Screening maize (*Zea mays* L.) for aluminium resistance – Contribution to the selection for adaptation to acid soils

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ABSTRACT

Aluminium toxicity in combination with soil acidity represents a major growth-limiting factor for plants in many parts of the world. Nevertheless, between 8 and 26 million hectares of maize are planted on acid soils. Breeding of maize cultivars with tolerance to soil acidity has been suggested as an environmentally compatible, relatively inexpensive, and permanent means of increasing maize yields on such soils. Progress in breeding could be accelerated if quick screening techniques could be used during the selection process. Screening in hydroponics is an attractive approach for the breeding of Al resistance. Aluminium-induced callose formation might be used as a physiological marker for Al-induced injury. However, solution culture experiments cannot be extrapolated to the field condition without validation. Therefore, it is necessary to combine field-screening with laboratory-screening techniques based on physiological parameters of resistance. The objectives of this work were aimed to study the following questions:

(i) Do limitations in screening for Al resistance exist by homogeneous application of Al to the root system ?

(ii) Can the screening be simplified by the use of excised root tips instead of the whole plant ?

(iii) How can maize inbred lines be assessed in Al resistance using callose formation and root elongation ?

(iv) Can Al-induced callose formation predict adaptation to an acid Al-toxic soil ?

(i) Using a split-root system 9-day-old seedlings of two maize cultivars differing in Al resistance were exposed to a spatially varied Al (0, 10 µM) and P supply (1, 25 µM). An overall low P concentration (1 µM P) in the nutrient solution led to an increase in root elongation and root dry matter as well as a decrease in shoot P content. Aluminium-induced inhibition of root elongation was higher for the Al-sensitive cultivar Lixis than for the Al-resistant cultivar BR201M. Root tips exposed to Al expressed a higher Al content and callose formation than those without Al exposure. Heterogeneous Al application led to a compensatory growth reaction for

the Al-sensitive cultivar Lixis only with enhanced root growth in the Al-free compartment. In contrast, the Al-resistant cultivar BR201M did neither exhibit compensatory growth nor a reaction of Al avoidance.
The findings presented indicate that an assessment of Al resistance based on root elongation does not depend whether Al is supplied homogeneously to the root system or heterogeneously to parts of the root system.

(ii) In subsequent experiments root tips of intact plants and excised root tips of 10 maize **cultivars** were exposed to 25 µM Al in nutrient solution. The cultivars could be classified concerning their Al resistance more reliably by Al-induced callose formation than by Al-induced inhibition of root elongation. Significant correlations between Al-induced callose formation, inhibition of root elongation ($r^2 = 0.80$***) and Al contents ($r^2 = 0.64$**) in root tips were found for intact plants only. Al-induced callose formation could be confirmed as a suitable indicator of Al sensitivity in intact maize seedlings. In contrast, no such relationships existed for excised root tips. Furthermore, Al-induced callose formation in excised root tips did neither correspond to inhibition of root elongation nor Al-induced callose formation of intact plants although in earlier studies cultivar-specific differences in Al resistance were found on the cellular and protoplast level. An additional glucose supply to excised root tips enhanced Al-induced callose formation and root tip elongation but did not improve characterisation of cultivars in Al resistance. The results presented suggest that certain Al exclusion mechanisms expressed in root apices of intact plants might be less active in excised root tips. Therefore, the use of excised root tips appears not possible in characterising maize germplasm in Al resistance.

(iii) **Inbred lines** of maize were screened for Al resistance using root elongation as assessed by prestaining and Al-induced callose formation after 48 h. Lines could be separated into Al-resistant and Al-sensitive on the basis of root elongation much better than by callose formation. The reason for this unexpected result was studied. Aluminium-induced callose formation followed a cultivar-specific kinetic, reaching a peak after 6 - 12 h of Al exposure. Thereafter, callose contents decreased in some of the maize inbred lines until only marginal amounts were found after 24 h. In contrast, in one inbred line Al-induced callose formation continued to increase up to 48 h. Al-induced callose formation and inhibition of root elongation

were positively correlated after 12 h (r^2 = 0.49*) as well as after 24 h (r^2 = 0.55*) of Al treatment. Differences in potential of callose formation were of minor importance than callose disappearance, possibly because of degradation. Results presented emphasise the importance to select the best treatment duration for the differentiation of maize inbred lines.

(iv) In Colombia, maize cultivars and inbred lines were analysed for Al-induced callose formation of root tips in nutrient solution prior to transplantation to an acid or non-acid soil. Plant growth as well as grain yield were significantly reduced on the acid Al-toxic site as compared to the non-toxic site. Grain yield of lines as well as of cultivars showed highly significant negative correlations with anthesis silking interval (ASI) (r_{lines} = -0,68***, $r_{cultivars}$ = -0.77**), positive correlations with plant height (r_{lines} = 0.63***, $r_{cultivars}$ = 0.83***) and ear height (r_{lines} = 0.70*, $r_{cultivars}$ = 0.69***) in the acid soil environment. For the cultivars, Al-induced callose formation could be significantly related to relative yield ($r_{cultivars}$ = -0.79*) and relative above-ground dry matter ($r_{cultivars}$ = -0.84**) at maturity. However, for inbred lines no such correlation was found. This may be attributed to a significant influence of the kernel weight on plant height at 2 weeks after sowing which in addition significantly limited grain yield on both sites. The results demonstrate that a non-acid control is needed to evaluate whether inherent growth limiting parameters exist which might be seen only on the non-acid site or in an comparison between the sites. For inbred lines the parameter plant height might be a useful trait for the estimation of grain yield of lines.

The results presented demonstrate that maize seedlings can be transplanted from nutrient solution into the soil for evaluation of plant performance. Transplanting might be also used for other root traits, which can be more easily assessed in nutrient solution. Evaluation of Al-induced callose formation during 12 h in nutrient solution is effective for a preliminary screening of maize cultivars. This system should be especially useful ins studies of inheritance of Al resistance where seed supply is small and where it is desirable to save individual plants after screening for transplanting into pot or field for further observations and/ or seed multiplication.

Keywords: Aluminium, Screening, *Zea mays*

ABBREVATIONS

Al	aluminium
ASI	anthesis silking interval
AAS	atomic absorption spectrometer
asl	above sea level
°C	degree Celsius
CIAT	Centro Internacional de Agricultura Tropical
CIMMYT	Centro Internacional de Mejorarmiento de Maize y Trigo
cm	centimeter
cv	cultivar
d	day
df	degree of reedom
Fig	figure
FW	fresh weight
Geno	genotype
GF	graphite furnace
Glc	glucose
h	hour
min	minute
MSD	minimum significant difference
MSQ	mean squares
n	number of observations
n.d.	not detected
n.s.	non significant
P	probability level
PE	pachyman equivalents
r	correlation coefficient
rel	relative
rpm	rounds per minute
SD	standard deviation
Tab	table

Transpl	transplanting
TTC	triphenyl-tetrazolium chloride
upw	ultra pure water
WAS	weeks after sowing
w/v	weight/ volume
x g	gravity
λ	wavelength

INTRODUCTION

Globally about 30 % of the ice-free land is constrained for crop production by soil acidity (Sanchez and Salinas, 1981; von Uexküll and Mutert, 1995; Eswaran *et al.*, 1997). Going along with decreasing soil pH (< 5.2) the extractable soil Al rises (Nair and Prenzel, 1978; Fox *et al.*, 1985, 1991; Johnson and McBride, 1991) leading to Al toxicity, a major growth-limiting factor for plants (Foy, 1974; Foy *et al.*, 1978; Malavolta *et al.*, 1981; Adams, 1984; Kamprath, 1984; Naidu *et al.*, 1991; Spehar, 1994). Nevertheless, between 8 (Pandey and Gardner, 1992) and 26 (von Uexküll and Mutert, 1995) million hectares of maize, the third most important cereal grown in the world, are planted on acid soils, especially in South and Central America (Baligar *et al.*, 1997). The investments for appropriate technology to circumvent the problem of soil acidity, particularly subsoil acidity, are immense (Farina, 1997; Foy and da Silva, 1991). In the poorer countries of the world, even though technology is available (Fox *et al.*, 1991; Quaggio *et al.*, 1991; Carvalho and van Raij, 1997), it is not implemented due to the lack of available funds and means to transfer technology to the resource-poor farmer (van Raij, 1991; McLay and Ritchie, 1993; Eswaran *et al.*, 1997). Therefore, selection for avoidance of ion toxicities (Al, Mn) appears to be of great potential in adapting plants to an unfavourable acid soil environment (Clark and Duncan, 1991; Baligar and Fageria, 1997). The development of maize cultivars with tolerance to soil acidity has been suggested as an environmentally compatible, relatively inexpensive, and permanent means of increasing maize yields on such soils (Foy, 1976; Bennet *et al.*, 1986; Pandey and Gardner, 1992). In maize, genetic variation for resistance to soil acidity (Granados *et al.*, 1993) and Al (Rhue *et al.*, 1978; Magnavaca et al. 1987; Furlani and Furlani, 1991) has been reported. Both traits are heritable (Rhue *et al.*, 1978; Miranda *et al.*, 1984; Kasim *et al.*, 1990; Lima *et al.*, 1992). Hence, substantial progress can be made in the breeding of maize cultivars with improved resistance to soil acidity (Ceballos *et al.*, 1995).

Breeding programmes include field testing of a large number of varieties. The tests are laborious, time consuming and need to be repeated to minimise effects of uncontrolled environmental factors (Spehar, 1994). Because Al resistance ratings are more repeatable and less expensive in hydroponics as compared with other techniques, hydroponics screening is an attractive approach for the breeding of Al tol-

erance (Garland Campbell and Carter, 1990; Blamey *et al.*, 1991). Laboratory screening methods are normally more rapid and less expensive than field screening. They also allow assessment of more germplasm in a given period of time, reduce the amount of germplasm to be tested in the field, and are season-independent (Duncan *et al.*, 1983). Furthermore, the nutrient-solution technique is useful to evaluate the isolated effect of Al in the plant, in contrast to field evaluations where a complex of factors related to nutrient and water availability, as well as climate effects, may interfere with the plant responses to Al stress (Bahia Filho *et al.*, 1997). Nevertheless, it must be kept in mind that solution culture experiments are synthetic and results cannot be extrapolated to the field without verification (Edmeades *et al.*, 1995). Therefore, it is necessary to combine field-screening with laboratory-screening techniques based on physiological parameters of toxicity or resistance (Scott and Fisher, 1993).

One prerequisite for screening in Al-toxic solution is the adaptation of plant roots to low pH. High H^+-concentration inhibited elongation of several plant species including maize (Foy, 1984; Johnson and Wilkinson, 1992; Yan *et al.*, 1992; Rufty *et al.*, 1995). As plant roots of maize are able to adapt to low pH (Yan *et al.*, 1992), an incrementally decrease of the solution pH is necessary. Furthermore, studies on Al toxicity should be conducted in test solutions of low ionic strength that approximate the soil solution's composition, ionic strength and Al activity (Pavan and Bingham, 1982; Blamey *et al.*, 1991; Edmeades *et al.*, 1995).

In nutrient solution plant growth is better correlated with solution Al activity than nominal Al concentration (Blamey *et al.*, 1983; Alva *et al.*, 1986a, b; Baligar *et al.*, 1991; Wheeler *et al.*, 1992a). However, the identity of the toxic species (monomeric vs. polymeric Al) remains a matter of speculation (Kinraide, 1991; Kinraide and Ryan, 1991). The polymeric cation might be more phyto-toxic than was reported for the monomeric form (Blamey *et al.*, 1983; Alva *et al.*, 1986b). It was even reported to compensate differences in Al resistance between maize cultivars (Comin *et al.,* 1999). Work of Parker *et al.* (1988, 1989) and (Kinraide, 1991) suggested that Al_{13} is likely to be present or even dominating in all of the hydroxy Al systems and is probably the source of most of the toxic effects of Al in these systems. For the present, it may be reasonable to conclude that Al_{13} and Al^{3+} are rhizotoxic, even though the evidence for the latter is confined to wheat (Kinraide, 1991).

Aluminium-caused morphological abnormalities of the root system might be explained by an inhibitory effect on either cell division or cell extension (Clarkson, 1965; Morimura *et al.*, 1978; Horst and Klotz, 1990; Horst et al., 1983). The resulting Al toxicity symptoms are the inhibition of root elongation (Horst, 1987; Horst and Klotz, 1990; Horst *et al.*, 1990; Foy, 1988; Zhang and Jessop, 1998), root thickening and inhibition of lateral roots (Horst, 1987; Blancaflor *et al.*, 1998; Larsen *et al.*, 1997) and root hairs (Hecht-Buchholz *et al.*, 1990). The root system as a whole appears coralloid, with many small stubby and brittle lateral roots but no fine branching (Foy, 1974; Furlani and Clark, 1981; Pavan and Bingham, 1982). Furthermore, destruction of the root epidermal and cortical cells results in disintegration of the outer root surface area (Hecht-Buchholz and Foy, 1981; Hecht-Buchholz *et al.*, 1990).

While reduction in root growth is a common symptom of Al toxicity, it takes a number of hours or days to become detectable (Kinraide *et al.*, 1985; Wissemeier *et al.*, 1987, 1992; Ownby and Popham, 1989; Godbold and Kettner, 1991; Horst *et al.*, 1992; Tice *et al.*, 1992; Ryan *et al.*, 1993). However, the first reduction in elongation might not be sensitive enough to distinguish a large range of cultivars in their sensitivity to Al. A minimum of 24 h of Al exposure or even more seemed to be required (Horst, 1987; Horst *et al.*, 1990, 1997; Ahlrichs *et al.*, 1990).

Nonetheless, reduced growth must result from physiological or biochemical changes that should be detectable within minutes of exposure. Such changes could provide a more sensitive indicator for Al toxicity. Formation of callose is a common plant response to a variety of stresses, including mechanical, biophysical, chemical, and biological injury (Fincher and Stone, 1981). The callose synthesising enzyme 1,3-ß-glucan-synthase is present in the plasma membrane of all living plant tissue (Moore *et al.*, 1972; Anderson and Ray, 1978; Fincher and Stone, 1981, Jounneau *et al.*, 1991).

Increased synthesis of callose has been observed upon exposure to excess of a variety of elements, including B (McNairn and Currier, 1965), Co, Ni, Zn (Peterson and Rauser, 1979) and Mn (Wissemeier *et al.*, 1992, 1993; Wissemeier and Horst, 1992). Aluminium-induced synthesis of callose has also been documented in protoplasts of *Avena sativa* (Schaeffer and Walton, 1990), root tips of *Picea abies* (Jorns *et al.*, 1991; Wissemeier *et al.*, 1998) and *Glycine max* (Wissemeier *et al.*, 1987; Horst *et al.*, 1992; Wissemeier and Horst, 1995), and leaves and roots of

Triticum aestivum and *Zea mays* (Llugany *et al.*, 1994; Schreiner *et al.,* 1994; Horst *et al.*, 1997). Aluminium-induced callose formation can be detected as early as after 30-90 min (Wissemeier *et al.,* 1987; Wissemeier and Horst, 1995) in root tips of Al-exposed plants. Wissemeier *et al.* (1992) suggested that callose formation might be used as a physiological marker for stress in plants and could possibly be developed into a sensitive marker for Al-induced injury (Horst *et al.*, 1997). It is not to be seen as a mechanism of Al resistance, with which is dealt elsewhere (Marschner, 1991; Taylor, 1991, 1997; Horst, 1995; Kochian, 1995; Keltjens, 1997). The objectives of this work were to answer the following questions:

Do limitations in screening for Al resistance of maize exist by using homogeneous distribution of Al to the roots ? (Chapter I)
Can the screening for Al resistance be simplified by the use of excised root tips instead of the whole plant ? (Chapter II)
Can maize inbred lines be assessed in Al resistance using callose formation and root elongation ? (Chapter III)
Can Al-induced callose formation predict adaptation to an acid soil ? (Chapter IV)

1 Aluminium avoidance - Limitation of the screening of maize cultivars (*Zea mays* L.) for Al resistance in solution culture?

Abstract

This study was conducted to examine the effects of a locally varied aluminium and phosphate exposure on growth of maize seedlings (*Zea mays* L.) under controlled conditions in a nutrient solution experiment. Nine day-old seedlings of two maize cultivars differing in Al resistance were exposed to a spatially varied Al (0, 10 µM) and P supply (1, 25 µM) for 4 days using a split-root system. An overall low P concentration (1 µM P) in the nutrient solution led to an increase in root elongation and root dry matter as well as a decrease in shoot P content. Supplying 25 µM P to only one side of the root system also resulted in a lower shoot P content.

Aluminium supply led to a decrease in root elongation and root dry matter. The Al-sensitive cultivar Lixis was more affected by Al exposure than the Al-resistant cultivar BR201M. Callose formation in root tips increased in response to Al by a factor of 2.8 and 12 in BR201M and Lixis, respectively. A compensatory growth reaction was found for the Al-sensitive cultivar Lixis only: Enhanced root growth in the Al-free compartment led to a total root dry matter equal or higher than under homogeneous Al-free conditions at the same level of P supply. The Al-resistant cultivar BR201M did neither exhibit compensatory growth nor a reaction of Al avoidance.

The results presented indicate that assessment of Al resistance based on root elongation was not changed by applying Al heterogeneously to the roots. Screening for Al resistance in maize seedlings is therefore not limited by using homogeneous solutions. Compensatory growth might be more important for an Al-sensitive than an Al-resistant cultivar and might explain why some Al-sensitive cultivars could possibly perform better in the field than in nutrient solution.

Introduction

Plant roots are characterised by very high adaptability. Their growth and development involves complex interactions with both, the soil environment and the shoot (Marschner, 1996). Roots are able to respond to the heterogeneous soil environment by improving root growth in more favourable pockets (Russell, 1973; Fransen *et al.*, 1999; Kerley *et al.*, 2000), described as plastic response of the root system (Feldman, 1984; Fitter, 1994, 1996) or adaptation in the uptake of a specific nutrient (de Jager, 1984; Jungk *et al.*, 1990; Sattelmacher and Thoms, 1995; Lainé *et al.*, 1995). Though this reaction is well established for several plant species it might not be true for others (Fitter, 1994; Wijesinghe and Hutchings, 1997). Additionally, nutrients like K, Mg, S, Zn, or Mn may not induce such changes (Drew, 1975; de Jager, 1982; Nable and Loneragan, 1984; Webb and Loneragan, 1990; Scott and Robson, 1991; Brouder and Cassman, 1994). Cereals were shown to be able to adapt to a localised supply of N and P by promoting root growth, especially of lateral roots, in enriched spots and layers (Drew *et al.*, 1973; Drew and Saker, 1975; Anghioni and Barber, 1988; Granato and Raper, 1989). In general, those roots provided with a local supply of nutrients (N, P) show an enhanced growth compared to sufficiently supplied controls. In contrast, the remaining root system tends to grow less (Robinson, 1994, 1996). The elongation of the main axis remained unaffected (Granato and Raper, 1989). Such a change in growth distribution is described as compensatory or correlative growth (Crossett *et al.*, 1975; Drew and Saker, 1975; Scott Russell, 1977; Brouwer, 1981) as well as correlative inhibition (Gersani and Sachs, 1992). This morphological adaptation of roots might not only be true for exploiting nutrient or water-rich soil zones but also for coping with adverse environmental conditions. Aeration of solution, temperature changes and trimming of roots led to compensatory growth in maize (Crossett *et al.*, 1975; Brouwer, 1981) and Cu toxicity in wheat (Adalsteinsson, 1994). In contrast, salinity though decreasing root as well as shoot growth of citrus (Zekri and Parsons, 1990), or high concentrations of NH_4^+ supply in maize (Schortemeyer and Feil, 1996), did not induce compensatory growth when parts of the root system were affected.

Hairiah *et al.* (1992) reported a phenomenon that they called Al avoidance for velvet bean (*Mucuna pruriens*), grown in hydroponics with a split-root system. It was

different from other mechanisms of adaptation to Al toxicity (Miyasaka *et al.*, 1989; Marschner, 1991; Keltjens, 1997). According to Hairiah *et al.* (1992) Al avoidance described for an Al-resistant *Mucuna* species preferential development of those roots not exposed to Al, accompanied by an enhanced growth inhibition of roots in contact with Al. The phenomenon occurred although *Mucuna* roots tolerated Al under a homogeneous Al exposure (Hairiah *et al.*, 1990). The reaction in the hydroponic split-root system was similar to the plant response to acid subsoil in the field, overlaid by non Al-toxic surface soil: Roots hardly penetrated into the acid subsoil when the less acid topsoil was present. But when the topsoil was removed, roots grew unimpeded into the subsoil (Hairiah *et al.*, 1991). Further split-root experiments revealed that Al avoidance was related to a local response of the plant to differences in P supply. With an increasing P supply no Al avoidance could be observed (Hairiah *et al.*, 1992). In addition, the form of N applied also affected the occurrence of the Al avoidance reaction. Hairiah *et al.* (1994) could not show any Al avoidance when applying NO_3NH_4 instead of NO_3^- only.

Since compensatory growth due to a localised supply of nutrients was found in many plant species (Robinson, 1994), the question arose whether a similar Al avoidance reaction described for *Mucuna* could also be found in maize seedlings. Young seedlings are generally screened for Al resistance with homogeneous Al supply to the entire root system (Foy *et al.*, 1967; Howeler and Cadavid, 1976; Fageria and Baligar, 1993; Horst *et al.*, 1997). A possible Al avoidance mechanism might contribute to misleading results in screening for Al resistance.

The objective of this study was to clarify (i) whether compensatory root growth occurred in maize seedlings differing in Al resistance in a split-root system under varying Al and P supplies, (ii) whether Al-resistant maize cultivars respond to inhomogeneous Al supply to the root by Al avoidance as *Mucuna* modified by P supply.

Materials and methods

Plant material and growth conditions

Seedlings of maize (*Zea mays* L.) were grown in a growth chamber under controlled environmental conditions of a 16/ 8 h day/ night cycle, 27/ 25 °C day/ night temperature, 75 ± 5 % relative air humidity and a photon flux density of 230 µmol m^{-2} s^{-1} photosynthetic active radiation (Sylvania Cool White, 195 W, Philips, Germany) as measured in mid plant height. Two maize cultivars differing in Al resistance were compared in two separate experiments in 1996 and 1997:

Cultivar BR201M was classified as Al-resistant and Lixis as Al-sensitive (Horst *et al.*, 1997). Seeds were germinated between sheets of filter paper, soaked with 1 mM $CaSO_4$ solution in an upright position (sandwich technique). The tap root was excised as well as later occurring adventitious roots, to allow better growth of mesocotyl roots. According to Brouwer (1981) the excision of adventitious roots upon appearance allowed unrestricted growth of the basal seminal roots. Furthermore, excision did not affect adequate nutrient supply to the shoot (Jeschke *et al.*, 1997). Three days after germination, seedlings were mounted onto the split-root system, using two plastic containers (7 l each) fixed together, with mesocotyl roots evenly spread to each side into a different container (Fig. 1.1). Lids prevented loss of solution and shaded roots from light. The nutrient solution was constantly aerated. Plants were cultivated for additional 5 days in nutrient solution containing 1 µM P, to minimise influence of the P reserves in the kernel (0.6 % in dry matter, data not shown). The composition of the nutrient solution was [in µM]: KNO_3 400, $CaSO_4$ 250, NH_4NO_3 200, $MgSO_4$ 100, Fe-EDDHA 20, H_3BO_3 8, $MnSO_4$ 1, $ZnSO_4$ 0.2, $CuSO_4$ 0.2, $(NH_4)_6Mo_7O_{24}$ 0.1, KH_2PO_4 1.

On the fifth day the pH was lowered incrementally to 4.3 over a period of 16 h. The pH was constantly observed during the experiment and, if necessary, adjusted using 0.1 M HCl or 0.1 M KOH. The nutrient solution was renewed every two days. The experiments lasted 4 days.

Experimental design

At the beginning of the experiment, the P supply in the compartments was adjusted to 1 µM and 25 µM, in order to produce P-deficient plants as well as P-sufficient plants. Two Al concentrations (0 vs. 10 µM) added as $AlCl_3$ from an Al atomic spectroscopy standard solution ($AlCl_3$ 6 H_2O, 1000 mg l^{-1}, Fluka, Germany) were used. Previous experiments had shown that for maize a concentration of 10 µM Al is sufficient to inhibit root elongation (Horst *et al.*, 1997). The spatial distribution of Al as well as P is shown in Fig. 1.1. Ten possible combinations were generated.

