3 a	95.6±60.5 b
Grain yield [g]	110.3±71.5 a	39.8±30.3 b

Comparison of means for each variable between the Al-toxic and non-toxic site. Same letters indicate no differences between means according to Tukey-test at p = 5%, n = 12.

Tab. 3: Correlation of phenotypical traits for 9 maize cultivars. Above diagonal for Al-toxic soil, below for non-toxic soil.

	Plant height	Ear height	Total above-ground dry matter	Grain yield	ASI
Plant height		0.96 ***	0.89 **	0.83 **	-0.66
Ear height	0.71 *		0.81 **	0.70 *	-0.70
Total above-ground dm	0.79 *	0.51		0.96 ***	-0.67
Grain yield	0.60	0.20	0.89 ***		-0.77 *
ASI	-0.01	0.12	-0.41	-0.73	

Significant at * <0.05, **<0.01, ***<0.001 probability level.

Similar correlations between agronomic traits were found at both sites. Generally, relationships were closer at the acid Al-toxic site S. Quilichao (Tab. 3). At the Al-toxic site, ASI was closely negatively correlated with grain yield, plant height, ear height and total dry matter. Additionally, ear height was significantly negatively correlated with grain yield. Since effects of Al toxicity on growth are generally expressed relative to a control where no Al is applied (Blum, 1988; Howeler and Cadavid, 1976) relative values (parameter at Al-toxic site in percentage of non-toxic site) were calculated for plant height, ear height, above-ground dry matter as well as grain yield and correlated to Al-induced callose formation in nutrient solution.

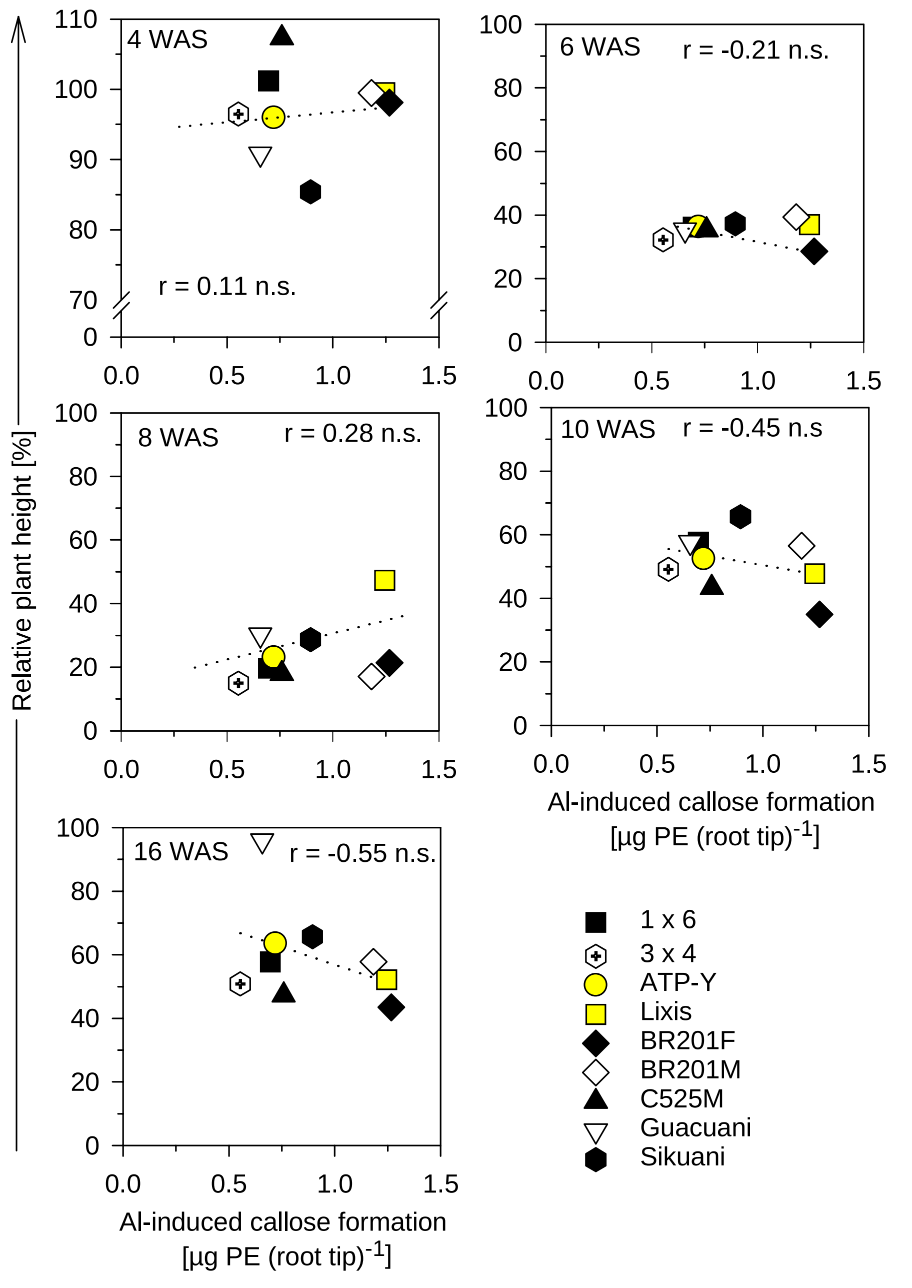

Fig. 2: Al-induced callose formation related to relative plant height at different stages of plant development. Cross 3x4 excluded from correlation analysis.