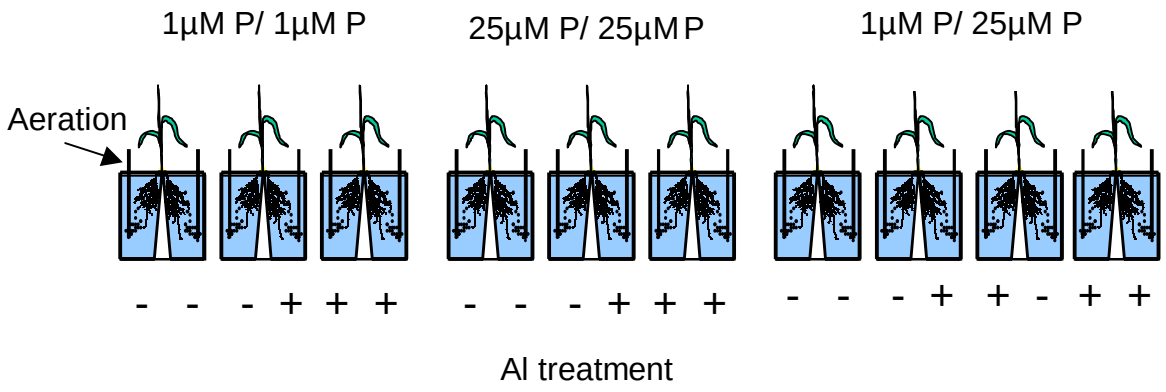

Fig. 1.1: Experimental set up (split-root containers). Combination of P (1 µM and 25 µM) and Al (0 µM and 10 µM) supply to nutrient solution (pH = 4.3) on either side of the root system.

Root-growth quantification

Root growth was determined by marking roots 3 cm behind the apex with a waterproof marker (Staedtler Lumocolor 317, Staedtler, Germany) which did not affect root growth as was verified in preliminary studies. After 4 days, root elongation was measured in each treatment, and 1 cm root tips were excised for callose (n = 9) or Al determination (n = 6). Root fresh weight was determined for both halves of the root system. Dry weights of shoots and roots were measured after drying at 65 °C until constant weights were reached.

Callose quantification

At harvest, plant roots were rinsed with deionised water, 1 cm root tips were excised using a razor blade, and fixed immediately in ethanol (96 %). For analysis, tips were rinsed with deionised water and transferred to Eppendorf cups containing 1 ml of 1 M NaOH solution and stored at -20 °C overnight. After defrosting, samples were homogenised with an ultrasonic device (Bandelin sonoplus HD70 with microtip MS 72 D, Bandelin Electronics, Berlin, Germany) for 45 s. To extract callose homogenised samples were heated at 80 °C in a water bath for 30 min. Thereafter, cooled samples were centrifuged at 10^3 x g for 15 min (Sorvall MC12V, Du Pont de Nemours & Co. Inc., Newton, Conn., USA). Callose was then determined according to Kauss (1989) and Köhle *et al.* (1985), using waterblue as stain (waterblue, Fluka, Seelze, Germany). The callose sirufluor complex was measured with a Hitachi F 2000 fluorescence spectrometer (Hitachi, Tokyo, Japan; excitation λ = 394 nm, emission λ = 484 nm, slit 10 nm, Voltage U = 700 V). Pachyman (Calbiochem, Deisenhofen, Germany) was used as calibration standard. Hence, callose content was expressed as pachyman equivalents (PE) per root tip.

Analysis of mineral elements

Root tips were analysed for Al, Mg, Ca, and K. One cm root tips were wet ashed in 2 ml ultrapure HNO_3 (65 %) in teflon centrifuge tubes (TFEP, Nalgene, Rochester, NY, USA) at 135 °C. The acid was evaporated and the residue dissolved in 2 ml diluted ultrapure HNO_3 (dilution 1:3 with ultrapure water, upw). For the determination of Al an aliquot of 200 µl was taken from this sample and diluted to a total volume of 2 ml with upw. Measurements were conducted using a graphite furnace AAS (Unicam 939 QZ, Analytical Technology, Cambridge, UK) at a wavelength of λ = 309.3 nm.

Magnesium, Ca, and K were detected by flame (Acetylene-air) with a Hitachi Z 8000, Zeeman-AAS (Hitachi Ltd., Tokyo, Japan) in the presence of Cs/La chloride according to Schinkel (1991) at a wavelength of λ = 766.5 nm (K), λ = 422.7 nm (Ca) and λ = 285.2 nm (Mg) in diluted aliquots of 100 µl of the wet-ashed samples.

The phosphate content in root tips was determined colorimetrically using an aliquot of 0.5 ml of the wet ashed sample by the method of Douglas and Field (1975). Samples were analysed with a photometer (Lambda 15, Perkin Elmer, Wellesley, MA, USA) at λ = 600 nm.

Shoots were analysed for P in a dry ashed sample (200 mg of ground dry matter) using the method of Gericke and Kurmies (1952). Measurements were conducted at a photometer (Lambda 15, Perkin Elmer, Wellesley, MA, USA) after 30 min using a wavelength of λ = 425 nm.

Analysis of nutrient solution

Monomeric Al in the nutrient solution was determined using the pyrocatecholviolet (PCV) method of Kerven *et al.* (1989), in samples of 3 ml nutrient solution with a photometer (Lambda 15, Perkin Elmer, Wellesley, MA, USA) at λ = 580 nm after 60 sec. Standard solutions were prepared in the range of 0 - 35 µM Al.

The P concentration in the nutrient solution was determined using the molybdenumblue-method according to Murphy and Riley (1962) with a sample volume of 8 ml. Measurements were conducted at a photometer (Lambda 15, Perkin Elmer, Wellesley, MA, USA) using a wavelength of λ = 882 nm.

Statistical analysis

The experiment had a completely randomised design. Each treatment was replicated twice, containing a number of 8 plants per split-root container. Three main groups were separated according to the P supply: high P on both sides (25/ 25 µM), low P on both sides (1/ 1 µM) and varying P(1/ 25 µM) supply on each side. Pre-chosen comparisons were conducted at the level of each P supply separately. To reduce complexity each side of the root was seen as independent to allow comparisons between each side of the root system. After analysis of variance (Proc GLM) the means were compared using the Tukey-test. Data were statistically analysed using SAS 6.10 (SAS Institute Inc., Cary, NC, USA).

Results

According to the analysis of the nutrient solution a supply of 10 µM Al resulted into a monomeric Al concentration of 8 ± 1.59 µM during the experiments. Phosphorus depletion in the nutrient solution occurred within two days at 25 µM P supply and within less than 1 day at 1 µM P supply.

Root elongation

Analysis of variance revealed a significant effect of Al and P treatment on root elongation, while no interactions were found between the two parameters (Fig. 1.2). The influence of P supply was most clearly expressed in the treatments without Al application. A low P supply enhanced root elongation. These results were obtained for both cultivars, though less clearly expressed in Lixis (Fig. 1.2B). No effect of P could be observed when the two root halves of one plant were subjected to different P supplies. On both sides, root elongation was similar for the plants uniformly supplied with high P indicating that root elongation is controlled by the P status of the shoot rather than by the external P supply.

The elongation of the main root axis was inhibited on the side where Al was applied. For the Al-resistant cultivar BR201M (Fig. 1.2A) grand mean of root elongation over all P treatments was reduced by 17 % compared to 42 % for cultivar Lixis (Fig. 1.2B) clearly reflecting the differences in Al resistance of the cultivars.

The growth reduction caused by Al took place at all P levels, regardless whether Al was applied uniformly to both root halves or non-uniformly to the roots. However, in both cultivars relative growth reduction (0 µM Al = 100 %) was more severely at uniform application of low P to both sides of the root system because of the higher root elongation of the controls without Al supply.

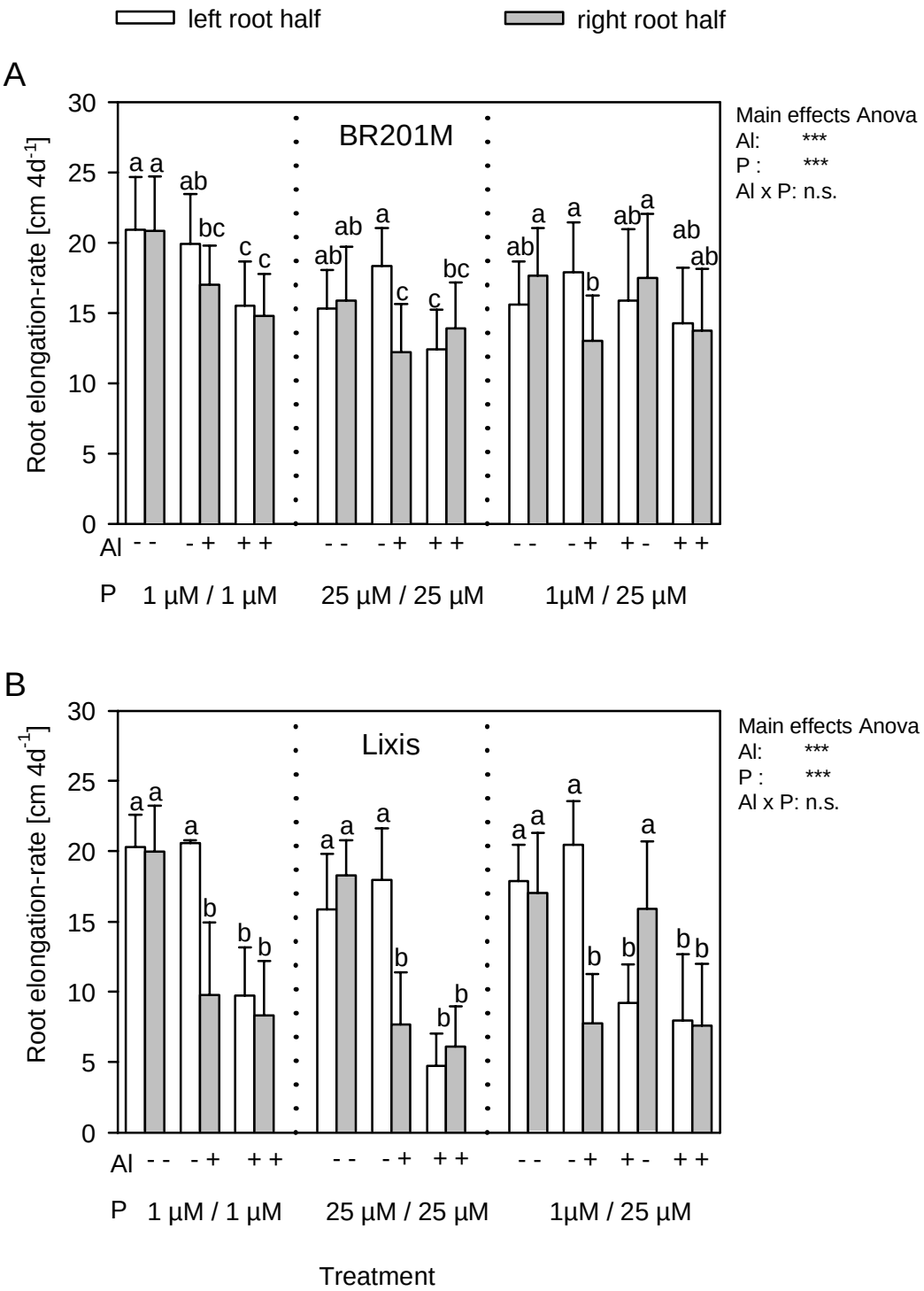

Fig. 1.2: Effect of Al (0 or 10 µM) and P supply (1 or 25 µM) on root elongation in a split-root system after 4 days for (A) the Al-resistant cultivar BR201M, and (B) the Al-sensitive cultivar Lixis. Plants were pre-cultured for 5 days in nutrient solution at 1 µM P. Bars represent means ± SD, n = 14. Means showing similar letters are not significantly different at $P < 0.05$, Tukey–test.

Within the three treatment groups (1/ 1 µM P, 25/ 25 µM P, 1/ 25 µM P) Al application to only one root half did not lead to a partial shift in the root elongation in favour of the non-treated half: Inhibition of root elongation was the same regardless whether Al was applied to both or only to one of the root halves. In addition, no enhancement of root elongation was found on the sides without Al supply.

Dry matter partitioning

Not only elongation of the primary root axis, but also growth of secondary roots of both cultivars as represented in the root dry weight was affected by the Al and P concentrations (Fig. 1.3). The analysis of variance showed a significant effect of the Al as well as the P supply. A significant interaction of the P and Al supply was found. The Al-sensitive cultivar Lixis produced the highest root dry matter. A low P supply enhanced the dry matter production of both cultivars, but the effect was more pronounced in cultivar Lixis. In the absence of Al, variation of the P supply between the root halves led to similar P effects in each root half. The Al application led to an overall reduction of root dry matter in both cultivars. Under conditions of low P supply root weight was reduced by 22 % for Al-resistant BR201M (Fig. 1.3A) and 35 % for Al-sensitive Lixis (Fig. 1.3B), indicating that root dry weight was a less suitable indicator of genotypical differences in Al resistance. At high a P supply both cultivars did not respond significantly to Al. A local application of Al to one root half only enhanced root dry weight on the Al-free half of the root system in the Al-sensitive cultivar Lixis. However, this effect was only significant at 25 µM P supply. Consequently, total root dry weight per plant was similar or even higher (Table 1.1) than in controls not treated with Al. This stimulating effect of a local Al supply on root growth of the Al-free side could not be observed in the Al-resistant cultivar BR201M.

In contrast to root dry weight, shoot dry weight was not affected by Al supply (Table 1.1). A trend towards lower shoot weights at 1 µM P supply was only found for cultivar BR201M, although its shoot weights were generally lower than those of cultivar Lixis. Plants expressed P-deficiency symptoms (purple colouring of leaves) at low P supply. Plants with high P supply to only one root half were not different from those with uniform high P supply and did not express P deficiency symptoms.

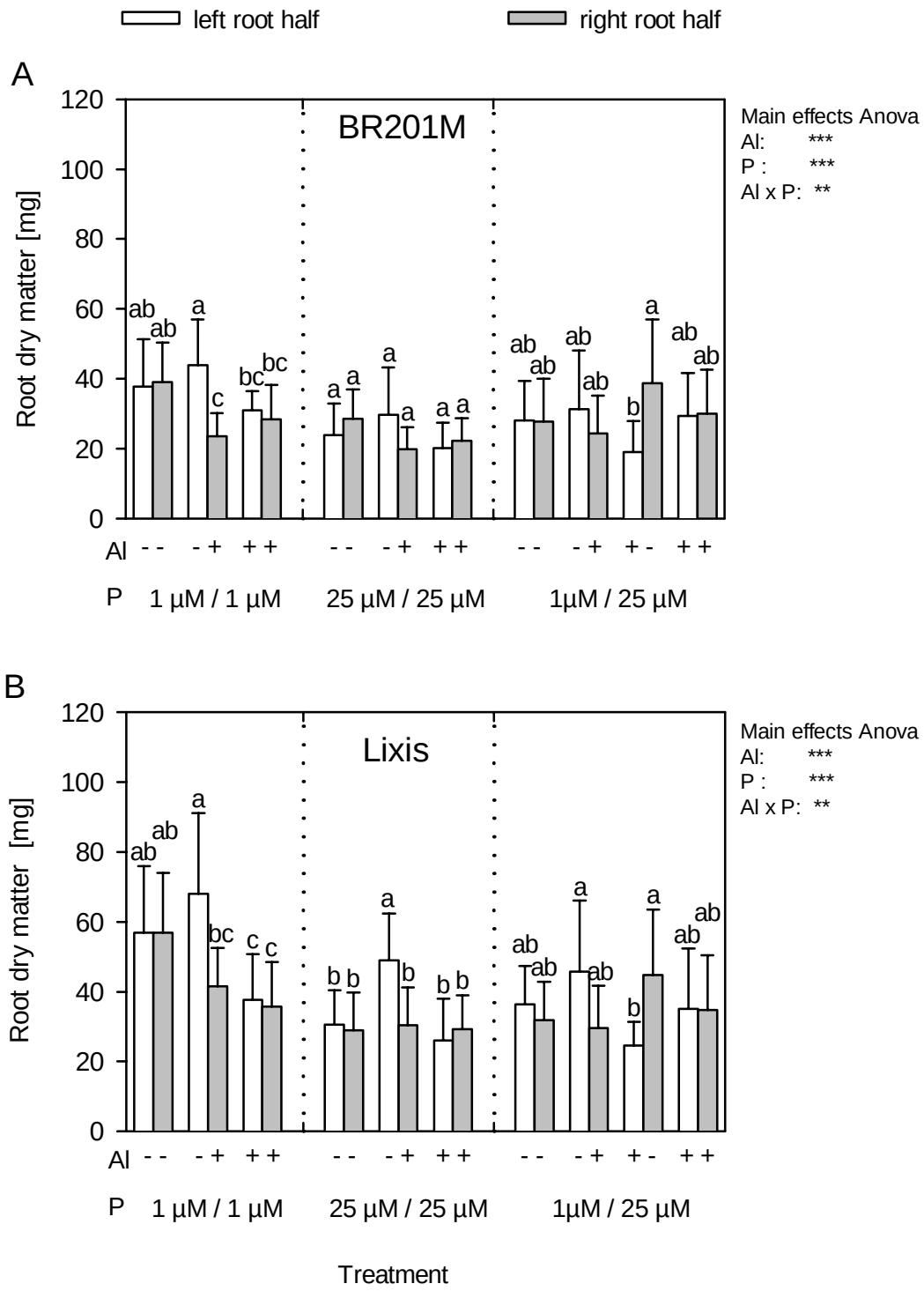

Fig. 1.3: Effect of Al (0, 10 µM) and P supply (1, 25 µM) on dry matter production of roots in a split-root system after 4 days for (A) Al-resistant BR201M, (B) Al-sensitive Lixis. Plants were pre-cultured for 5 days in nutrient solution at 1 µM P. Bars represent means ± SD, n = 14. Means showing similar letters are not significantly different at $P < 0.05$, Tukey–test.

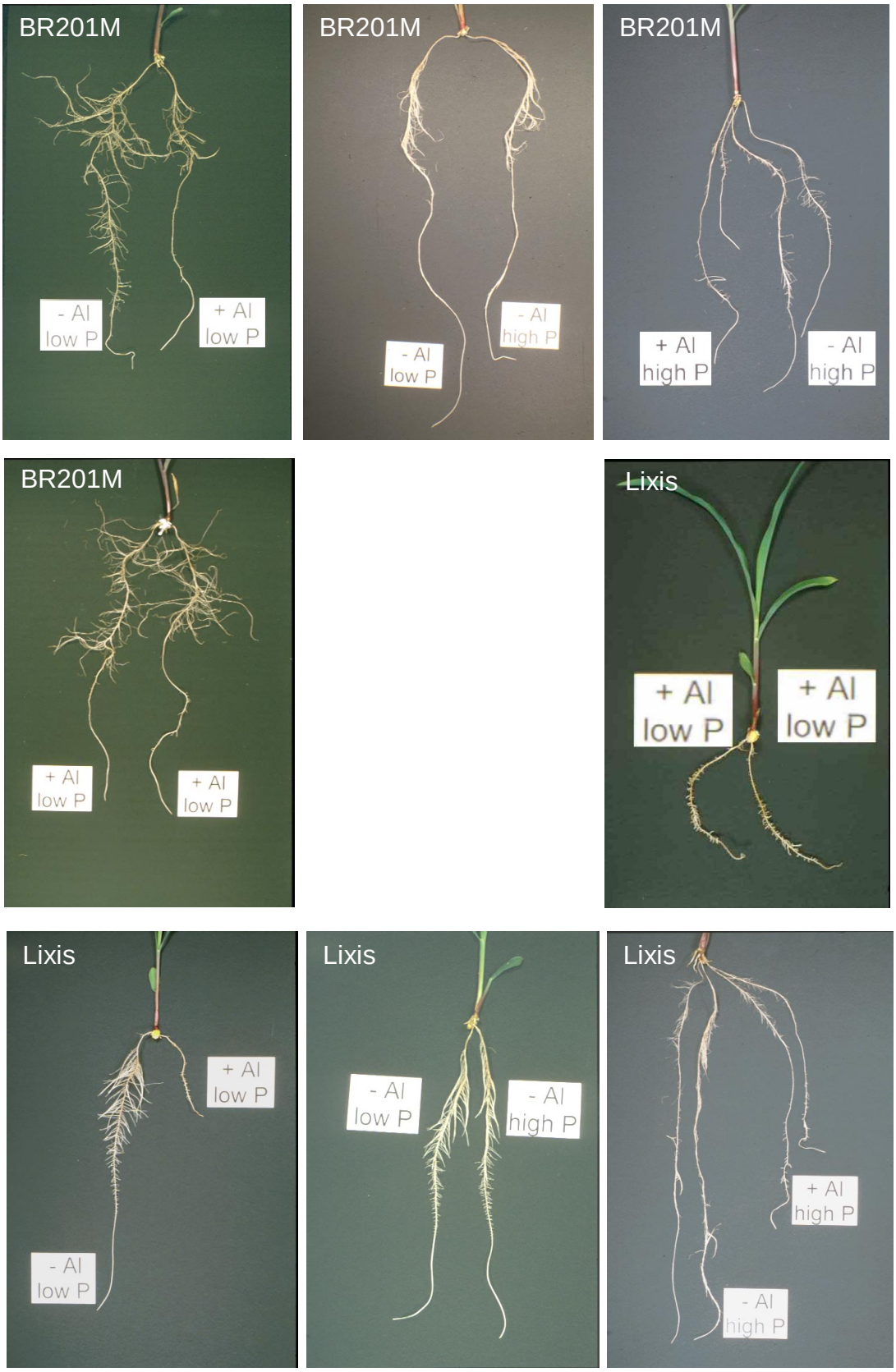

Fig. 1.4: Root development of an Al-resistant (BR201M) and Al-sensitive (Lixis) maize cultivar exposed to a varied Al (0, 10 µM) and P supply (1, 25 µM) in a split-root system in nutrient solution (pH = 4.3) for 4 days.

In agreement with the expression of P deficiency symptoms P contents in the shoot dry matter were much lower compared to high and intermediate P supply (cv. Lixis). When low and high P was supplied to either root half, the application of Al did not significantly affect shoot P contents.

Tab. 1.1 Root dry matter, shoot dry matter and P content in shoot dry matter (DM) of the Al-sensitive cultivar Lixis and Al-resistant cultivar BR201M after 4 days exposure of seminal roots to a spatially distributed P (1, 25 µM) and Al supply (0, 10 µM). Plants were pre-treated with 1 µM P for 5 days. Comparison of means were conducted for each P supply separately. Means showing similar letters are not significantly different at $P < 0.05$; Tukey-test, n = 14.* MSD refers to an overall comparison including all P levels.

Cultivar	Distribution of Al, P		Root	Shoot	
	P [µM]	Al	Dry matter [mg plant^{-1}]	Dry matter [mg plant^{-1}]	P content. [mg (g DM)$^{-1}$]
BR201M	1/ 1	- / -	76.8 a	245.4 a	2.15 a
		- / +	67.7 ab	252.7 a	2.08 a
		+ / +	59.5 b	299.1 a	2.17 a
	25/ 25	- / -	52.6 a	315.4 a	5.82 a
		- / +	49.6 a	322.8 a	5.14 a
		+ / +	43.3 a	294.2 a	5.43 a
	1/ 25	- / -	55.9 a	308.0 a	3.84 a
		- / +	55.9 a	307.6 a	3.64 a
		+ / -	57.6 a	321.6 a	3.43 a
		+ / +	59.9 a	341.2 a	3.57 a
				84.6*	0.967*
Lixis	1/ 1	- / -	113.6 a	345.6 a	1.70 a
		- / +	109.5 a	403.5 a	1.76 a
		+ / +	73.3 b	364.3 a	1.68 a
	25/ 25	- / -	59.5 b	347.0 a	5.41 a
		- / +	79.3 a	448.4 a	5.00 a
		+ / +	55.2 b	391.6 a	5.76 a
	1/ 25	- / -	68.2 a	400.3 a	3.50 a
		- / +	75.4 a	464.7 a	2.98 b
		+ / -	69.3 a	369.4 a	3.87 a
		+ / +	69.8 a	423.9 a	3.25 ab
				141.5*	1.198*

Callose formation in root tips

Callose formation as an indicator for Al injury was enhanced in root tips treated with Al. The P level had no effect. Absolute callose formation in the Al-treated root tips was only slightly higher in Lixis (Fig. 1.5B) than in BR201M (Fig. 1.5A). However, callose formation of the controls not treated with Al was higher in cultivar BR201M than in cultivar Lixis. Consequently, Al-induced callose formation was lower in BR201M (2.8- fold increase) than in Lixis (12- fold increase), confirming the higher Al resistance of cultivar BR201M.

Aluminium and P contents in root tips

Aluminium contents were much higher in Al-treated root tips than in controls (Fig. 1.6). There were no clear differences between the cultivars and P treatments. Only in cv. BR201M there was a tendency of lower Al contents at high P supply. In agreement with the lower Al-induced callose formation, Al contents were lower in root tips of plants receiving high and low P supply to either root half, in cultivar Lixis. Results for Ca, Mg, and K contents of the root tips are shown in the appendix. The supply of P had only little effect on the P contents detected. Split P application did not lead to a consistent higher P content at the half supplied with high P concentrations. However, overall low P supply led to a lower P content in root tips compared to roots with an overall high P supply (Fig. 1.7).

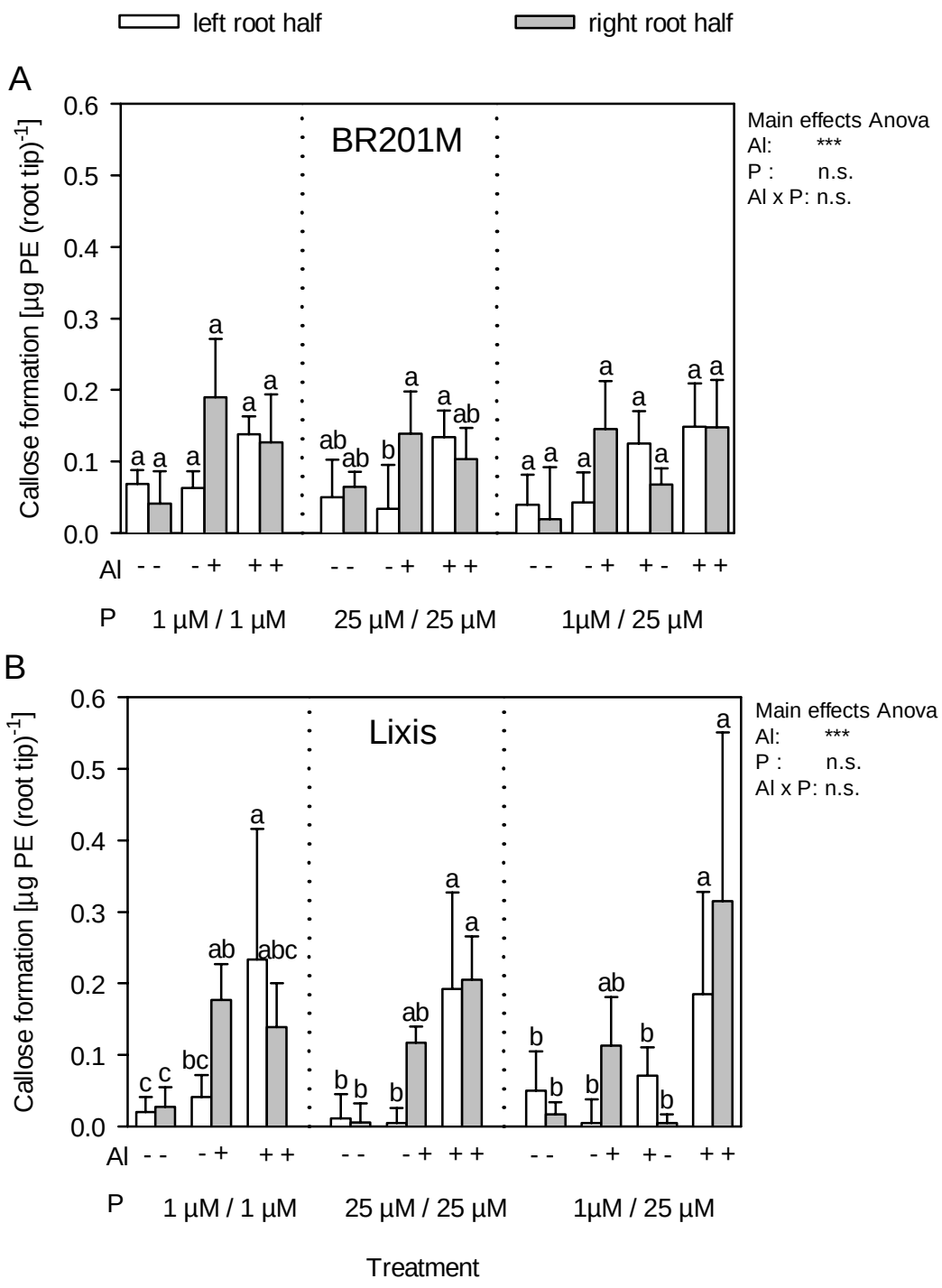

Fig. 1.5: Effect of Al (0, 10 µM) and P supply (1, 25 µM) on callose formation in 1 cm root tips in a split-root system after 4 days for (A) Al-resistant BR201M, (B) Al-sensitive Lixis. Plants were pre-cultured for 5 days in nutrient solution with 1 µM P. Bars represent means ± SD, n = 9. Means showing similar letters are not significantly different at $P < 0.05$, Tukey-test.

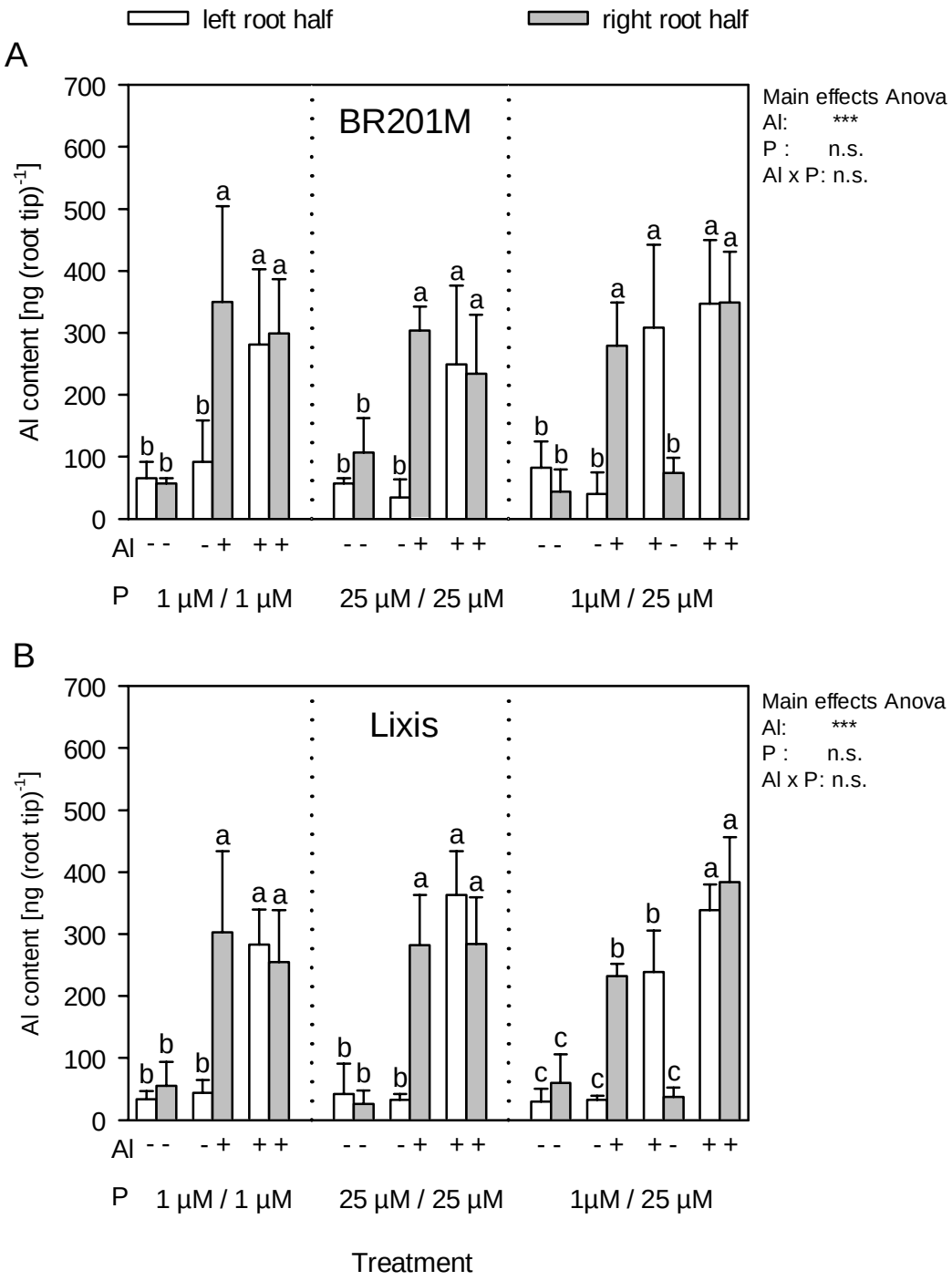

Fig. 1.6: Effect of Al (0, 10 µM) and P supply (1, 25 µM) on Al contents in 1 cm tips of seminal roots in a split-root system after 4 days for (A) Al-resistant BR201M, (B) Al-sensitive Lixis. Plants were pre-cultured for 5 days in nutrient solution at 1 µM P. Bars represent means ± SD, n = 6. Means showing similar letters are not significantly different at $P < 0.05$, Tukey-test.

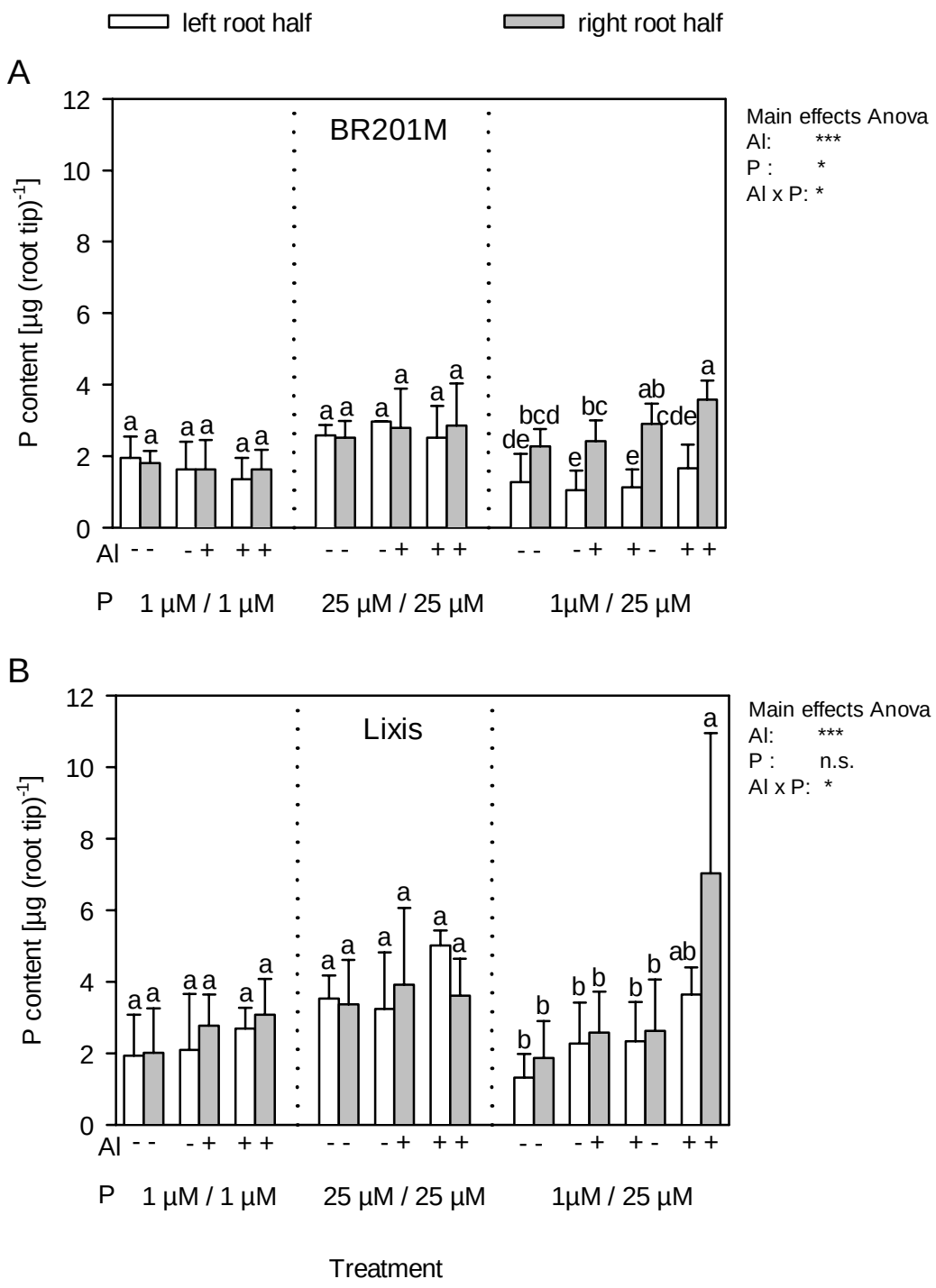

Fig. 1.7: Effect of Al (0, 10 µM) and P supply (1, 25 µM) on P contents in 1 cm tips of seminal roots in a split-root system after 4 days for (A) Al-resistant BR201M, (B) Al-sensitive Lixis. Plants were pre-cultured for 5 days in nutrient solution at 1 µM P. Bars represent means ± SD, n = 6. Means showing similar letters are not significantly different at $P < 0.05$, Tukey-test.

Discussion

In this study seminal roots of maize were exposed to a varied Al and P supply. It is known that plant roots react to heterogeneous distribution of soil resources by root proliferation in the most nutrient or water-rich zones (Scott Russell, 1977; Fitter, 1994; Marschner, 1996). A similar reaction might be also observed when roots are encountering growth-limiting conditions, e.g. under Al toxicity. This growth reaction might be furthermore restricted to low P status of the plant (Hairiah *et al.,* 1990).

Effects of a split P supply on plant development

The local application of P to only one part of the root system has a stimulating effect on root growth of cereals (Drew, 1975; de Jager, 1982) as well as other plant species (Borkert and Barber, 1983; Brouder and Cassman, 1994). In contrast to results reported by several authors (Drew, 1975; Drew and Saker, 1978; de Jager, 1982), in this study 1 µM P application to the one and 25 µM P to the other root half, did not enhance root dry weight or length on the side with higher P supply.

Compensatory growth in maize and in soybean were found only when less than 50 % or 25 % of the root system was supplied with P, respectively (Anghioni and Barber, 1980; Borkert and Barber, 1983). McClure (1972) applied P to 3 vertical compartments along the root of maize and wheat plants. A significant increase of root growth was found only when the uppermost compartment was supplied with P. No decrease was observed at applications to one of the other two compartments. It therefore appeared, that the magnitude of the compensatory reaction depended on the portion of the root system subjected to a localised P supply, the P demand of the root system in contact with the higher P supply and hence on the P nutritional status of the shoot (Raghothama, 1999).

However, at uniformly low P supply to the entire root system root elongation and total root dry weight were enhanced by 30 % and 29 % (BR201M), and by 18 % and 54 % (Lixis) compared to high P supply, respectively (Fig 1.2, Table 1.1). These results were in agreement with a reported 50 % increase in root weight of maize plants after 4 days of low P supply (Anghioni and Barber, 1980; de Jager, 1982).

A split P supply (1/ 25 µM) resulted in shoot P contents (Tab. 1.1) ranging between the amounts found in plants homogeneously supplied with 25 µM P or 1 µM P as it was also described by de Jager (1984). The P contents were at the lower end of the sufficiency range (Jones, 1974 c.f. Reuter and Robinson, 1986). They might explain the observed lack of compensatory root growth in this P treatment and support the view that a growth response to low P supply is contributed to shoot P status (Marschner *et al.*, 1996). The shoot P contents at uniform 25 µM P supply were similar to those found by other authors (McClure, 1972; Anghioni and Barber, 1980). They were within the range calculated as adequate (0.3 - 0.5 %) for maize plants smaller than 30 cm (Jones, 1974 c.f. Reuter and Robinson, 1986). Phosphorus contents at 1 µM P supply were below this range indicating P deficiency in both cultivars. The conditions for an induction of Al avoidance as described by Hairiah *et al.* (1993) were therefore met in this treatment.

Effects of a split Al supply on plant development

As expected, Al application did not influence shoot weights of either cultivar. Aluminium toxicity affects the root first. Shoot development is only reduced at later stages of growth (Rajaram *et al.*, 1991).

In agreement with other studies (Llugany *et al.*, 1995; Horst *et al.*, 1997), the Al-sensitive cultivar Lixis showed a higher reduction in root elongation (Fig. 1.2) than the Al-resistant cultivar BR201M. The magnitude in inhibition of root elongation depended on the external P supply and was higher at 25 µM P (70 % inhibition Lixis, 27 % BR201M) than at 1 µM P supply (40 % Lixis, 16 % BR201M).
However, a split application did neither induce Al avoidance in the Al-resistant cultivar BR201M nor compensatory growth in either cultivar when compared to homogeneous Al exposure, regardless of P supply: No enhanced inhibition of elongation of the roots in contact with Al, nor enhanced root elongation of Al-free root occurred. It might be concluded that as reported for a localised nutrient supply (Drew and Saker, 1975; de Jager, 1982), compensatory root growth is not expressed in the elongation of the main axis.

However, different results were found for root dry matter (Fig. 1.3). The Al-sensitive cultivar Lixis responded to a localised Al supply with compensatory root growth: Root dry matter production at the Al-free side was enhanced (Fig. 1.3B) following a preferential growth under more favourable conditions (Gersani and Sachs, 1992). This additional growth of cultivar Lixis led to a similar or even higher total root weight than in controls (0 µM Al applied to both sides, Table 1.1). The additional growth might have derived from an increased lateral root growth as described for Cu toxicity (Adalsteinsson, 1994) and for a localised nutrient supply (Drew, 1975; Drew and Saker, 1975; de Jager, 1982; Sattelmacher and Thoms, 1995). Such root reactions occur rapidly and were shown to result from changes in the size of first order-laterals, second-order laterals or overall changes in growth rate (Robinson, 1994). Assimilate partitioning between axes of a split-root system is proposed to be responsive to the total sink demand of the root (Chaillou *et al.*, 1994). Since roots are supposed to compete for carbohydrates (Brouwer, 1981; Granato and Raper, 1989) lateral root initiation for cultivar Lixis on the Al-free side represented a stronger sink than the side exposed to Al.

The magnitude of the observed compensatory growth depended on the P supply. It was more clearly expressed at high than at low P supply. At a low P supply root growth is enhanced due to shoot-P deficiency (Marschner *et al.*, 1996). The compensatory growth was calculated in comparison to a control in which root growth was generally enhanced by low P supply. Therefore, the magnitude of compensatory growth was lower than in the treatment with high P supply (Fig. 1.3B) where shoot P content was sufficient and Al toxicity the only growth limiting factor. Hence, for cultivar Lixis the observed compensatory growth effects were most pronounced at high P supply leading to an overcompensation in total root dry matter with a 1.3- fold increase as compared to the control (Table 1.1). The variation in P supply (1 µM and 25 µM P) led to an intermediate reaction, because root dry matter in the controls ranked in-between the two other P treatments.

Contrasting observations could be made for the Al-resistant BR201M: At all P and Al levels total root weight remained unaffected. A preferential growth of the Al-free side might have existed in tendency only (Fig. 1.3A). Neither was a phenomenon of Al avoidance found for Al-resistant BR201M, nor compensatory growth. As a matter of the cultivars' inherent Al resistance, inhibition of root elongation was only

small. It was therefore concluded that the sink strength remained at similar levels also at heterogeneous Al supply. Hence, no shift in growth patterns occurred.

Callose formation after 4 days of Al treatment was unexpectedly low (Fig. 1.5). The differences between the cultivars were only visible on a relative basis. These findings were in contrast to other reports of Al-induced callose formation of those two cultivars (Horst *et al.*, 1997), but might be explained by the late date of sampling (see also chapter III). A degradation of callose might have led to the detected values below the expected order of magnitude. Hairiah *et al.* (1993) demonstrated with *Mucuna*, that upon heterogeneous Al supply the root exposed to Al was more stressed than when the total root system was supplied with Al. As Al-induced callose formation is an indicator of stress (Kauss, 1996), it was expected to find higher callose formation under heterogeneous supply especially in the root exposed to Al. However, this was not the case. From the findings presented it could not be concluded that those roots of the Al-resistant cultivar exposed to heterogeneous Al suffered more from Al toxicity than under homogeneous Al supply. Especially for the Al-resistant cultivar BR201M this would have been expected according to the findings of Hairiah *et al.* (1992).

In contrast to the findings in *Mucuna*, no Al avoidance reaction was found for the Al-resistant maize cultivar BR201M. However, the observed compensatory growth for the Al-sensitive cultivar Lixis was in agreement with reports of changes in root development of cereals under unfavourable conditions (Brouwer, 1981; Adalsteinsson, 1994). Furthermore, the observed reaction depended on plant P status. Since compensatory growth has been observed in many species it seems to be the rule rather than the exception (Gersani and Sachs, 1992; Robinson, 1994). Therefore, Al toxicity might also induce compensatory growth in the Al-resistant cultivar BR201M at higher Al concentrations than used in this study.
These results point out that a change in the growth pattern of roots is not necessarily a new mechanism of reported tolerance mechanisms (Keltjens, 1997; Marschner, 1991, 1996). It might be a consequence of environmental heterogeneity expressed at cultivar-specific threshold levels and in case of Al toxicity depending on the cultivars' Al resistance. It therefore appears that the findings of Hairiah *et al.*

(1992) represented compensatory growth rather than a special case of Al resistance.

The findings clearly indicate that characterisation in Al resistance of the two cultivars did not depend on the use of heterogeneous or homogeneous Al supply. An occurrence of compensatory growth as indicated from our results might be more important for an Al-sensitive than an Al-resistant cultivar and explain why some sensitive plants perform better under field conditions than in nutrient solution. Consequently, screening of maize in homogeneous nutrient solution is not limited.

2 Assessment of Al resistance of maize cultivars (*Zea mays* L.) using intact plants and excised root tips

Abstract

Maize cultivars (*Zea mays L.*) were evaluated in their Al resistance using intact plants and excised root tips exposed to 25 µM Al in nutrient solution of low ionic strength at pH 4.3. Aluminium supply increased callose formation and Al content in root tips of intact plants as well as in excised root tips.

Using intact plants cultivars could be characterised in their Al resistance by means of Al-induced callose formation, Al-induced inhibition of root elongation as well as Al contents in the root tips. Furthermore, significant correlations between Al-induced callose formation and Al content in root tips (r^2 = 0.64**) and inhibition of root elongation (r^2 = 0.80***) were found. In contrast, excised root tips did not show a significant Al-induced inhibition of root elongation and no correlation with Al-induced callose formation was found. While the average Al-induced callose formation was similar for root tips of intact plants and excised root tips (0.4 µg PE segment^{-1}), average Al content in excised root tips was up to 1.5 - fold higher than in tips of intact plants after 24 h of Al treatment. Aluminium-induced callose formation found in excised root tips did neither correspond to Al-induced callose formation nor to inhibition of root elongation of intact plants. The addition of 10 mM glucose to the incubation medium led to a significant increase in the elongation of excised root tips when no Al was supplied. Furthermore, a 2- 3- fold increase in Al-induced callose formation in comparison to roots without glucose supply (Glc) was found. Staining with triphenyl-tetrazolium-chloride (TTC) revealed increased viability of these root segments. However, these effects of glucose supply did not improve the characterisation of cultivars in Al resistance.

The results presented suggest that certain Al-exclusion mechanisms expressed in root tips of intact plants might be non-operational in excised root tips. The limitations under the described conditions consolidate the viewpoint that excised root tips might not be an adequate means of replacing the work with intact plants in characterising maize germplasm.

Introduction

The most frequently measured effect of Al toxicity in plants is the inhibition of root elongation in nutrient solution (Rengel, 1996 and references therein). The observation of Al-induced inhibition of root elongation in maize is possible after 30 - 90 min of Al exposure (Llugany *et al.*, 1995). Cultivar-specific differences using a large scale of cultivars were observed after 24 h (Horst *et al.*, 1997). Genotypic differences in Al-induced callose formation have been also found in several crop species and could be related to inhibition of root elongation in nutrient solution (Wissemeier *et al.*, 1992; Zhang *et al.*, 1994; Horst *et al.*, 1997; Massot *et al.*, 1999). The magnitude of Al-induced callose formation depended on the Al concentration (Llugany *et al.*, 1994; Schreiner *et al.*, 1994; Staß and Horst, 1995; Horst *et al.*, 1997) and could be detected before the inhibition of root elongation (Zhang *et al.*, 1994; Wissemeier and Horst, 1995). Aluminium sensitivity was also expressed in protoplasts and in cell suspension cultures by the induction of callose synthesis (Staß and Horst, 1995; Wagatsuma *et al.*, 1995; Horst *et al.*, 1997). Therefore, the induction of callose formation has been proposed as a physiological marker of Al injury to be used in screening for Al resistance (Wissemeier *et al.*, 1987; Horst *et al.*, 1997).