A severe reduction in plant height was firstly observed at 6 WAS. Al-induced callose formation in nutrient solution was not correlated to relative plant height at early stages (Fig. 2). Only at 10 WAS and at harvest (16 WAS) a negative correlation was found. However, significant correlations were found between Al-induced callose formation and relative grain yield as well as total above-ground dry matter (Fig. 3).

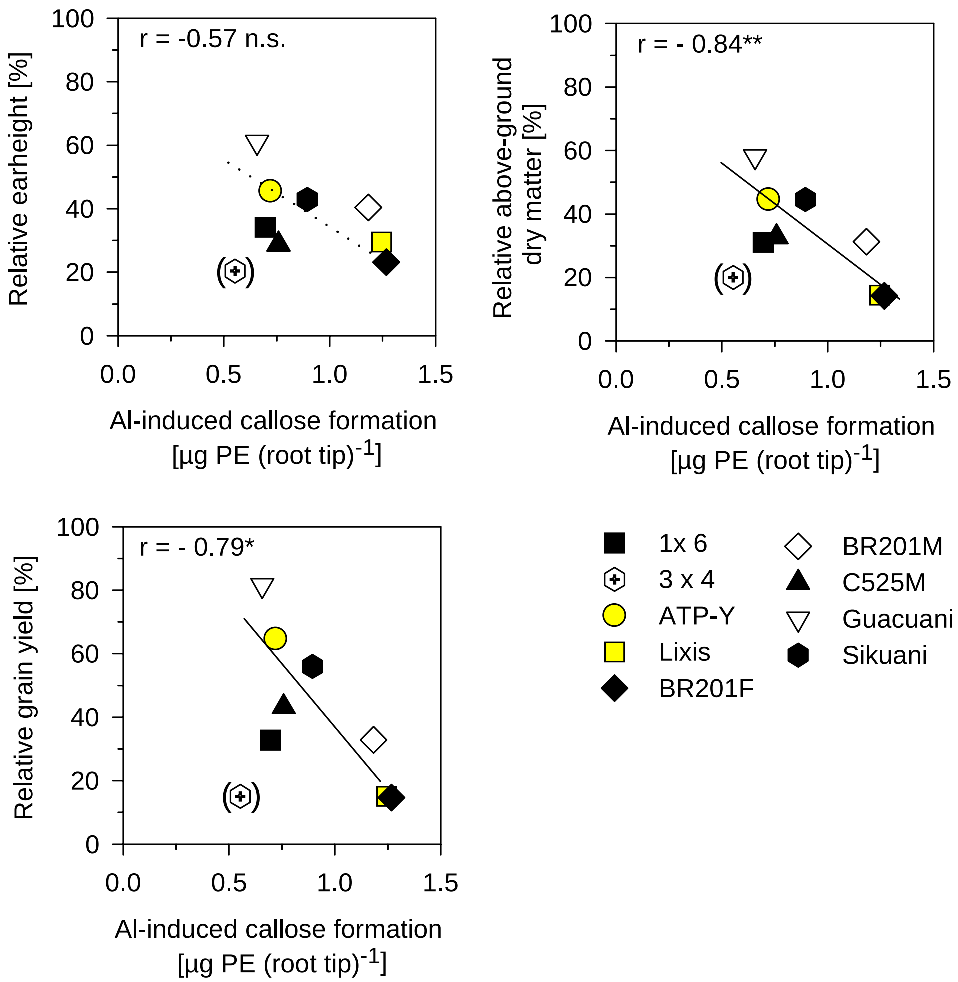

Fig. 3: Al-induced callose formation related to relative ear height, relative above-ground dry matter and relative grain yield of transplanted maize cultivars. Cross 3x4 excluded from correlation analysis.

Discussion

The results presented clearly demonstrate that individual plants can be successfully transplanted from nutrient solution into the field nearly without losses. Furthermore, this method allows a direct comparison of individual plants with regards to Al-resistance of the seedling and subsequent growth and yield on an Al-toxic field site.

Delay of silking, lower ear heights, as well as reduced yields with an increased level of acidity stress have been reported earlier (Duque-Vargas *et al*., 1994; Granados *et al*., 1993; Pandey *et al*., 1994; Salazar *et al*., 1997). Delay of flowering and thus lengthening of the life cycle as well as a higher ASI might have contributed to reduced plant fertility: Grain yield in the acid soil environment showed highly significant negative correlation with ASI (r = -0.77**). Positive correlations with plant height (r = 0.83***) and ear height (r = 0.69***) were consistent with those reported by several authors (Duque-Vargas *et al*., 1994; Granados *et al*., 1993; Salazar *et al*., 1997).