Xia and Saglio (1988) proposed the use of excised root tips in kinetic studies instead of protoplasts, due to their easier use and the elimination of possible artefacts. Excised root tips have been found to remain viable up to 200 h after excision (Brouquisse *et al.*, 1991). As Al-toxicity effects are primarily confined to the root apex (Bennet *et al.*, 1984; Ryan *et al.*, 1993; Horst, 1995; Blancaflor *et al.*, 1998; Sivaguru and Horst, 1998; Sivaguru *et al.*, 1999), root tips have been used in several studies regarding Al toxicity (Huett and Menary, 1979; Macklon and Sim, 1981; Malavolta *et al.*, 1981; Samuels *et al.*, 1997).

Wheat cultivars and lines could be characterised in Al resistance by means of organic anion exudation and Al uptake using excised root tips (Ryan *et al.*, 1995a, b; Zhang and Taylor, 1989, 1991; Zhang *et al.*, 1995). It was therefore assumed that the use of excised root tips could also facilitate screening for Al resistance in maize, with Al-induced callose being used as parameter.

Materials and methods

Plant material and growth conditions

The experiments were conducted with seeds of 11 maize (*Zea mays* L.) cultivars differing in Al resistance as well as in adaptation to acid, Al-toxic soils (Tab. 2.1).

Tab. 2.1: Origin of maize cultivars and classification according to adaptation to acid soils and Al resistance.

No	Cultivar	Origin/ Donator	Classification	
			Al resistance	Adaptation to acid soil
1	SA-4, P102-1	Colombia, CIMMYT	Al-resistant	tolerant
2	SA-4, P102-2	Colombia, CIMMYT	Al-sensitive	sensitive
3	SA-5, P102-3	Colombia, CIMMYT	Al-resistant	tolerant
4	SA-5, P102-4	Colombia, CIMMYT	Al-sensitive	sensitive
5	C525M	Brazil, Embrapa	Al-resistant	tolerant
6	BR201F	Brazil, Embrapa	Al-sensitive	
7	BR201M	Brazil, Embrapa	Al-resistant	
8	ATP-Y	Cameroon, IRA	Al-resistant	tolerant
9	HS701B	France, INRA	Al-sensitive	
10	Lixis	Germany, Force Limagrain	Al-sensitive	
11	Helix	Germany, Force Limagrain	Al-sensitive	

Seeds were germinated for 3 d between sheets of filter paper, soaked with 1 mM $CaSO_4$ solution, in an upright position. Seeds of a similar size were used to assure a similar initial physiological stage (Bockstaller and Girardin, 1994; Pommel, 1990). Afterwards, the plants were transferred to 22 l plastic pots with constantly aerated nutrient solution. At this stage coleoptiles of seedlings were 2 - 4 cm long and generally slightly opened. The tap root had a length of approximately 7 to 15 cm.

The composition of the nutrient solution was [µM]: KNO_3 400, $CaSO_4$ 250, NH_4NO_3 200, $MgSO_4$ 100, Fe-EDDHA 20, KH_2PO_4 10, H_3BO_3 8, $MnSO_4$ 1, $ZnSO_4$ 0.2, $CuSO_4$ 0.2, $(NH_4)_6Mo_7O_{24}$ 0.1.

The seedlings were grown in a growth chamber under controlled environmental conditions of a 16/ 8 h day/ night cycle, 27/ 25 °C day/ night temperature, 75 ± 5 %

relative air humidity and a photon flux density of 230 µmol m^{-2} s^{-1} photosynthetic active radiation (Sylvania Cool White, 195 W, Philips, Germany) as measured in mid plant height.

The plants were adapted for 24 h to the nutrient solution. In the following 24 h, the pH was decreased from 5.8 to 4.3 in steps of 0.3 units using a pH-stat device keeping the pH constant during the experiment by adding 0.1 M HCl or 0.1 M KOH. The final pH of 4.3 was reached not later than 16 h before the start of the experiments.

The plants were divided into 2 groups, one for the use as intact plants and the other as source of excised root tips for incubation.

Experiment I

A. Intact plants (cultivars no. 1 – 10) were transferred to nutrient solution containing 0 and 25 µM Al as used for screening purposes in previous studies (Horst *et al.*, 1997). For each cultivar the Al treatment was repeated 5 times, each replicate containing 10 seedlings. Three out of 10 seedlings were harvested for callose determination after 24 h. Root tips of 1 cm length were cut and transferred into Eppendorf cups containing ethanol (96 %). For Al analysis root tips of 0.5 cm length were placed into Eppendorf cups with ultrapure water (upw.) and stored at -20°C.

B. Parallel to the experiment with intact plants, 2 cm root tips were cut from the pre-cultured plants and placed into Erlenmeyer flasks (8 root tips per flask) containing 200 ml nutrient solution (see above) at pH 4.3. The flasks were placed on a gyratory shaker (80 rpm) for 24 h at 26 °C in darkness. After the incubation at 0 or 25 µM Al (4 replicates), root elongation was measured against a 1 mm scale and root tips were transferred into Eppendorf cups containing ethanol (96 %). Previous experiments revealed that callose formation was most pronounced in the first apical 5 mm (Wissemeier and Horst, 1995; Sivaguru and Horst, 1998). Thus, for callose analysis root tips stored in ethanol were cut 5 mm behind the apex, also avoiding contamination by wound callose deposits at the end of the excised root tips resulting from previous injury (Jaffe *et al.*, 1985).

Twelve root tips (0.5 cm length), 3 from each replicate were placed into Eppendorf cups with upw. and stored at -20°C for Al analysis .

Experiment II

In a following experiment root tips (cultivars no. 1 – 10) were incubated for 24 h at different levels of Al supply (0, 10, 25 and 50 µM Al) with or without the addition of glucose (10 mM). Vitality of root tips was determined by staining with triphenyl-tetrazolium-chloride (TTC) (Larcher, 1969; Altman, 1976). Root tips were infiltrated in TTC solution (0.1 % w/ v in deionised water) at 30 – 50 mbar to allow penetration of the TTC into the root tissue. Thereafter they were incubated for 1 h at 30 °C in the dark. Only in living cells the colourless TTC salt is reduced to insoluble red coloured formazan due to enzymatic processes (Jensen *et al.*, 1951). Staining of the root tips was recorded on slights. Glucose-fed, non-vital root tips did not stain (not shown).

Experiment III

Excised root tips of the Al-sensitive cultivars Lixis and HS701B and the Al-resistant cultivars ATP-Y and C525M were incubated for 24 h in 0 and 25 µM Al and an additional glucose supply of 0 or 10 mM. Callose formation was determined after 0, 4, 8, 16 and 24 h.

Experiment IV (N. Schmohl 1998, Institute of Plant Nutrition, Univ. Hannover)

Root tips of intact plants and excised root tips of the Al-sensitive cultivar Helix were analysed for Al and Al-induced callose formation after short term exposure to 50 µM Al for 3 h in nutrient solution. Additionally, excised tips were placed in ethanol (96 %) to induce cell death, thereafter rinsed in upw. and placed into nutrient solution containing 0 or 50 µM Al.

Calculation of the Al-induced inhibition of root elongation

Root elongation was determined by measuring root length at the beginning and the end of the experiment using a 1 mm scale. The difference between both lengths was defined as root elongation during the treatment period. Aluminium-

induced inhibition of root elongation was calculated as described by Sapra *et al.* (1982):

$$\text{Al-induced inibition of root elongation} = 100 - \left(\frac{RL_{+Al}}{RL_{-Al}} * 100 \right) [\%]$$

RL_{-Al}: root elongation at 0 µM Al

RL_{+Al}: root elongation at 25 µM Al

Analysis of nutrient solution

Monomeric Al in the nutrient solution was determined using the pyrocatecholviolet (PCV) method of Kerven *et al.* (1989), in samples of 3 ml nutrient solution with a photometer (Lambda 15, Perkin Elmer, Wellesley, MA, USA) at λ = 580 nm after 60 sec. Standard solutions were prepared in the range of 0 - 35 µM Al.

Callose quantification

At harvest plant roots were rinsed with deionised water, 1 cm root tips were excised using a razor blade, and fixed immediately in ethanol (96 %). For analysis, tips were rinsed with deionised water and transferred to Eppendorf cups containing 1 ml of 1 M sodium hydroxide solution and stored at -20 °C overnight. After defrosting samples were homogenised with an ultrasonic device (Bandelin sonoplus HD70 with microtip MS 72 D, Bandelin Electronics, Berlin, Germany) for 45 s. To extract callose homogenised samples were heated at 80 °C in a water bath for 30 min. Thereafter, cooled samples were centrifuged at 10^3 x g for 15 min (Sorvall MC12V, Du Pont de Nemours & Co. Inc., Newton, Conn., USA). Callose was then determined according to Köhle *et al.* (1985) and Kauss (1989) using waterblue as stain (waterblue, Fluka, Seelze, Germany). The callose sirufluor complex was measured with a Hitachi F 2000 fluorescence spectrometer (Hitachi, Tokyo, Japan; excitation λ = 394 nm, emission λ = 484 nm, slit 10 nm, Voltage U = 700 V). Pachyman (Calbiochem, Deisenhofen, Germany) was used as calibration standard. Hence, callose content was expressed as pachyman equivalents (PE) per root tip.

Analysis of mineral elements

Root tips were analysed for Al using GFAAS (Unicam 939 QZ graphite furnace atomic absorption spectrophotometer, Analytical Technologies Inc., Cambridge, U.K.). Root tips of 0.5 cm length were wet ashed in 2 ml ultrapure HNO_3 (65 %) in teflon centrifuge tubes (TFEP, Nalgene, Rochester, NY, USA) at 135 °C. The acid was evaporated and the ash was dissolved with 2 ml of a diluted ultrapure HNO_3 (dilution 1:3 with upw.). For the determination of Al an aliquot of 200 µl was taken from this sample and diluted to a total volume of 2 ml with upw. The measurements were conducted using a graphite furnace AAS (Unicam 939 QZ, Analytical Technology, Cambridge, UK) at a wavelength of λ = 309.3 nm.

Results

In a first approach (experiment I, II) intact plants and excised root tips of cultivars with contrasting response to Al and soil acidity were evaluated for their response to 25 µM Al supply in nutrient solution. Within the nutrient solution used for intact plants monomeric Al concentration was detected at 17.4 ± 1.9 µM. In the incubation medium of excised root tips monomeric Al was detected at a concentration of 19.7 ± 1.07 µM; the pH remained constant (pH = 4.35 ± 0.05) during the course of experiment. Significant cultivar-specific differences were found for root elongation of intact plants at 0 µM and 25 µM Al supply (Fig. 2.1). The application of Al led to a significant decrease in root elongation (Fig. 2.1A). Root elongation in the presence of Al differed between cultivars by a factor of two or more. Those cultivars classified as Al-resistant (C525M, ATP-Y, BR201M) had the highest growth rate at 25 µM Al in comparison to other cultivars (P102-1, BR201F), classified as Al-sensitive. In contrast, excised root tips showed a much lower elongation rate than intact roots ranging from 2 - 3 mm d^{-1}. The presence of Al did not significantly inhibit root elongation and no differences between the cultivars were observed (Fig. 2.1B).

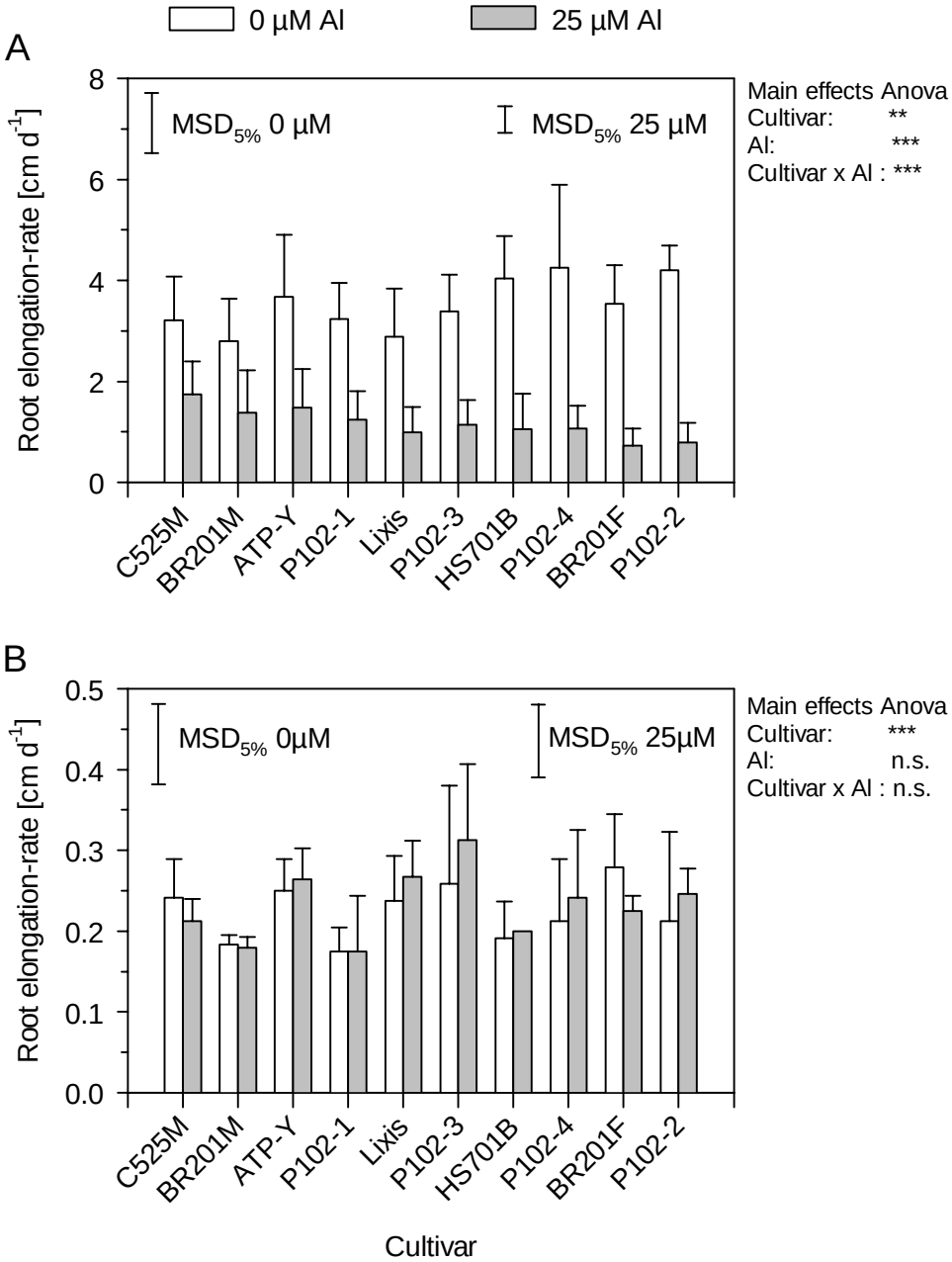

Fig. 2.1: Effect of Al supply (0, 25 µM) on root elongation-rate of different maize cultivars grown in nutrient solution, at pH = 4.3. Bars represent means ± SD for (A) intact plants, treatment duration 48 h, MSD at 0 µM Al = 1.174, MSD at 25 µM Al = 0.647, at $P < 0.05$, Tukey-test, n = 20 (B) Excised root tips, treatment duration 24 h, MSD at 0 µM Al = 0.097, MSD at 25 µM Al = 0.101, at $P < 0.05$, Tukey-test, n = 15.

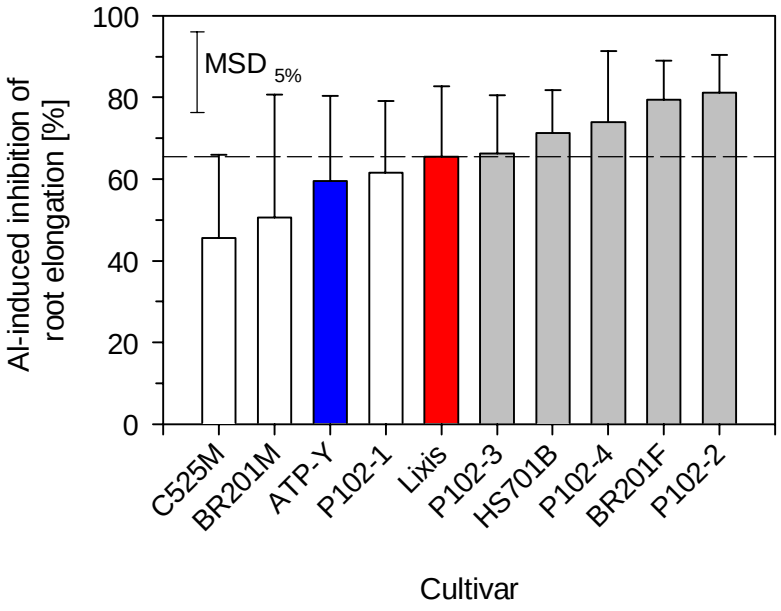

Fig. 2.2: Aluminium-induced inhibition of root elongation of intact maize seedlings of different cultivars grown in nutrient solution for 48 h at 25 µM Al, pH = 4.3. Bars represent means ± SD, MSD = 19.52 at $P < 0.05$, Tukey-test, n = 20. Dotted line refers to the overall mean.

Consequently, only for roots of intact plants an Al-induced inhibition of root elongation was found (Fig. 2.2). The inhibition of root growth ranged from 46 % (C525M) to 81 % (P102-2) with an overall mean of 65 %. The cultivars reacted according to their prior classification for Al resistance (Tab. 2.1): The Al-resistant cultivars C525M, BR201M, ATP-Y and P102-1 showed the lowest (< 60 %) whereas the Al-sensitive cultivars P102-2, P102-4, BR201F showed the highest (> 75 %) inhibition of root elongation. However, the Al-sensitive cultivar Lixis showed a lower inhibition than expected, whereas the cultivar ATP-Y showed higher inhibition than known from previous experiments (Horst *et al.*, 1997).

Al-induced callose formation

Aluminium application led to an increase in callose formation in the root tips of intact plants. A wide variation was found among the cultivars leading to significant

differences in Al-induced callose formation (Fig. 2.3A). The separation of the cultivars according to their Al-resistance was much better than on the basis of inhibition of root elongation: Statistical differentiation was more distinct using Al-induced callose formation as a parameter, e.g. cultivar ATP-Y and cultivar Lixis could be clearly separated as Al-resistant and Al-sensitive, respectively (Fig. 2.3A intact plants). The calculation of callose formation based on root tip fresh weight did not lead to a different pattern in the ranking of the cultivars or a better differentiation between Al-sensitive and Al-resistant cultivars (Fig. 2.3B intact plants).

Aluminium treatment induced callose formation in excised root tips, too. In contrast to their small rate of root elongation (Fig. 2.1B), significant differences in Al-induced callose formation were found between the cultivars (Fig. 2.3A excised root tips). The overall mean of Al-induced callose formation was similar to root tips of intact plants with 0.4 µg PE per segment. However, the observed cultivar-specific response was not in agreement with the differences found in intact plants, e.g. cultivars ATP-Y and Lixis could not be separated according to their differential Al sensitivity. Callose formation in excised tips of the cultivars C525M, BR201M and ATP-Y was higher than in intact plants whereas other cultivars produced less callose (Lixis, HS701B, P102-4). Therefore, only the cultivar P102-1 (Al-resistant) and P102-2, P102-4, BR201F (Al-sensitive) could be clearly separated according to their reported and above-shown response to Al (Fig. 2.2). In contrast to results achieved with intact plants the cultivars Lixis and P102-3 were rated similar in Al-resistance as C525M, ATP-Y and BR201M. Relating Al-induced callose formation to root fresh weight (FW) (Fig. 2.3B excised root tips) rather than to root length did not change the characterisation of cultivars, but their differentiation was even less pronounced.

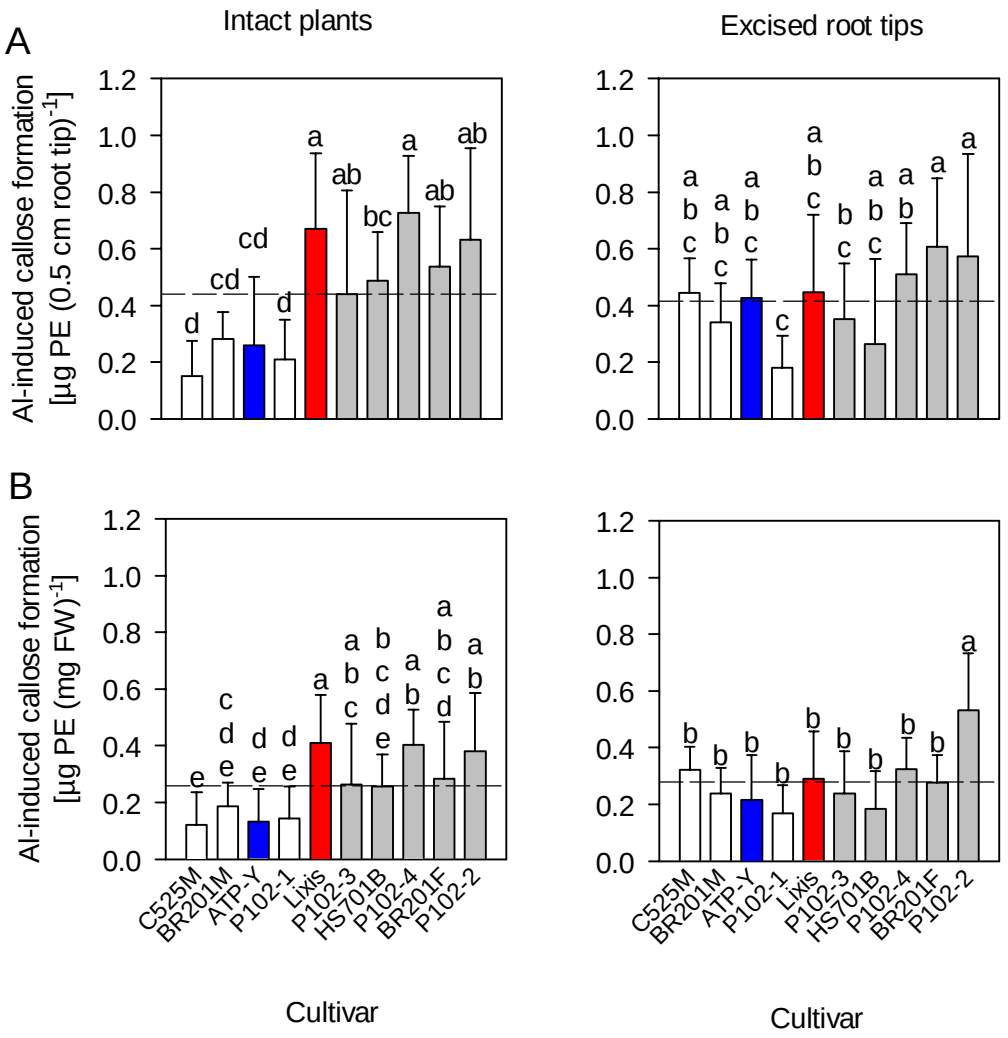

Fig. 2.3: Aluminium-induced callose formation in root tips (0.5 cm) of intact maize seedlings and excised root tips of different cultivars grown in nutrient solution at 25 µM Al supply for 24 h at pH = 4.3. Bars represent means ± SD. Means with similar letters are not significantly different at $P < 0.05$, Tukey-test, n = 15. A: based on 0.5 cm root tip length. B: based on mg FW. The dotted lines refer to overall means.

Aluminium content in root tips

As expected, the application of Al led to a significant increase in Al contents in root tips (Fig. 2.4).

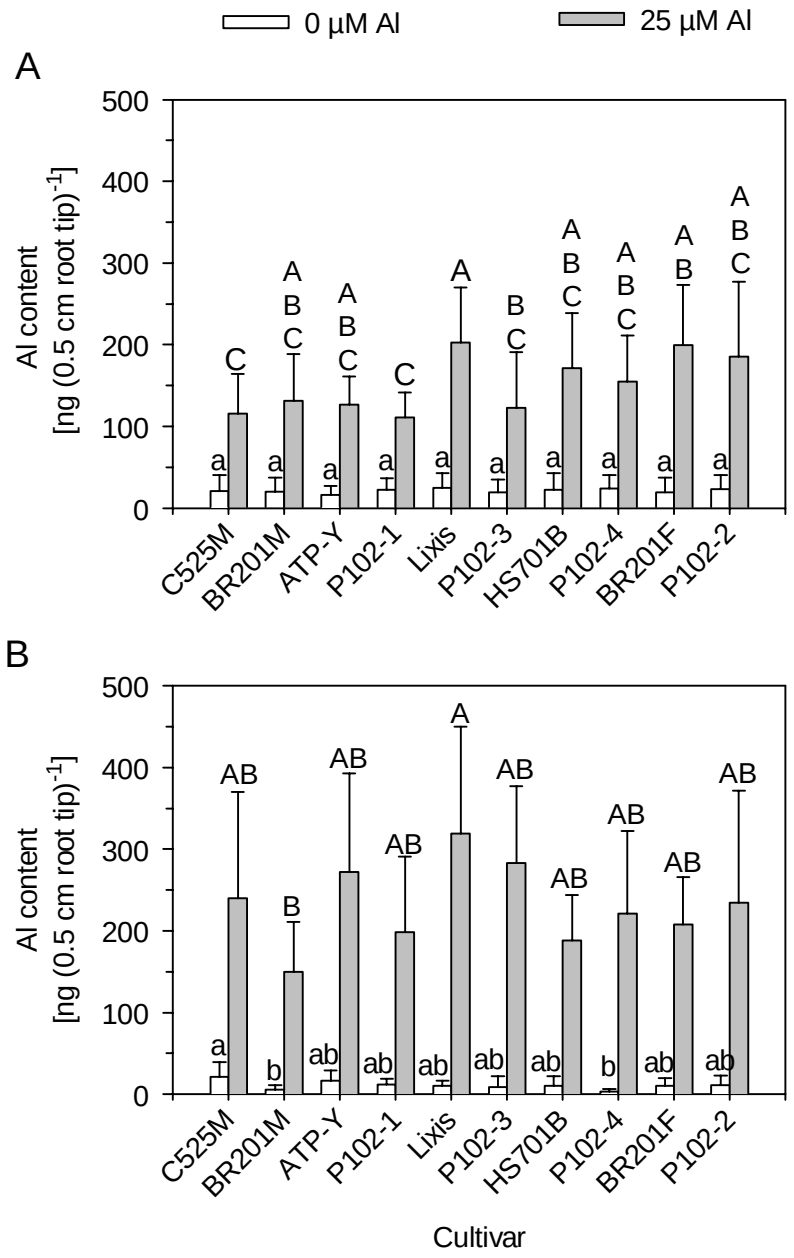

Fig. 2.4: Aluminium content in root tips (0.5 cm) of (A) intact maize seedlings after 48 h and (B) excised root tips after 24 h of different cultivars grown in nutrient solution at two levels of Al supply (0, 25 µM), pH = 4.3. Bars represent means ± SD. Capital letters are used for comparison between cultivars at 25 µM Al, small letters at 0 µM Al supply. Means with similar letters are not significantly different at $P < 0.05$, Tukey-test, n = 15 (A), n = 12 (B).

Generally, the root tips of intact plants of Al-resistant cultivars showed lower Al contents than Al-sensitive cultivars, though these differences were not strongly pronounced (Fig. 2.4A).

In excised root tips (Fig. 2.4B) Al contents were generally higher than in root tips of intact plants. Owing to a high variability only 2 cultivars, BR201M and Lixis, could be statistically separated into low Al accumulating and high Al accumulating, respectively. For all other cultivars not even a tendency of lower Al contents of Al-resistant cultivars existed.

Relationships between Al-induced callose formation, root elongation and Al contents

Significant correlations between the parameters observed were only found for root tips of intact seedlings but not for excised root tips of the studied cultivars (Fig. 2.5): The Al content was negatively correlated with root elongation ($r^2 = 0.65$**). Also, a significant positive correlation ($r^2 = 0.64$**) between Al content was found and Al-induced callose formation (Fig. 2.5B) as well as between inhibition of root elongation and Al-induced callose formation ($r^2 = 0.80$***) (Fig. 2.5C).

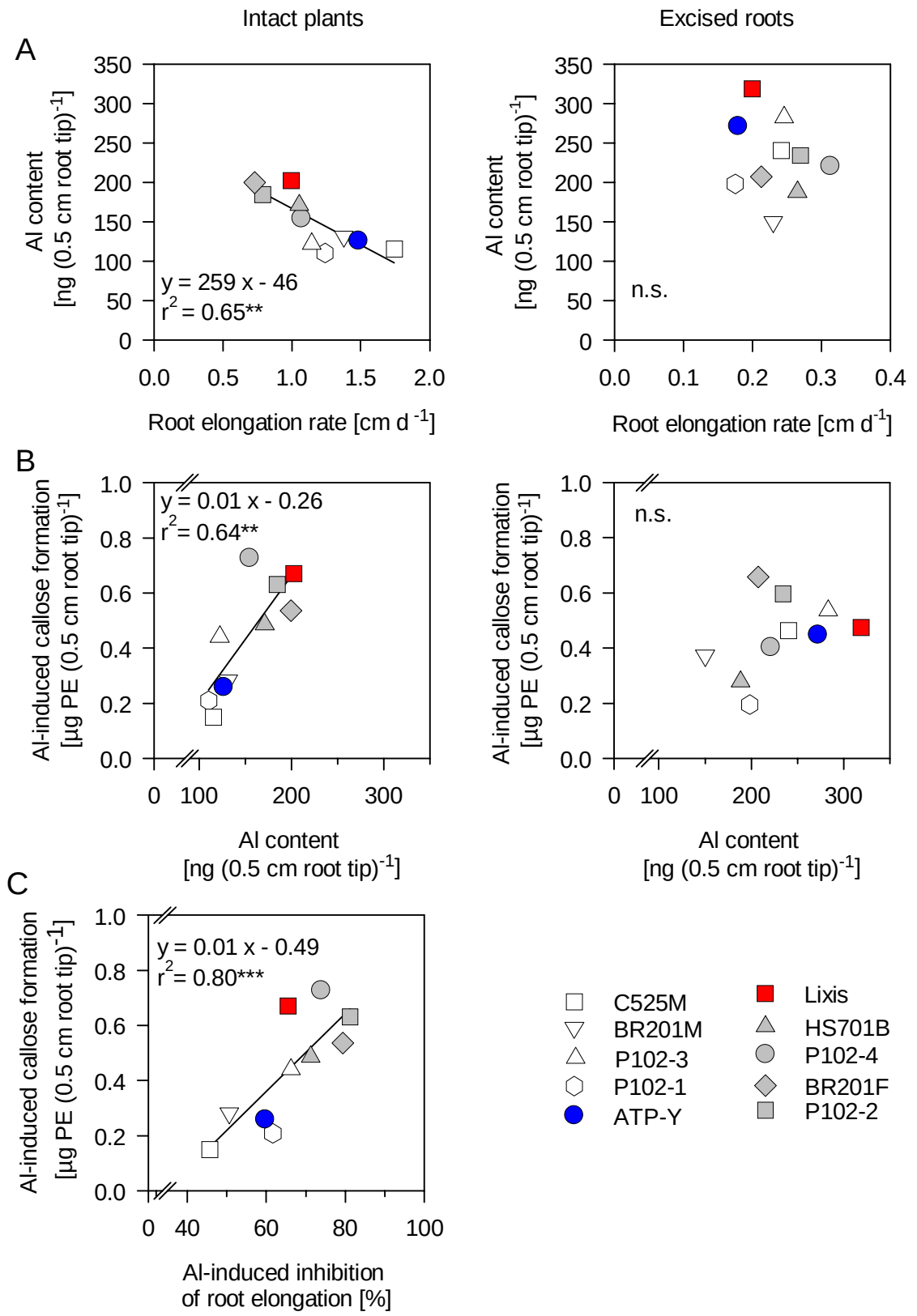

Fig. 2.5: Relationships between root elongation-rate and Al contents of root tips (A), Al contents and Al-induced callose formation in root tips (B), Al-induced inhibition of root elongation and Al-induced callose formation (C) in root tips of intact seedlings or excised root tips of maize cultivars. Root tips of intact plants and excised root tips were exposed to 25 µM Al in nutrient solution, pH = 4.3. *, **, *** denote probability levels < 0.05, 0.01, and 0.001, n.s. > 0.05.

Relationships between intact plants and excised root tips

In order to examine relationships between intact plants and excised root tips Al-induced callose formation (Fig. 2.6B) and Al contents (Fig. 2.6A) were compared between both systems. Neither significant relationships between excised root tips and tips of intact plants in Al-induced callose formation (Fig. 2.6A) nor Al contents in the root tips were present (Fig. 2.6B). While in cultivars C525M and ATP-Y Al-induced callose formation in root tips of intact plants was lower than in excised tips, in cultivars Lixis and P102-4 an opposite observation was made (Fig. 2.6A).

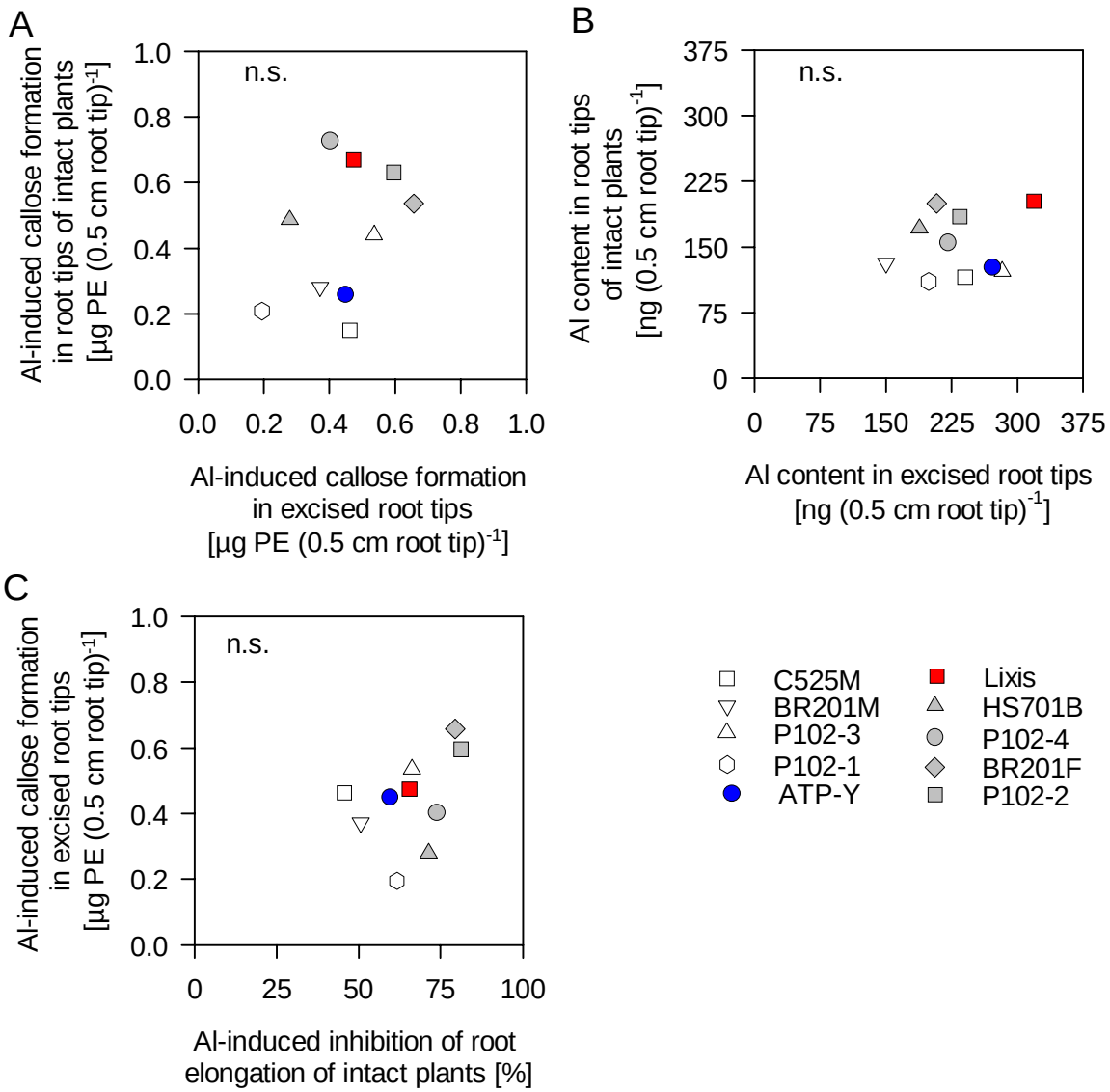

Fig. 2.6: Relationships between Al-induced callose formation (A) and Al contents (B) of intact and excised root tips of different maize cultivars. (C) shows the relationship between Al-induced inhibition of root elongation of intact plants and Al-induced callose formation in root apices of excised root tips of the same maize cultivars. Root tips of intact plants and excised root tips were exposed to 25 µM Al in nutrient solution, pH = 4.3. n.s. denotes $P > 0.05$.

Furthermore, Al-induced callose formation in excised root tips did not correspond to Al-induced inhibition of root elongation of intact plants (Fig. 2.6C) and thus did not indicate their differences in Al resistance.

Effects of additional glucose supply

To examine, whether the weak elongation of root tips and lower callose formation in excised root tips of some cultivars was due to a limited carbohydrate availability or to a low Al concentration in the incubation medium, the excised root tips were incubated in nutrient medium with increasing Al supply as well as in the presence of glucose (Glc). The concentration of monomeric Al was 6.1 ± 0.5, 17.1 ± 1.4, and 33.8 ± 9.4 µM after incubation at 10, 25 and 50 µM Al supply, respectively. Addition of glucose to the 25 µM Al supply did not alter the monomeric Al concentration, detected at a concentration of 16.6 ± 6.1 µM Al.

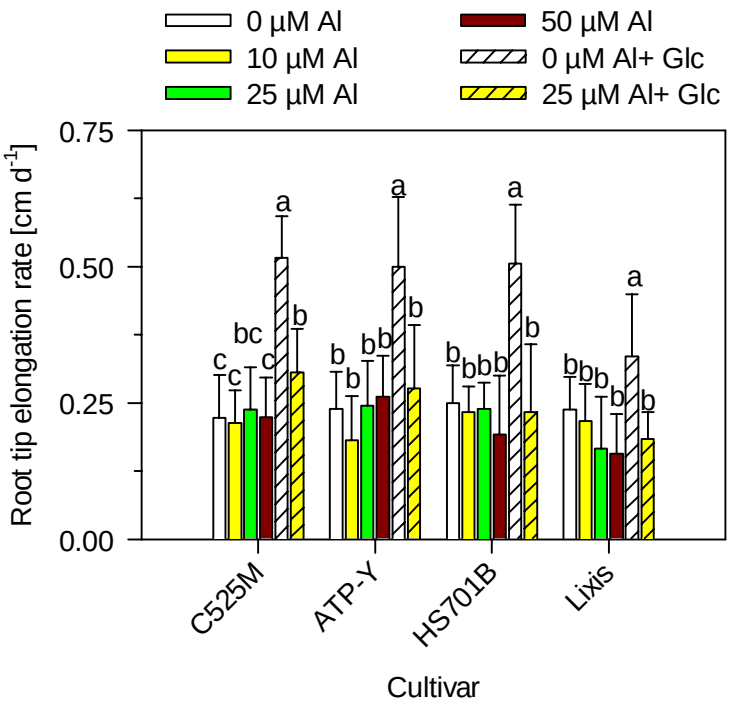

Fig. 2.7: Elongation-rate of excised root tips (2 cm) of different maize cultivars incubated in nutrient solution with varying levels of Al (0, 10, 25, 50 µM) and glucose (0, 10 mM) supply. Bars represent means ± SD. Means with similar letters are not significantly different at $P < 0.05$, Tukey-test, n = 20.

The increase of Al supply to the nutrient medium did not lead to a significant inhibition of root elongation in absence of glucose (Fig. 2.7). However, when 10 mM glucose was applied an inhibition of root elongation by Al became apparent. The supply of glucose strongly enhanced root elongation in the absence of Al confirming the assumption that in the excised roots metabolism was substrate-limited. Differences between the cultivars in response to Al in the presence of glucose were significant. However, these differences in absolute root elongation did not result in a cultivar-characteristic Al-induced inhibition of root elongation. All cultivars were similarly inhibited in root elongation by 41 - 54 % with no significant differences between the cultivars.

Not only root elongation of the excised root tips, but also Al-induced callose formation could have been substrate-limited in some cultivars. Increasing the Al concentration in the medium as well as applying glucose at 25 µM Al enhanced callose formation (Tab. 2.2). The glucose supply led to a similar or even higher level of callose formation than at 50 µM Al supply. However, the standard deviation was also increased. Neither increasing the Al supply nor glucose supplementation improved the separation of the cultivars according to their differential Al sensitivity expressed in intact plants.

Tab. 2.2: Aluminium-induced callose formation in excised and incubated root tips of different maize cultivars exposed to a varying Al and glucose supply for 24 h. Small letters indicate comparisons within one level of Al concentration to demonstrate cultivar-specific differences. MSD is stated at the bottom of the columns for each cultivar to indicate differences in treatment effects. Tukey-test, $P < 0.05$, n = 15.

	Al-induced callose formation [µg PE (0.5 cm root tip)$^{-1}$]			
	Cultivar			
Al conc. [µM]	C525M	ATP-Y	HS701B	Lixis
10	0.288 ± 0.08 c	0.418 ± 0.22 bc	0.641 ± 0.15 a	0.582 ± 0.19 ab
25	0.478 ± 0.08 c	0.657 ± 0.20 bc	1.034 ± 0.30 a	0.758 ± 0.19 b
50	0.522 ± 0.15 b	0.961 ± 0.29 a	1.175 ± 0.46 a	1.066 ± 0.32 a
25+Glc	0.944 ± 0.34 a	0.822 ± 0.72 a	1.343 ± 1.16 a	1.023 ± 0.66 a
MSD_{column}	0.174	0.421	0.660	0.399

The addition of glucose to the incubation medium led to changes of the medium pH during the incubation period as well. Those changes were most pronounced in the treatment without Al supply, where the pH rose more than one unit to pH = 5.5. A small increase in pH was also measured upon Al supply (25 µM) combined with glucose addition. After 16 h of incubation the pH reached 4.7 but monomeric Al concentration did not change. First signs of microbial colonisation of the incubation with glucose were visible. The positive effect of glucose supplementation on root growth and callose formation indicated that vitality of the excised roots tips was lost with increasing incubation time. Vitality staining of the root tips incubated for 24 h confirmed this assumption (Fig. 2.8). Therefore, it was assumed that shortening of the incubation time could lead to a better differentiation between the cultivars. Additionally, an increase in pH and possible growth of micro organisms might be excluded. Consequently, the time of incubation was varied and in addition, 10 mM glucose was added to the incubation medium (Fig. 2.9).

Fig. 2.8: Root tips of cultivar ATP-Y after incubation for 24 h and staining with TTC for vitality. Vital tips stained red. (a) 0 µM Al/ 0 mM Glc; (b) 10 µM Al/ 0 mM Glc; (c) 25 µM Al/ 0 mM Glc; (d) 0 µM Al/ 10 mM Glc; (e) 25 µM Al/ 10 mM Glc; (f) 50 µM Al/ 0 mM Glc.

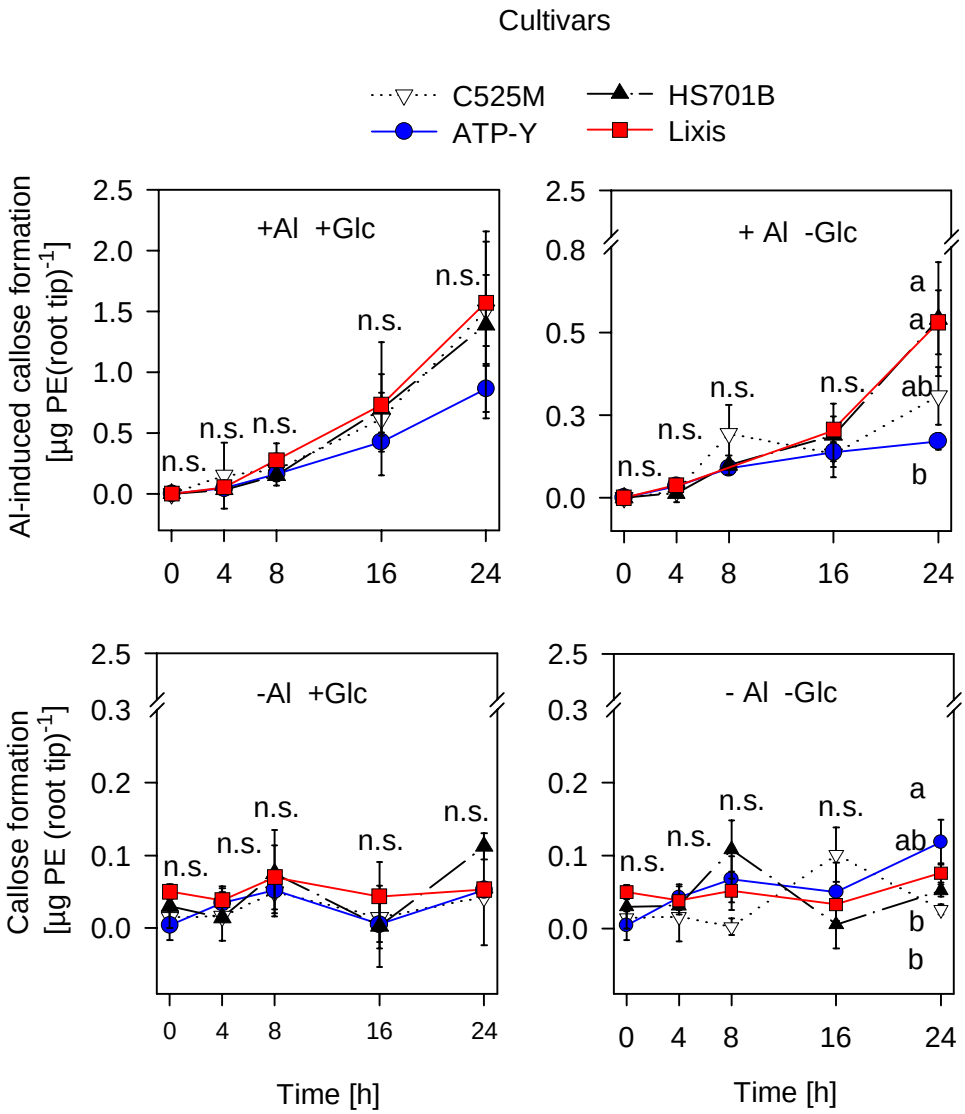

Fig. 2.9: Kinetics of Al-induced callose formation of excised root tips of 4 maize cultivars differing in Al resistance incubated in nutrient solution (0, 25 µM Al, pH = 4.3) with and without additional glucose supply (10 mM). Means with similar letters are not significantly different at $P < 0.05$ according to Tukey-test, n = 20.

The detected monomeric Al concentration of 19.0 ± 2 µM was independent of glucose supply. Callose formation at 0 µM Al was below 0.12 µg PE segment^{-1} over the whole time course of the experiment regardless of glucose supply (Fig. 2.9). In contrast, Al-induced callose formation was strongly time-dependent with or without glucose supply. After 4 h of Al supply callose contents were very low and then increased steadily over the course of the experiment. Significant cultivar-specific

differences were only found without glucose application after 24 h of incubation. The additional glucose supply led to an increased callose formation that was about 2-fold higher than without glucose supply. However, no cultivar-specific differences could be found, although the Al-resistant cultivar ATP-Y showed the lowest Al-induced callose formation. Neither a shortening of the incubation period nor the addition of glucose led to a satisfying differentiation between the Al-resistant cultivars ATP-Y, C525M and Al-sensitive cultivars Lixis and HS701B.

In the last experiment the response to Al of root tips of intact plants and excised root tips was studied in more detail in an Al-sensitive maize cultivar (Fig. 2.10). Aluminium contents were higher in the apical than in the more basal root segments. Aluminium contents in apical root sections were higher in excised compared to intact roots. In agreement with the Al concentration, Al-induced callose formation was higher in excised than in root tips of intact plants and showed a steep decreasing gradient from the apex to the base with a maximum in the 1 - 2 mm sections.