Al-induced callose differed significantly between cultivars. In agreement with other reports, Al-induced callose formation classified cultivar Lixis as Al-sensitive and ATP-Y as Al-resistant (Horst *et al*., 1997). However, low callose formation of the cross 3x4 accompanied by unusual root growth in nutrient solution was in contrast to its poor performance on acid soil in this and previous studies (De Leon, 1998; personal communication). The curling of roots might be associated with decreased capacity for callose synthesis from callose synthase which is assumed to be associated with cellulose synthase in the plasma membrane (Delmer, 1987). However, it might be also the case that for this cross Al-toxicity on the acid soil was not the most important growth limiting factor. Unusual elongation and/ or low callose formation might be due to the inbreeding process which the parental inbred lines have passed.

Aluminium-induced callose formation was not correlated to plant height at early stages after transplanting to the field. Marked differences in plant development between the Al-toxic and non-toxic sites were observed not earlier than 6 WAS. This might be due to the fact that early Al-toxic effects following germination might have been excluded by sowing in peat substrate and transplanting at 2 WAS. Also, since Al primarily affects the root tips, effects on shoot development could be expressed only at later stages of growth when the root size increasingly gains importance for sustaining shoot growth through supply of water, nutrients and phytohormones. Consequently, Al injury is mostly expressed when shoot elongation is at its maximum rate following 6 WAS. Therefore, genotypic differences will be expressed only at later growth stages when exploitation of the subsoil becomes increasingly important. This suggestion is confirmed by the significant correlations between callose formation and relative grain yield (r = - 0.79*) as well as relative above-ground dry matter (r = - 0.84**) at maturity. Negative relations between Al-induced callose formation in nutrient solution and grain yield on an acid Al-toxic soil were also reported for *Triticale* (Oettler *et al*., 1997). Results presented, indicate that the developed screening system can contribute to the acceleration of the breeding process for adaptation of maize to acid Al-toxic soils.

References

Blum A, ed. (1988). "Plant breeding for stress environments", pp 1-213. CRC Press, Boca Raton, Florida.

Delmer DP (1987) Cellulose biosynthesis. *Ann Rev Plant Physiol* 38: 259-290.

Duque-Vargas J, Pandey S, Granados G, Ceballos H, and Knapp E (1994) Inheritance of tolerance to soil acidity in tropical maize. Crop Sci 34: 50-54.

Foy CD (1976) General principles involved in screening plants for aluminum and manganese tolerance. In: Wright MJ and Ferrari AS (eds) Plant adaptation to mineral stress in problem soils, pp 256-267. Cornell University Agricultural Experimental Station.

Foy CD, Carter Jr. TE, Duke JA, and Devine TE (1993) Correlation of shoot and root growth and its role in selecting for aluminum tolerance in soybean. J Plant Nutr 16: 305-325.

Granados G, Pandey S, and Ceballos H (1993) Response to selection for tolerance to acid soils in a tropical maize population. Crop Sci 33: 936-940.

Hanson WD and Kamprath EJ (1979) Selection for aluminium tolerance in soybeans based on seedling-root growth. Agron J 71: 581-586.

Horst WJ, Püschel AK and Schmohl N (1997). Induction of callose formation is a sensitive marker for genotypic aluminium sensitivity in maize. Plant Soil 192: 23-30.

Howeler RH and Cadavid LF (1976) Screening of rice cultivars for tolerance to Al-toxicity in nutrient solutions as compared with a field screening method. Agron J 68: 551-555.

Kerven GL, Edwards DG, Asher CJ, Hallman PS, and Kokot S (1989) Aluminium determination in soil solution. II. Short-term colorimetric procedures for the measurement of inorganic monomeric aluminium in the presence of organic ligands. Aust J Soil Res 27: 91-102.

Köhle H, Jeblick W, Poten F, Blaschek W, and Kauss H (1985) Chitosan-elicited callose synthesis in soybean cells as a Ca^{2+} -dependent process. Plant Physiol 77: 544-551.

Menzies, NW, Kerven, GL, and Edwards, DG (1992) Determination of total soluble aluminum in soil solution using pyrocatechol violet, lanthanum and iron to discriminate against micro-particulates and organic ligands. Commun. Soil Sci Plant Anal 23: 2525-2545.

Oettler G, Geiger R, Wiethölter S, Gaus CS, and Hesemann, CU (1997) Methodische und genetische Untersuchungen zur Aluminium-toleranz von Triticale. Vortr Pflanzenzücht 38: 41-56.

Pandey S, Ceballos H, Magnavaca R, Bahia Filho AFC., Duque-Vargas, J, and Vinasco LE (1994) Genetics of tolerance to soil acidity in tropical maize. Crop Sci 34: 1511-1514.

Salazar FS, Pandey S, Narro L, Perez JC, Ceballos H, Parentoni SN, and Bahia Filho AFC (1997) Diallel analysis of acid tolerant and intolerant tropical maize populations. Crop Sci 37: 157-1462.

Sapra VT, Mebrahtu T, and Mugwira LW (1982) Soybean germplasm and cultivar aluminum tolerance in nutrient solution and Bladen clay loam soil. Agron J 74: 687-690.

Uexküll von HR and Mutert E (1995) Global extent, development and economic impact of acid soils. Plant Soil 171: 1-15.