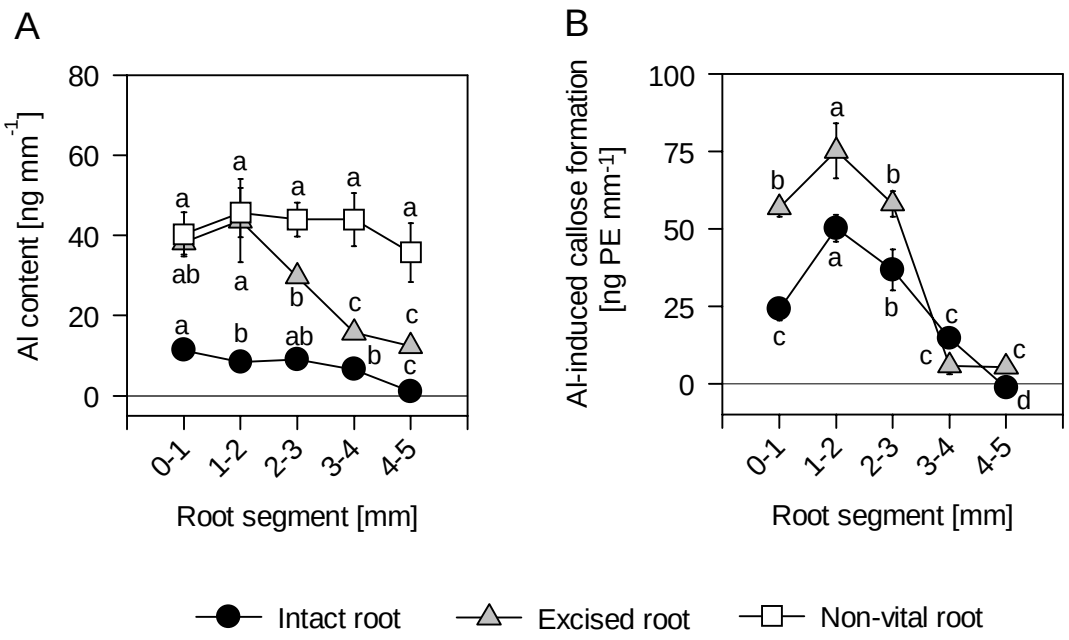

Fig. 2.10: Aluminium content (A) and Al-induced callose formation (B) of apical root segments of *Zea mays* (cv. Helix) of intact plants or excised root tips incubated (viable or dead) in nutrient solution, pH 4.3 with 50 µM Al for 3 h. Aluminium content at 0 µM was subtracted from the one at 50 µM. Similar letters indicate no significant differences between means according to Tukey-test at $P < 0.05$, n = 12.

Discussion

Intact plants

Inhibition of root elongation is one of the first reactions of plants to Al-toxicity and has been used to assess Al sensitivity of cereals (Furlani and Clark, 1981; Sapra *et al.*, 1982; Sasaki *et al.*, 1994; Horst *et al.*, 1997). Intact plants of maize cultivars could be clearly separated in their Al resistance by inhibition of root elongation (Fig. 2.2). The cultivars BR201F and Lixis were classified as Al-sensitive, while C525M and ATP-Y were rated Al-resistant, confirming previous reports on the cultivars characterisation (Llugany *et al.*, 1994, 1995; Poschenrieder *et al.*, 1995; Horst *et al.*, 1997; Comin *et al.*, 1999). The cultivars could also be separated on the basis of Al-induced callose formation in root tips per root-tip length (Fig. 2.3). This was expected, since callose formation was induced by Al in protoplasts and cell suspension culture (Staß and Horst, 1995; Wagatsuma *et al.*, 1995) as well as in root tips of intact plants (Wissemeier *et al.*, 1987, 1998; Zhang *et al.*, 1994; Budíková, 1999).

Aluminium-stressed roots were reported to be thicker than non-stressed controls (Haug, 1984; Wright *et al.*, 1989; Blancaflor *et al.*, 1998). However, an increased weight did not seem to affect differentiation of cultivars. The ranking of cultivars in callose formation was independent of their root fresh weight. An explanation is given by the fact that callose is mainly synthesised in the outer cortical layers of the root apex (Wagatsuma *et al.*, 1987, 1995; Wissemeier *et al.*, 1987, 1992; Wissemeier and Horst, 1995; Sivaguru and Horst, 1998), where Al is mostly accumulated in the rhizodermis and cortex area (Marienfeld *et al.*, 1995, 2000). The contribution of these outer cells to callose formation is most important but is only small when compared to the weight of the tips. Therefore, the use of the root segment length instead of its weight as a basis for Al-induced callose formation is justified.

The significant correlation between Al-induced callose formation and Al-induced inhibition of root elongation (Fig. 2.5C intact plants) was in agreement with findings by other authors (Llugany *et al.*, 1995; Horst *et al.*, 1997; Massot *et al.*, 1999). In this study Al-induced callose formation gave a more distinct differentiation of cultivars than inhibition of root elongation (Fig. 2.3A). However, this finding might be restricted to maize cultivars as Massot *et al.* (1999) reported that root elongation in

Phaseolus vulgaris was a more sensitive indicator of Al-sensitivity than Al-induced callose formation.

Aluminium-sensitive cultivars of maize also accumulated greater amounts of Al in the root tip region (Fig. 2.4A), which was in agreement with findings of other authors using the root tip for Al analysis (Horst *et al.*, 1990; Rincón and Gonzales, 1992; Delhaize *et al.*, 1993a; Lazof *et al.*, 1994; Massot *et al.*, 1999). As a result of cultivar-specific differences in Al resistance a highly significant relationship between Al contents and Al-induced inhibition of root elongation was found (Fig. 2.5, intact plants). However, contrasting results regarding the correlation between Al contents and root elongation have been reported for intact plants (Horst *et al.*, 1997; Massot *et al.*, 1999). An explanation might be found in the different lengths of root tips analysed. Long root segments might have a masking effect, neutralising differences in the Al contents of the tips (Blamey *et al.*, 1992; Rengel and Elliott, 1992), e.g., Horst *et al.* (1997) analysed root tips of 10 or 30 mm length, while in the present study only 5 mm root apices were used.

The results presented confirm that Al resistance is related to a reduced Al accumulation in the root tips (Fig. 2.4A). They further support the view that Al resistance might be determined by Al exclusion from cells critical to growth processes (Horst *et al.*, 1983; Lazof *et al.*, 1994; Samuels *et al.*, 1997). Furthermore, Al contents correlated with Al-induced callose formation (Fig. 2.5, intact plants) indicating that callose formation is directly related to the lesions caused by Al toxicity in the outer cell layers of the root tip (Hecht-Buchholz and Foy, 1981; Hecht-Buchholz *et al.*, 1990).

Excised root tips

In contrast to the results obtained from intact plants divergent results were obtained by the use of excised root tips. Callose formation was induced by Al in the excised root tips (Fig. 2.3B). However, the magnitude did neither reflect the genotypical differences in Al resistance found in intact plants nor the characterisation reported in the literature (Horst *et al.*, 1997). Unexpectedly, callose formation was not overall lower in excised root tips. Excised root tips of the Al-resistant cultivars C525M and ATP-Y synthesised higher amounts of callose than those of intact plants whereas root tips of the Al-sensitive cultivars P102-4, HS701B and Lixis

produced less callose when excised. Three reasons might account for this observation. Firstly, the observed differences might have resulted from substrate shortage in excised root tips. Secondly, results might have been due to an amplification of Al-toxicity effects by wounding, or thirdly, to an inactivation of an Al exclusion mechanism active in intact root tips only.

According to Saglio and Pradet (1980) the maize root does not have many carbohydrate reserves. Especially in the Al-sensitive cultivars this could have been the major limitation to root elongation and callose formation. Using excised maize roots (Brouquisse *et al.*, 1991; Dieuaide-Noubhani *et al.*, 1997) demonstrated a decrease in respiration rate immediately after excision, reaching 30 to 50 % of its initial value after 20 h. Following root excision, proteins and lipids were continuously degraded and virtually the only substrates for respiration and biosynthesis after 24 h of glucose starvation. Additionally, the concentration of nucleotides was reduced (Saglio *et al.*, 1980). Supply of exogenous sugars restored respiration in excised root tips (Morgan and Street, 1959; Saglio and Pradet, 1980; Bryce and Rees, 1985; Brouquisse *et al.*, 1991; Vucinic and Vuletic, 1995). In this study, the additional glucose feeding led to a significant increase in root segment elongation (Fig. 2.7) and a higher viability of excised root tips as revealed by TTC staining (Fig. 2.8). With glucose supplementation an Al-induced inhibition of root elongation of excised root tips was found. However, genotypical differences were only small (Fig. 2.9, Tab. 2.2). The supply of glucose also promoted Al-induced callose formation, since glucose is a substrate for glucane synthesis (Henry and Stone, 1982; Datema *et al.*, 1983; Delmer, 1987; Kauss, 1987a). However, without an additional Al supply glucose did not enhance callose formation. This observation indicates that excision itself did neither impede callose formation nor enhance callose formation. Nevertheless, the Al-resistant (ATP-Y, C525M) and Al-sensitive (Lixis, HS701B) cultivars showed no differences in Al-induced callose formation during the course of the experiment (Fig. 2.9). Excised root tips produced even higher amounts of callose than root tips of intact plants exposed to Al (Fig. 2.3). Another disadvantage of glucose supplementation was a significant rise in pH and microbial contamination of the incubation media. At a pH above 4.5 the concentration of monomeric Al decreased, which is reported to be of major importance for Al toxicity (Pavan and Bingham, 1982; Alva *et al.*, 1986b; Blamey *et al.*, 1983). Upon

decreasing the incubation period as practised by Zhang and Taylor (1989, 1991) and Zhang *et al.* (1995), hence avoiding the need of glucose supply, insufficient amounts of callose were formed by excised root tips for the expression of differences between Al-resistant and Al-sensitive cultivars (Fig. 2.9).

The lack of correlation between parameters obtained at intact plants and excised root tips might be also put down to the amplification of Al-toxicity effects by wounding. On the one hand, wound-induced depolarisation of the plasma membrane was reported (Mertz and Higinbotham, 1976; Chastain and Hanson, 1982; Stahlberg and Cosgrove, 1994; Meyer and Weisenseel, 1997). Depolarisation was also detected several mm away from the cut edge of cucumber epicotyls (Stahlberg and Cosgrove, 1994). Hence, in this study the root apex could have also been affected. The involvement of both H^+ and Ca^{2+} (Hush and Overall, 1989) as well as K^+ and Cl^- fluxes in the wound current were reported (Meyer and Weisenseel, 1997). On the other hand, the depolarisation of the root cell plasma-membrane potential is also reported upon Al exposure (Etherton *et al.*, 1983; Miyasaka *et al.*, 1989; Lindberg *et al.*, 1991; Huang *et al.*, 1992; Papernik and Kochian, 1997; Tabatake and Shimmen, 1997). Sasaki *et al.* (1995) suggested that the ability to maintain the integrity of the plasma membrane and the ability to recover its electrical balance is an important factor in determining Al resistance (Sasaki *et al.*, 1994, 1995). It is uncertain whether this phenomenon is specifically related to Al-sensitive or Al-resistant cultivars. Sivaguru *et al.* (1999) found a rapid depolarisation of the plasma membrane of the Al-sensitive maize cultivar Lixis, whereas Papernik and Kochian (1997) found it only in an Al-resistant but not in an Al-sensitive cultivar of wheat. Furthermore, also for Al toxicity an alteration of the Ca homeostasis in the cell is discussed (Kochian 1995, Rengel and Elliott, 1992; Rengel, 1996). Consequently, wounding-induced (Mertz and Higinbotham, 1976; Chastain and Hanson, 1982; Stahlberg and Cosgrove, 1994; Meyer and Weisenseel, 1997) or Al-induced alterations (Rengel *et al.*, 1995) of plasma-membrane properties combined with changes in Ca homeostasis, involved in callose formation (Kauss, 1987b, 1996; Waldmann *et al.*, 1988) could have resulted in higher callose formation especially in root tips of Al-resistant cultivars.

The lack of differences between Al-sensitive and Al-resistant cultivars in Al-induced callose synthesis in excised root tips could have also indicated a lower activity of an Al exclusion mechanism. This was unexpected because in wheat genotypes differences in Al resistance due to Al exclusion have been demonstrated in intact and excised root tips as well (Samuels *et al.*, 1997). In maize, however, Al exclusion might have been severely disturbed, since the Al content of excised root tips was much higher than of intact root tips not only after long-term (Fig. 2.4B) but also after short-term Al treatment (Fig. 2.10). Since it was demonstrated that the exclusion mechanism is under metabolic control (Zhang and Taylor, 1991; Zhang *et al.*, 1995; Basu *et al.*, 1997), Al exclusion might have failed in excised root tips. The substrate limitation of excised maize root tips has been discussed above.

Conclusively, use of Al-induced callose formation as an indicator for Al sensitivity was confirmed for intact plants. The assessment of cultivar-specific differences in Al resistance could not be improved by determining Al-induced callose formation in excised root tips. This might be explained by excision-induced callose formation and/or substrate limitation of metabolic processes involved in callose formation, Al exclusion and cell viability in general.

3 Characterisation of maize inbred lines (*Zea mays* L.) in Al resistance by assessment of root elongation and callose formation

Abstract

The prospects and limits of prestaining with neutral red was studied for assessing genotypic differences in Al-induced inhibition in roots of maize in nutrient solution at 0 and 25 µM Al. New root growth occurring after the staining could be distinguished from the stained portion of the pre-treated root system. In a first step the staining technique was calibrated using 3 maize cultivars as standards. Root measurements demonstrated cultivar-specific differences in inhibition of root elongation at 24 h as well as 48 h after Al exposure. These differences were more clearly expressed using the staining procedure than measurements of the whole root axis length at the beginning and the end of the treatment period. Furthermore, the staining with neutral affected roots less than the two measurements with a ruler as indicated by a higher total root length. Neutral red did not affect determination of monomer Al and Al-induced callose formation.

In a next step 110 maize inbred lines were exposed to nutrient solution containing Al in concentrations of 0 and 25 µM. After 48 h root elongation was measured and root tips were sampled for callose analysis. A large genotypic variation in inhibition of root elongation (10 - 70 %) as well as in Al-induced callose formation (0.05 - 1.5 µg PE root tip^{-1}) was found. The lines could be separated into Al-resistant and Al-sensitive on the basis of root elongation much better than by Al-induced callose formation. The relationship between both parameters was only small (r^2 = 0.16***). The reasons for this findings were studied. Aluminium-induced callose formation followed a cultivar-specific curvature within the experimental period of 48 h, reaching a peak after 6 - 12 h. Thereafter, callose contents in general decreased in the maize inbred lines until only marginal amounts were found after 24 h. However, in one inbred line Al-induced callose formation continued to increase up to 48 h. Al-induced callose formation and inhibition of root elongation were positively correlated at 12 h (r^2 = 0.49*) as well as after 24 h (r^2 = 0.55*), but not after 48 h of Al treatment. These relationships were even stronger when Al-induced callose forma-

tion was expressed relative to digitonin-induced callose formation, especially after 12 h ($r^2 = 0.85$***). The results obtained from callose formation and inhibition of root elongation were in agreement with regrowth of the same lines after exposure to various levels of Al supply for 48 h.

Introduction

Aluminium-induced callose formation has been found to serve as an indicator for Al stress in several crop species, showing a significant correlation with inhibition of root elongation (Wissemeier *et al.*, 1987; Horst *et al.*, 1997; Massot *et al.*, 1999). Aluminium-induced callose formation steadily increased over 48 h in wheat (Zhang *et al.*, 1994). In maize, cultivar-specific differences could be determined as long as after 36 h of Al treatment (Püschel 1996, personal communication). However, callose formation might be impaired in some cases (Larsen *et al.*, 1996). Elicitation of callose synthesis by digitonin is independent from described Al resistance mechanisms as changes in pH (Miyasaka *et al.*, 1989; Degenhardt *et al.*, 1998), the exudation of organic anions (Delhaize *et al.*, 1993b; Pellet *et al.*, 1995; Ma, 2000) or other chelators (Horst *et al.*, 1982; Basu *et al.*, 1994). Therefore, eliciting callose formation by another reagent differing in injury from Al toxicity effects should be suitable to reveal the potential for callose formation. Because of its capacity to induce callose synthesis (Waldmann *et al.*, 1988; Kauss *et al.*, 1991; Kauss and Jeblick, 1991; Messiaen *et al.*, 1995) digitonin should be suitable to reveal the potential for callose formation also in maize plants as demonstrated for suspension cultures before (Schmohl and Horst, 2000; Schmohl *et al.*, 2000).

Inhibition of root elongation, one of the first reactions to Al toxicity has often been used to assess Al resistance of plants (Clarkson, 1965; Furlani and Clark, 1981; Sapra *et al.*, 1982; Godbold and Kettner, 1991; Horst *et al.*, 1992, 1997; Sasaki *et al.*, 1994). It is generally accepted as a parameter of Al toxicity effects, regardless of the time at which readings are taken. First assessment of new germplasm might be therefore more appropriate using Al-induced callose formation combined with inhibition of root elongation as a generally accepted standard. However, measurements of root elongation required to measure at the beginning and the end of the treatment period and carry the risk of damaging the root. Schumacher *et al.* (1983) described a method using the vital stain neutral red chloride to measure

root elongation rate of *Phaseolus vulgaris* and *Glycine max* in nutrient solution. The staining did neither affect root weight, root-respiration rate nor shoot weight. To my knowledge, this method has not been used under the conditions necessary to assess Al resistance in maize. Particularly, it was not known whether the method might influence Al-induced callose formation or affect concentration of monomeric Al in the nutrient solution.

The following study describes a series of experiments in which (i) the usefulness of prestaining in screening was evaluated (ii) inbred lines were characterised in Al resistance by Al-induced inhibition of root elongation and callose formation and (iii) callose synthesising capacity and effects of treatment duration on callose formation were evaluated.

Materials and methods

Plant material and growth conditions

Seeds of maize (*Zea mays* L.) were germinated between sheets of filter paper, soaked with 1 mM $CaSO_4$ solution in an upright position using a sandwich technique for 4 days. Thereafter, morphologically similar seedlings were grown in nutrient solution under controlled environmental conditions of a 16/ 8 h day/ night cycle, 27/ 25 °C day/ night temperature, 75 ± 5 % relative air humidity and a photon flux density of 230 µmol m^{-2} s^{-1} photosynthetic active radiation (Sylvania Cool White, 195 W, Philips, Germany) as measured in mid plant height. At the fifth day the pH was adjusted gradually to 4.3 over a period of 16 h. The pH was kept constant using pH-stat during the experiment and, if necessary, adjusted using 0.1 M HCl or 0.1 M KOH. The composition of the nutrient solution was [µM]: KNO_3 400, $CaSO_4$ 250, NH_4NO_3 200, $MgSO_4$ 100, Fe-EDDHA 20, KH_2PO_4 10, H_3BO_3 8, $MnSO_4$ 1, $ZnSO_4$ 0.2, $CuSO_4$ 0.2, $(NH_4)_6Mo_7O_{24}$ 0.1.

Determination of the inhibition of root elongation

Root elongation was determined either by measuring root length at the beginning and end of the experiment using a 1 mm scale or using prestaining of roots with neutral red (0.5 g l^{-1} deionised water, pH 5.5 adjusted with 0.1 M HCl) according to

Schumacher *et al.* (1983): Roots of intact plants were placed into neutral red staining solution for 5 min, rinsed with deionised water, and transferred to pots containing nutrient solution with or without Al. Root elongation was then determined as the distance between the root apex and the beginning of the staining which was still visible after 4 days. Aluminium-induced inhibition of root elongation was calculated as described by Sapra *et al.* (1982):

$$\text{Al-induced inhibition of root elongation} = 100 - \left(\frac{RL_{+Al}}{RL_{-Al}} * 100 \right) [\%]$$

RL_{-Al}: root elongation at 0 µM Al

RL_{+Al}: root elongation at 25 µM Al

Analysis of nutrient solution

Monomeric Al in the nutrient solution was determined using the pyrocatecholviolet (PCV) method of Kerven *et al.* (1989), in samples of 3 ml nutrient solution with a photometer (photometer (Lambda 15, Perkin Elmer, Wellesley, MA, USA) at λ = 580 nm after 60 sec. Standard solutions were prepared in the range of 0 - 40 µM Al from an $AlCl_3$ atomic absorption standard stock solution.

Callose quantification

At harvest, plant roots were rinsed with deionised water, 1 cm root tips were excised using a razor blade, and fixed immediately in ethanol (96 %). For analysis, tips were rinsed with deionised water and transferred to Eppendorf cups containing 1 ml of 1 M sodium hydroxide solution and stored at -20 °C overnight. After defrosting, samples were homogenised with an ultrasonic device (Bandelin sonoplus HD70 with microtip MS 72 D, Bandelin Electronics, Berlin, Germany) for 45 s. To extract callose, homogenised samples were heated at 80 °C in a water bath for 30 min. Thereafter, cooled samples were centrifuged at 10^3 x g for 15 min (Sorvall MC12V, Du Pont de Nemours & Co. Inc. Newton, Conn., USA). Callose was then determined according to Kauss (1989) and Köhle *et al.* (1985) using waterblue as stain (waterblue, Fluka, Deisenhofen, Germany). The callose sirufluor complex was measured with a Hitachi F 2000 fluorescence spectrometer (Hitachi, Tokyo, Japan; excitation λ = 394 nm, emission λ = 484 nm, slit 10 nm, Voltage

U = 700 V). Pachyman (Calbiochem, Deisenhofen, Germany) was used as calibration standard. Hence, callose content was expressed as pachyman equivalents (PE) per root tip.

Experiment I

A. Seeds of three cultivars of known Al resistance (ATP-Y, C525M, Al-resistant; Lixis Al-sensitive) were germinated as described above and placed into nutrient solution, containing Al in concentrations of 0 and 25 µM Al for 24 and 48 h. Root growth was measured either with a ruler or neutral red prestaining. Additionally, root tips of 0.5 cm length were cut for callose analysis.

B. In the following root elongation and Al-induced callose formation in root tips were determined after 48 h in Al treatment at 25 µM for 110 inbred lines. Root growth of inbred lines was less than of 1.5 to 2 cm d^{-1} as reported for maize (Konzak *et al.*, 1976 and own observation). Therefore, root elongation was measured after 48 h using prestaining with neutral red.

Experiment II

A set of 8 out of the 110 inbred lines, characterised in their adaptation to an Al-toxic soil by CIMMYT Colombia (Tab. 3.1), and the cultivars Lixis (Al-sensitive) and ATP-Y (Al-resistant) were exposed to Al (0, 25 µM) or to 10 µM of digitonin in nutrient solution to determine the potential for callose formation. Root elongation was measured after 6, 12, 24 and 48 h of Al treatment using a ruler. At the same time root tips of 0.5 cm length were cut for Al-induced and digitonin-induced callose analysis.

Tab. 3.1: Characterisation of 8 inbred lines in their adaptation to an acid Al-toxic soil in Colombia.

No	Line	Pedigree	Adaptation to soil acidity
1	66	SA-4 C2HC7-1-5-1-3-4-7-BB	tolerant
2	14	SA-4 C2HC7-1-4-1-2-1-7-BB	tolerant
3	21	SA-4 C2HC7-1-4-1-2-5-2-BB	susceptible
4	28	SA-4 C2HC7-1-4-1-2-10-5-BB	susceptible
5	70	SA-4 C2HC7-1-5-1-3-7-5-BB	moderately tolerant
6	98	SA-5 C2HC1-3-9-2-1-3-4-BB	moderately tolerant
7	68	SA-4 C2HC7-1-5-1-3-7-3-BB	moderately susceptible
8	1	SA-4 C2HC7-1-4-1-1-1-1-BB	moderately susceptible

Additionally, the same cultivars/ lines were exposed to increasing levels of Al supply (10, 20, 30, 40, 50, 60 µM) for 24 h. Thereafter, roots were rinsed with deionised water and transferred to Al-free solution. After 48 h root tips were stained with hematoxylin as described by Aniol (1984, 1990). Hematoxylin is binding to Al. The root regrowth was measured as the unstained portion of the root system using a 1 mm scale.

Experimental design and statistical analysis

A completely randomised design was used with two replicates for each Al and digitonin treatment. Data were statistically analysed using SAS 6.10 (SAS Institute Inc., Cary, NC, USA). Prechosen comparisons were used. After analysis of variance (Proc GLM) the means were compared using the Tukey-test or Dunnett-test with adjustment to multiple comparisons when necessary.

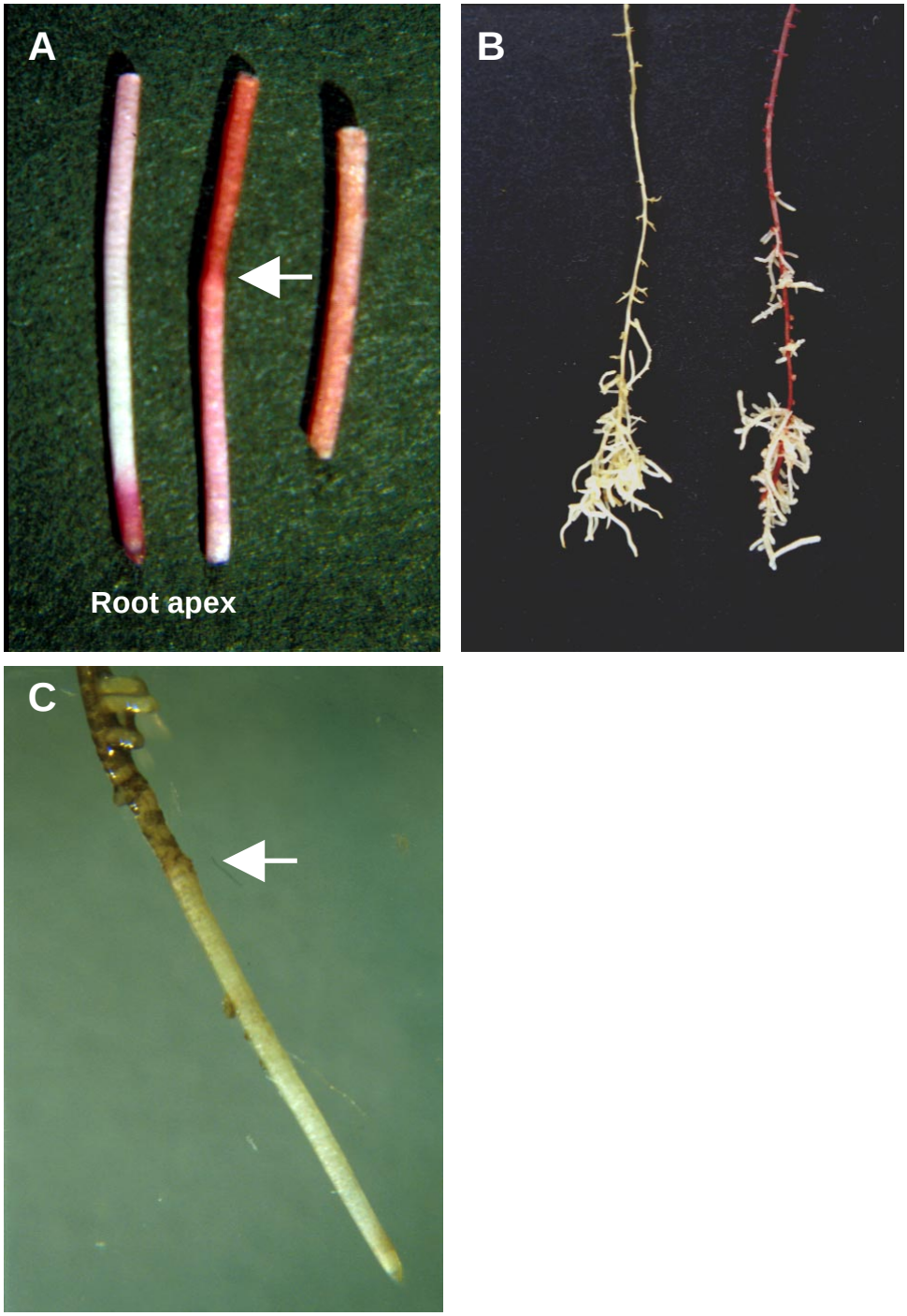

Fig. 3.1: (A) Neutral red-stained apical root segment. Root apex on the left handed side. Arrow indicates where readings for root elongation were made. (B) Unstained and neutral red-stained root after exposure to 25 µM Al for 4 days. (C) Hematoxylin staining of new root regrowth after 48 h in Al-free solution following the exposure to 25 µM Al for 24 h. Arrow indicates where readings for root elongation were made.

Results

Neutral red prestaining to determine root elongation

Prestaining was less time-intensive than using a ruler. The staining at the beginning of the experiments took about 10 min. In contrast, measuring root elongation of the same number of plants with a 1 mm scale took about 2.5 h. Erroneous assignment of measured lengths from the beginning and the end of the experiment did not take place because observations were only made once at the end of experiment. This led to a considerable decrease in the variation of root elongation (Fig. 3.2).

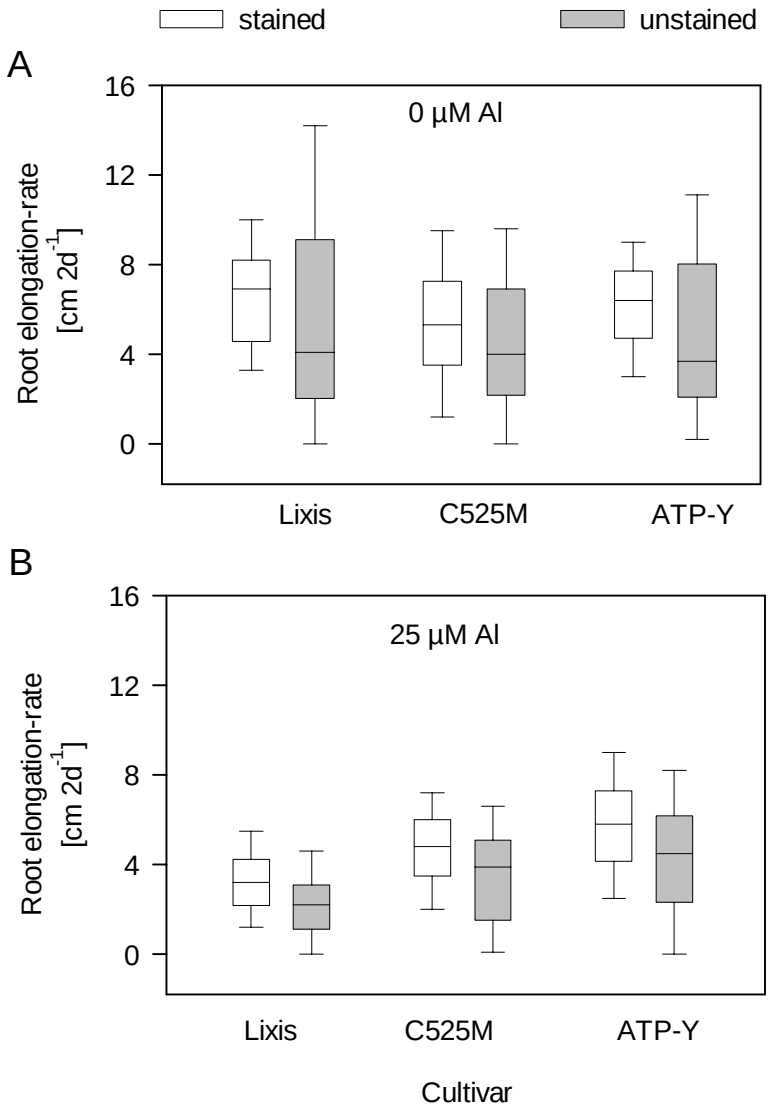

Fig. 3.2: Root elongation-rate of three maize cultivars at 0 and 25 µM Al in nutrient solution (pH = 4.3) with or without neutral red prestaining. Box whisker plots represent lower (0.25) and upper quartile (0.75) and median within the plotted boxes.

The staining led to a higher total root length which was most expressed at 0 µM Al (Fig. 3.3).

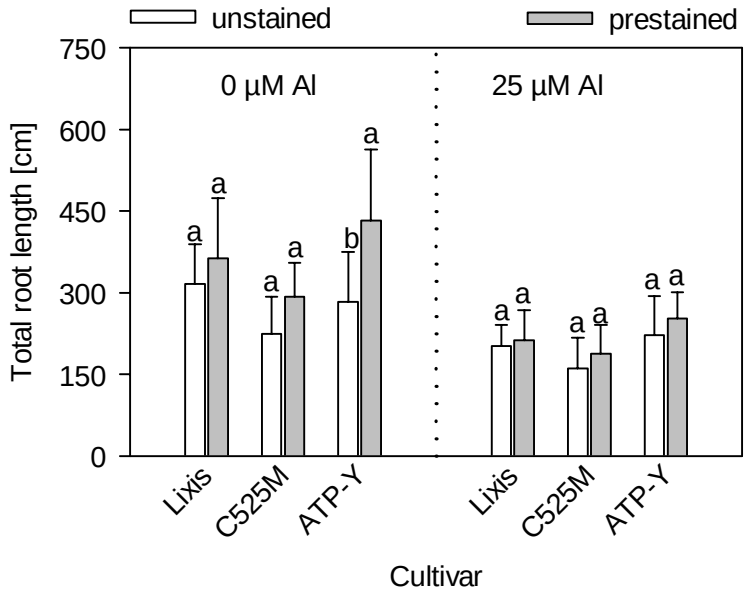

Fig. 3.3: Total root length of three maize cultivars 48 h after either pertaining with neutral red or left unstained and root length measured with a ruler at two levels of Al supply (0, 25 µM) in nutrient solution, pH = 4.3. Bars represent means ± SD, n = 20. Comparisons were conducted separately for each cultivar and Al concentration with Tukey-test at $P < 0.05$. Means with similar letters are not significantly different.

Dye residues of neutral red led to a visible colouring of the nutrient solution. This contamination was determined to be in the range of 0.2 - 0.32 µM neutral red. However, the intercept of the Al calibration curve was significantly increased only when neutral red concentration was above 0.32 µM. The slope of the regression curve remained similar.

Aluminium application inhibited root elongation significantly after 24 h and 48 h, irrespective of the neutral red staining. Tap and basal root did not differ significantly in the response to Al toxicity. Therefore, they were pooled within the comparisons of means (Fig. 3.4). The three cultivars used were assessed similarly in Al resistance using either method to determine root elongation. The cultivar Lixis

showed a higher inhibition of root elongation than the cultivars C525M and ATP-Y. Using prestaining, the two Al-resistant cultivars could be further differentiated: The cultivar ATP-Y proved to be more Al-resistant than C525M.

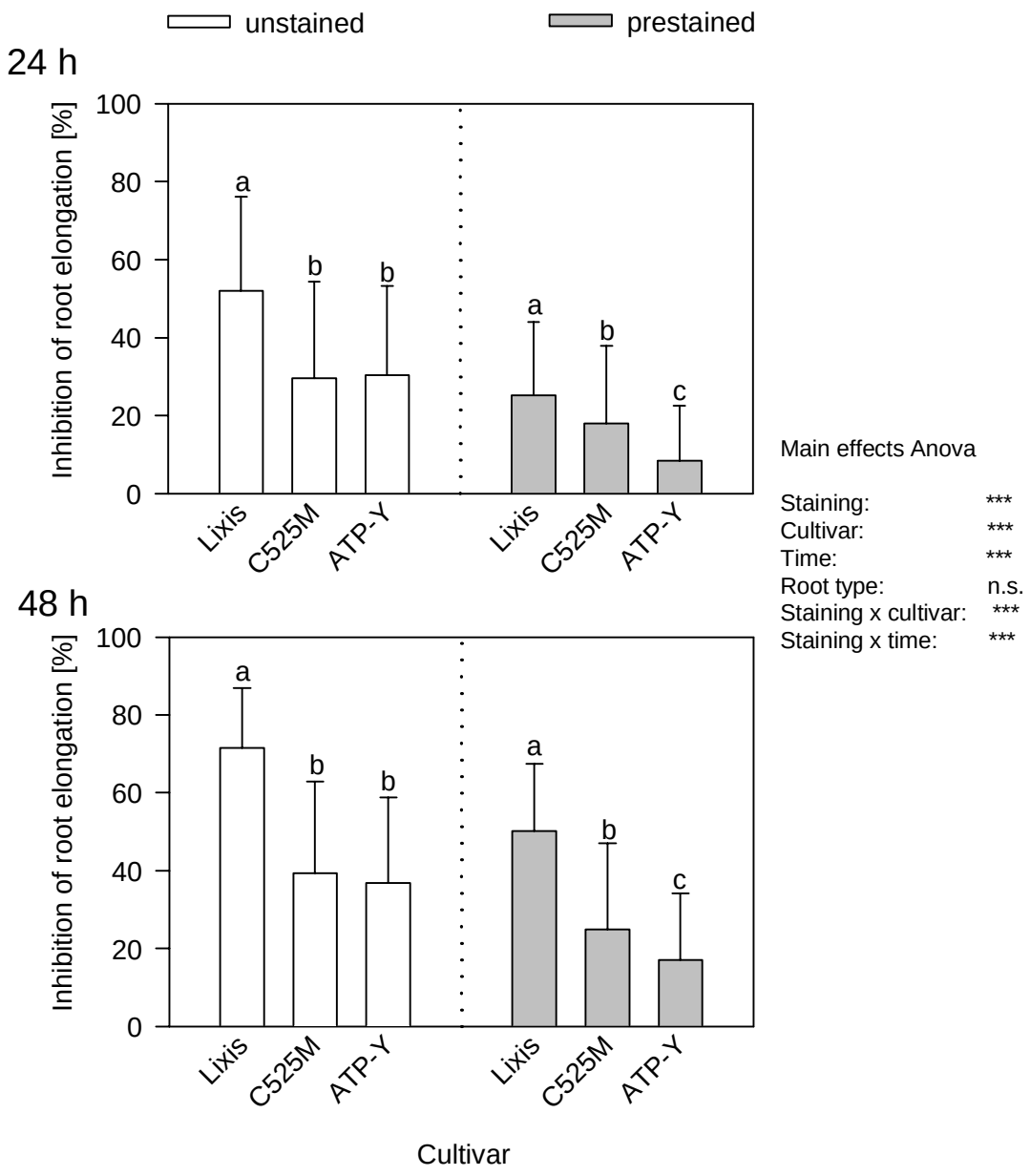

Fig. 3.4: Inhibition of root elongation of three maize cultivars in nutrient solution at 25 µM Al, pH = 4.3 after 24 h (A) and 48 h (B) of treatment using staining with neutral red or measurements with a ruler for estimation of root elongation. Bars represent means ± SD, n = 20. Comparisons of means between cultivars for each method separately with Tukey-test at $P < 0.05$. Means with similar letters are not significantly different.

Neutral red and Al-induced callose formation

Neither Al-induced callose formation nor determination was influenced by prestaining with neutral red (Fig. 3.5). Storing the root tips in ethanol entirely removed the dye. Aluminium-induced callose formation was similar in the order of magnitude, irrespectively of the method used for determining root elongation. The treatment duration (time) significantly affected Al-induced callose formation. Aluminium-induced callose formation was higher after 24 h than after 48 h of exposure to 25 µM Al. Nevertheless, the cultivars ranking in Al resistance remained unaffected. In agreement with the inhibition of root elongation the Al-sensitive cultivar Lixis showed a higher Al-induced callose formation than the Al-resistant cultivars C525M and ATP-Y. For these two cultivars Al-induced callose formation was only half that of Lixis.

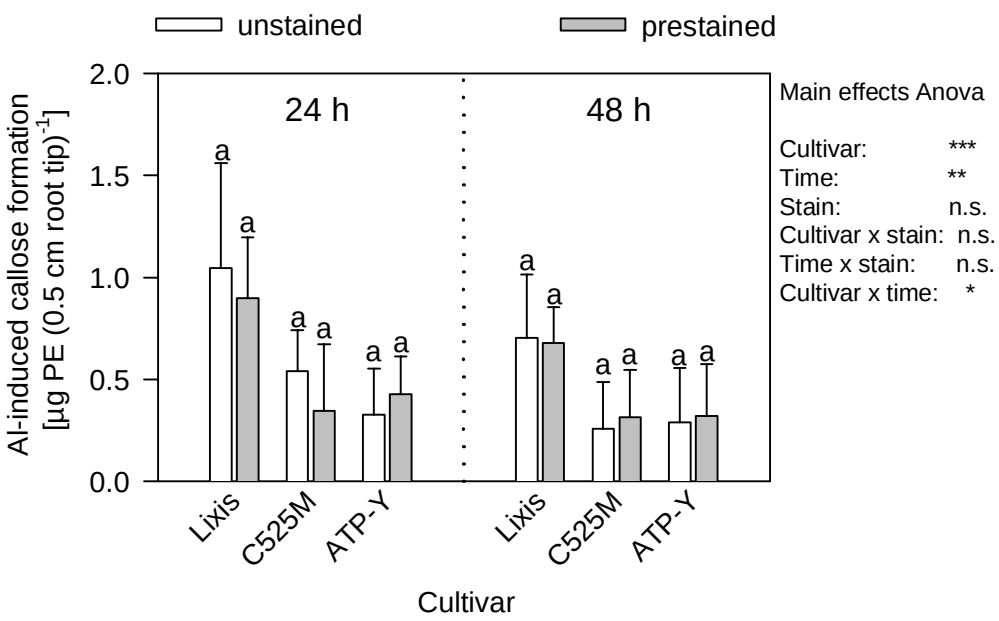

Fig. 3.5: Effect of neutral red prestaining of roots on Al-induced callose formation of three maize cultivars after 24 and 48 h in nutrient solution, pH = 4.3 with 25 µM Al. Bars represent means ± SD, n = 20. Comparisons of meanswere conducted between cultivars for 24 h and 48 h separately, using Tukey-test, $P < 0.05$. Means with similar letters are not significantly different.

Characterisation of inbred lines using inhibition of root elongation and Al-induced callose formation as parameters

Based on the results with pre-stained cultivars 110 lines were assessed in their sensitivity to 25 µM Al. A nominal concentration of 25µM Al resulted in a monomeric Al concentration of 21 ± 1.2 µM. A wide variation in the inhibition of root elongation assessed with the prestaining method was found for the inbred lines (Fig. 3.6). Generally, the reaction to Al did not depend on the root type (Fig. 3.6). A significant (r^2 = 0.47***) relationship between the root types, tap and basal roots, was found. Only three lines, namely line 83, 89 and 86, showed a divergent reaction. In these cases, inhibition of tap root elongation was more than 2-fold higher than that of basal roots. The exclusion of these 3 lines increased r^2 to 0.64***.

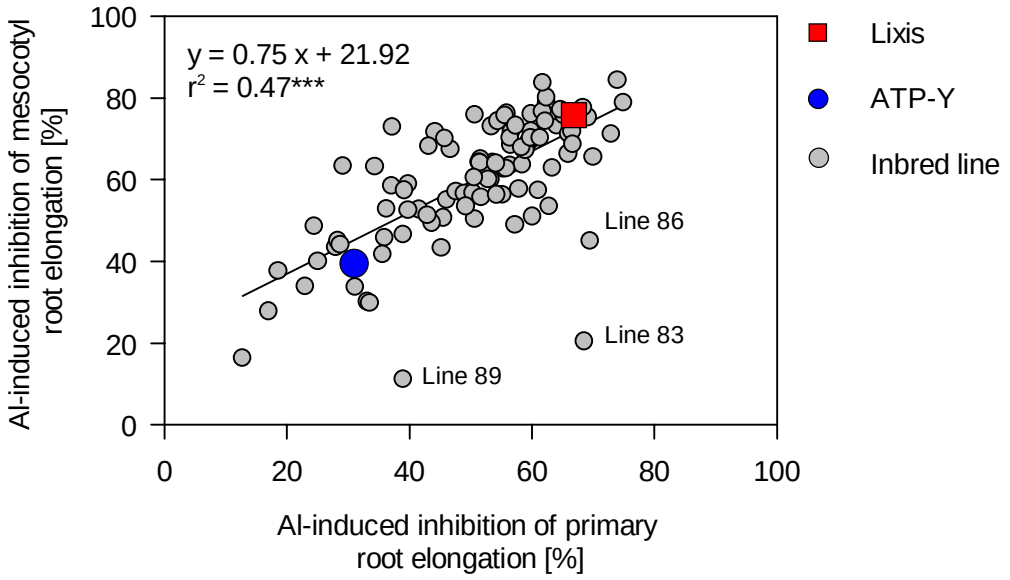

Fig. 3.6: Relationship between Al-induced inhibition of tap root and basal root elongation of inbred lines at 25 µM Al supply in nutrient solution (pH = 4.3) after 48 h. Al-resistant maize cultivar ATP-Y and Al-sensitive maize cultivar Lixis included as checks. *, **, *** denote probability levels < 0.05, 0.01, and 0.001, n.s. > 0.05.

Aluminium-induced inhibition of tap root elongation ranged from 6 % to 78 %. According to the inhibition of root elongation the inbred lines could be differentiated into several distinct groups as shown in table 3.2. The cultivars ATP-Y and Lixis

were clearly separated as Al-resistant and Al-sensitive, respectively. However, the previous assessment of the 8 inbred lines (lines 1, 14, 21, 28, 66, 68 70, 98) in their adaptation to an acid soil in Colombia did not correspond with the inhibition of root elongation in Al-toxic nutrient solution. Only inbred line 66 was rated Al-resistant in nutrient solution and was also characterised as adapted to acid soils. In contrast to their poor rating in adaptation to acid soil, the inbred lines 21, and 28 showed an Al-induced inhibition of root elongation that was significantly less than that of the Al-sensitive cultivar Lixis.

Tab. 3.2: Characterisation of Al resistance of inbred lines according to inhibition of root elongation in nutrient solution at two different levels of Al supply (0, 25 µM) at pH = 4.3 measured with neutral red prestaining based on primary root growth. The inbred lines are listed according to the magnitude of root length inhibition within the column.

Inhibition of root elongation	Inbred lines/ cultivars
10 – 20 %	27, 24, 29
21 – 30 %	13, 58, 21, 16, 18, 66, 25, **ATP-Y**
31 – 40 %	60, 17, 59, 80, 85, 64, 52, 23, 94, 89,63, 62, 2, 28
41 – 50 %	20, 57, 100, 53, 47, 65, 100, 53, 47, 65, 51, 73, 14, 31, 55, 84, 111
51 – 60 %	98, 108, 33, 44, 61, 99, 26, 110, 22, 70, 68, 39, 7, 19, 101, 112, 67, 109, 40, 93, 88, 37, 4, 3, 1, 42, 6, 105, 103, 106, 48, 30, 15, 41, 10, 95, 9, 8,
61 – 70 %	54, 3, 96, 102, 38, 77, 35, 82, 69, 11, 34,12, 91, 5, 78, 45, 72, 104, **Lixis**, 90, 83, 76, 8, 6, 4
> 70 %	92, 32, 36

A wide variation in Al-induced callose formation was found. The cultivars ATP-Y and Lixis were rated in agreement to previous studies in the Al-induced reduction of root growth and callose formation as Al-resistant and Al-sensitive, respectively. For the inbred lines a significant correlation of root-growth inhibition and Al-induced callose contents in root tips was found (Fig. 3.7). However, the relationship was not very strong: A number of inbred lines with a high inhibition of root growth showed only low Al-induced callose formation and vice versa.

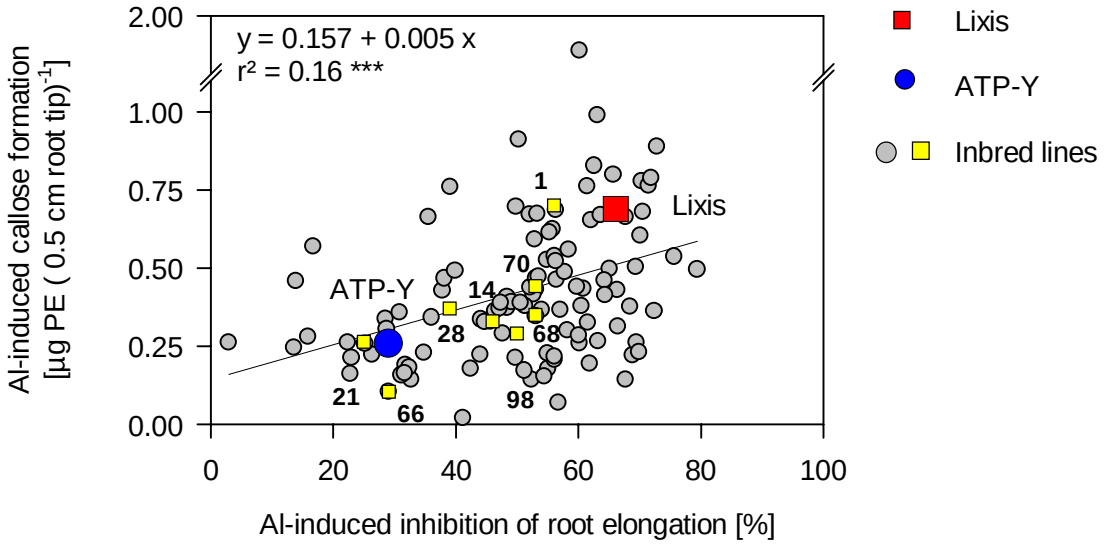

Fig. 3.7: Relationship between Al-induced inhibition of root elongation and Al-induced callose formation in root tips of intact plants of 110 maize lines subjected to 25 µM Al for 48 h in nutrient solution at pH = 4.3, n = 10. Cultivars ATP-Y (Al-resistant) and Lixis (Al-sensitive) were included as checks. Numbers refer to the inbred lines in tab. 3.1. *, **, *** denote probability levels < 0.05, 0.01, and 0.001, n.s. > 0.05.

Kinetics of Al-induced callose formation and its relationship to inhibition of root elongation

In a subsequent experiment kinetics of callose formation were studied in a subset of maize inbred lines and cultivars (Fig. 3.8). Aluminium-induced callose formation increased reaching a maximum between 6 and 12 h of Al exposure. Thereafter, it decreased in most of the lines (line 21, 66, 68, 70, 98). However, in some inbred lines the peak at 12 h was less pronounced (line 14, 28) or even not detectable; e.g. in line 1 highest Al-induced callose formation was found at 48 h and not at 12 h. The order of magnitude in Al-induced callose formation was similar for inbred lines and the two cultivars Lixis and ATP-Y.

Digitonin-induced callose formation was up to 3-fold higher than Al-induced callose formation. Especially in the cultivars ATP-Y and Lixis, digitonin-induced callose formation was higher than in the inbred lines (except line 68). In contrast to Al-

induced callose formation, no decrease in digitonin-induced callose formation occurred. Digitonin-induced callose formation followed a saturation curve, the increase after 12 h was negligible. After 6 h of Al treatment inhibition of root elongation did not show consistent differences between the cultivars. Cultivar ATP-Y and Lixis showed similar reductions in root elongation of 25 %. Following 12 h of Al treatment the rating of cultivars in their inhibition of root growth remained similar. The Al-sensitive cultivar Lixis showed a higher inhibition of root elongation than the Al-resistant cultivar ATP-Y.

Aluminium-induced inhibition of root elongation correlated significantly with Al-induced callose formation found after 12 h of Al treatment (Fig. 3.9). After 6 h and 48 h such a correlation did not exist. After 12 h of Al exposure the significant relationships between inhibition of root elongation and Al-induced callose synthesis could be improved when Al-induced callose formation was related to the plants potential in callose formation upon digitonin exposure (relative callose formation, digitonin = 100 %). The use of relative callose formation led to a similar ranking of the lines as when Al-induced callose formation was used. Differences in the ranking existed, but only for those lines expressing low callose formation. Those lines with high Al-induced callose formation and high inhibition of root elongation where rated similarly using either of the calculations.

The inbre lines 21 and 28 were rated Al-resistant according to Al-induced callose formation and inhibition of root elongation which was confirmed by the regrowth technique (Fig. 3.10). To allow a comparison between cultivars the lethal Al concentration was set at a regrowth rate lower than 5 mm $2d^{-1}$. According to the measurement of regrowth ATP-Y was rated Al-resistant and cultivar Lixis as Al-sensitive. Aluminium concentrations necessary to inhibit root regrowth of line 21 and 28 were much higher than for Al-sensitive cultivar Lixis and similar to the concentration for line 14, rated as tolerant in adaptation to acid soils.

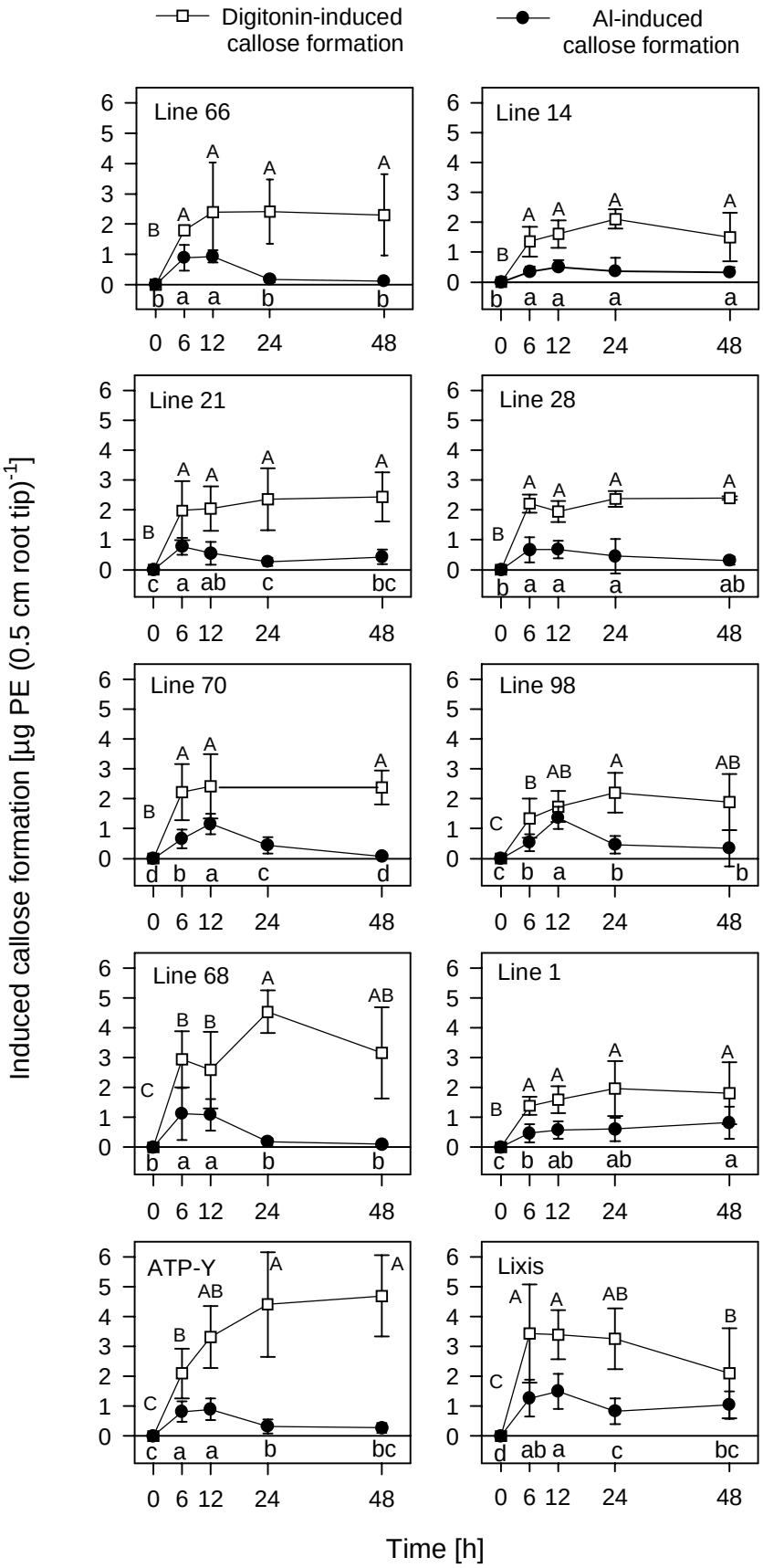

Fig. 3.8: Kinetics of Al- (25 µM) and digitonin-induced (10 µM) callose formation up to 48 h in root tips of 8 maize inbred lines in nutrient solution at pH = 4.3, n = 15. The cultivars ATP-Y (Al-resistant) and Lixis (Al-sensitive) were included as standards. Means with similar letters are not significantly different at $P < 0.05$, Tukey-test.

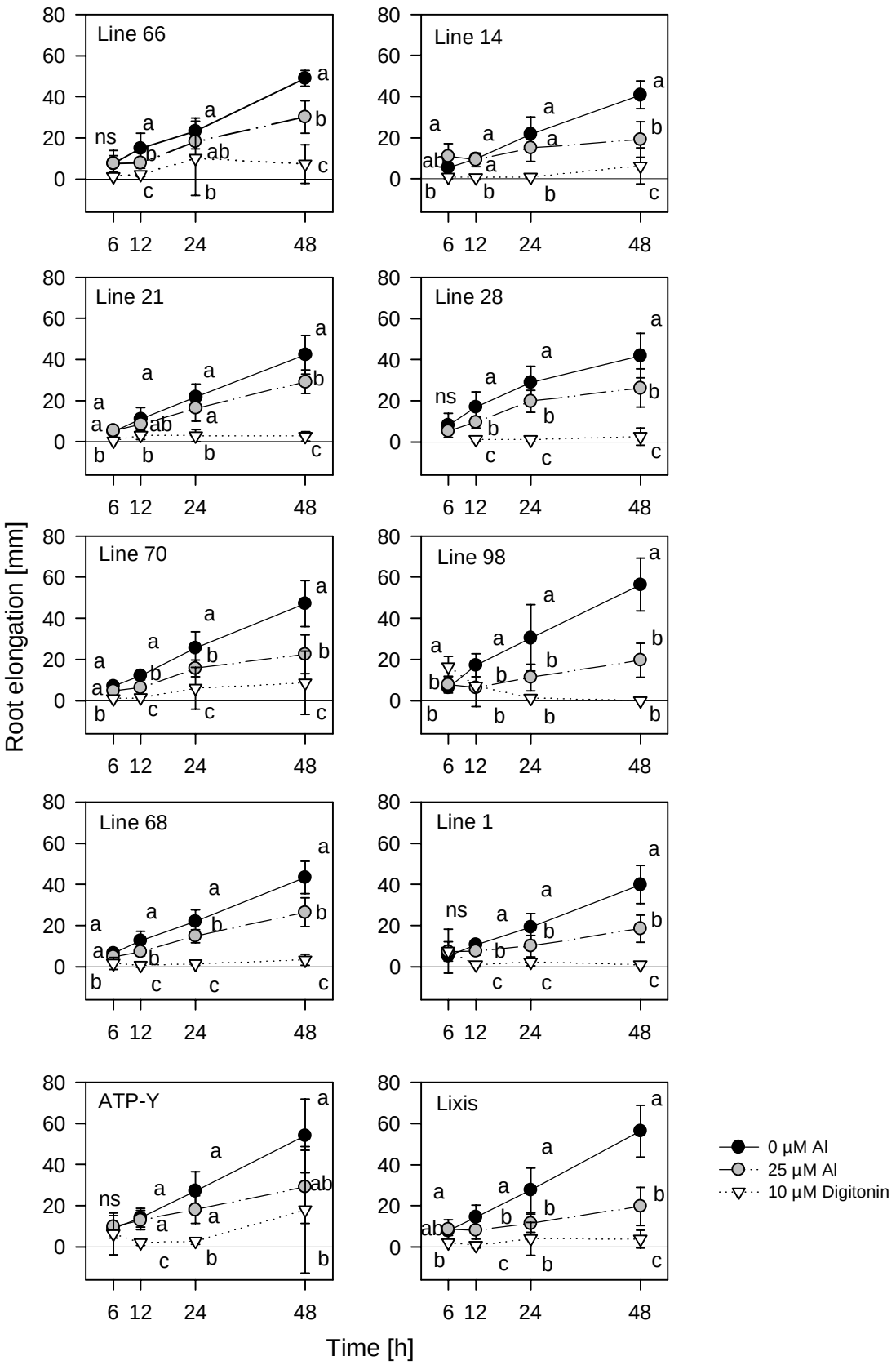

Fig. 3.9: Effect of 0 and 25 µM Al and digitonin supply (10 µM) on root elongation of intact maize cultivars and inbred lines in nutrient solution (pH = 4.3) after 6, 12, 24, and 48 h. Symbols represent means ± SD. Means with similar letters are not significantly different at $P < 0.05$, Tukey-test, n = 15.

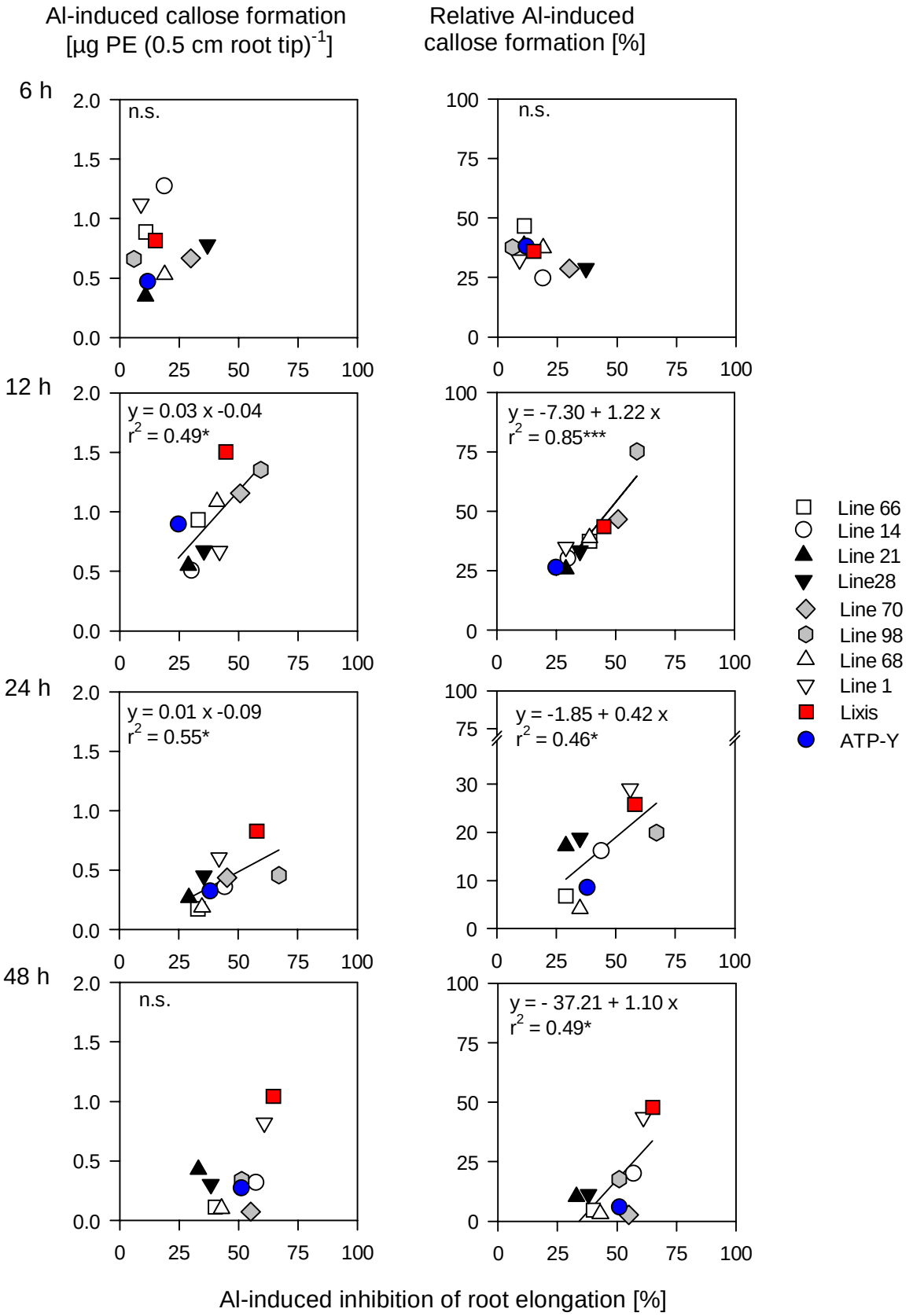

Fig. 3.10: Relationship between Al-induced inhibition of root elongation and Al-induced callose formation or relative callose formation (digitonin-induced = 100 %) of maize cultivars and inbred lines exposed to 25 µM Al in nutrient solution for 6, 12, 24, and 48 h. *, **, *** denote probability levels < 0.05, 0.01, and 0.001, n.s. > 0.05.

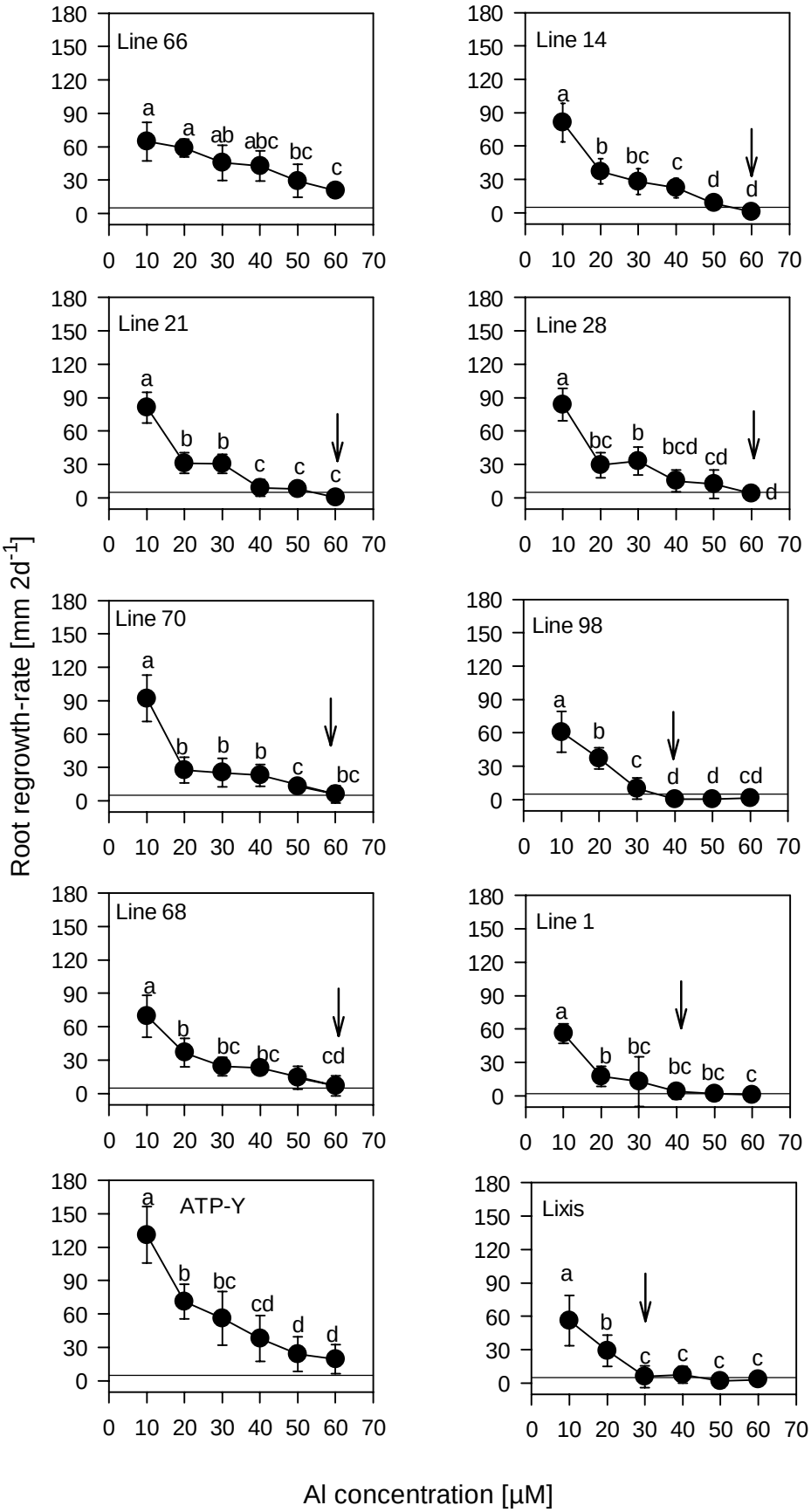

Fig. 3.11: Root regrowth of 8 inbred lines and two cultivars after 2 days following exposure to various levels of Al supply for 24 h. Arrows indicate Al concentration with root regrowth below 5 mm $2d^{-1}$. Comparisons were conducted for each cultivar between Al concentrations. Means with similar letters are not significantly different at $P < 0.05$, Tukey-test.

The maize lines were characterised similarily in Al resistance using either the regrowth technique or Al-induced inhibition of root elongation, indicated by a positive correlation. However, relationships were not significant (Tab. 3.3).

Tab. 3.3: Spearman rank-correlation coefficient for traits associated with Al resistance achieved with different methods. *, **, *** denote probability levels < 0.05, 0.01, and 0.001, n.s. > 0.05.

	Regrowth	Al-induced inhibition of root elongation at 25 µM		
		12 h	24 h	48 h
Regrowth after 2 days		0.58 n.s.	0.76*	0.57 n.s.
Al-induced callose formation at 12 h	0.23 n.s.	0.79*	0.49 n.s.	0.38 n.s.

Discussion

An adapted prestaining technique according to Schumacher *et al.* (1983) allowed the easy assessment of root elongation. Neither rhizotoxicity of Al in the solution, nor Al-induced callose formation were influenced by the staining itself or by dye contamination of the solution (Fig. 3.5). The described procedure also turned out to be more time-effective, allowing the assessment of a large number of germplasm within one trial. Furthermore, stress and damaging of roots seemed to be less. The total root length (Fig. 3.3) was higher using prestaining, explained by a smoother handling of plants in contrast to measurements with a ruler. Using a 1 mm scale, each plant was taken out of the nutrient solution twice, at the beginning and at the end of the experiment. Therefore, the chance of damaging and bending of roots was higher. A loss of roots could also result in erroneous assignment of the readings to a specific root. These problems did not arise using the prestaining method where elongation measurements are only made once at the end of the experiment.

Using prestaining a set of 110 inbred lines could be separated in Al resistance by inhibition of root elongation of the primary as well as the mesocotyl root (Tab. 3.2, Fig. 3.6). In the literature the focus has recently been directed towards the different

parts of the root system (Eshel and Waisel, 1996): A difference in physiology of each root sub-type would lead to a type-specific reaction to external influences. At least for short-term screening for Al resistance in maize in nutrient solution these reported differences in root type seemed to be less important than reported by Bushamuka and Zobel (1998). They described one maize cultivar which tap-root elongation was more inhibited than basal-root elongation, exhibiting different patterns in Al resistance for each root type. In our study only three out of 110 inbred lines had an inhibition of radicle markedly different from the inhibition of seminal roots. Therefore, possible differences in the inhibition of root elongation of these two root types might be negligible when testing a large number of genotypes.
The results presented (Fig. 3.4 and 3.7) clearly show that neutral red could be used successfully in the screening of maize inbred lines for Al resistance. Using prestaining measurements of root length for slow growing inbred lines was best after 48 h. Readings at 24 h were less precise due to a blurred staining of the root interface where new root growth occurred.

However, a combination of this method of root measurement and Al-induced callose formation was not successful when screening inbred lines (Fig. 3.7). The relationship found was too small to be sensitive enough for a screening. The statistical significance was mostly based on the high degree of freedom resulting from the 110 lines.
Although no decrease in Al-induced callose formation has been reported so far under continuous Al exposure, it can be assumed that degradation of callose had taken place. Upon a single stimulation, ultrasonic-induced callose formation has been reported to disappear (Currier and Webster, 1964, and references therein) when monitored over a time course of 8 days in cotton petioles. A similar observation was made upon single mechanical stimulation of bean stems by Jaffe *et al.* (1985). Callose formation increased within the first 6 h and thereafter gradually disappeared and was not detected after 3 days. In contrast to a single stimulus, a continuous gravitropic response stimulus did not result in a decrease of callose deposits within a time course of 48 h (Jaffe *et al.*, 1985). A disappearance of Al-induced callose in root tips has only been described after transfer of soybean seedlings to Al-free solution (Wissemeier and Horst, 1995) and not under continuous Al exposure. Zhang *et al.* (1994) demonstrated that Al-induced callose forma-

tion steadily increased when an Al-sensitive wheat cultivar was continuously subjected to Al for 48 h.

In contrast, the presented results demonstrate that Al-induced callose formation did not increase constantly over time (Fig. 3.8). Al-induced callose formation showed a steep increase within the first hours. However, depending on the cultivar, a decrease (line 1) or no decrease (line 70) was observed, too. A degradation in Al-induced callose formation might explain why after 48 h of Al exposure in the 110 inbred lines only low amounts of Al-induced callose could be detected. Consequently, the relationship between Al-induced callose formation and inhibition of root elongation was only weak (r^2 = 0.16***).

For the two cultivars ATP-Y and Lixis a degradation of callose could be also assumed. Aluminium-induced callose formation was lower after 24 h than after 12 h of in both cultivars. The values found after 24 h were similar to those reported by other authors (Llugany *et al.*, 1994; Horst *et al.*, 1997). However, the rating in Al resistance (Fig. 3.10) were not changed. As a matter of the enzymatic process of callose formation and possible degradation the time aspect should be considered when screening for Al resistance. Results achieved at any time (Basu *et al.*, 1997; Wissemeier *et al.*, 1998; Massot *et al.*, 1999; Gunsé *et al.*, 2000) without covering the time course of callose formation might lead to misinterpretation or show artefacts.

Since the capacity for callose formation might differ among genotypes, digitonin was used as a callose elicitor (Waldmann *et al.*, 1988) to assess callose-formation capacity. The independence of digitonin-induced callose synthesis from Al effects as well as its strong callose inducing capacity suggested the use of digitonin for demonstrating differences in the potential of callose synthesis. Saponin (Kauss and Jeblick, 1991) specificly inhibits the membrane associated Na^+/K^+ ATP-ase, responsible for ion transport (Richter, 1988). In cell cultures digitonin has been described as an effective elicitor of callose formation by inducing $^{45}Ca^{2+}$ uptake (Kauss and Jeblick, 1991; Kauss *et al.*, 1991) increasing cytosolic Ca^{2+} which is involved in the regulation of callose synthesis (Kauss, 1987a, 1996). The Al-induced callose formation was expressed relative to digitonin-induced callose formation, which was set to 100 % as an internal standard of callose-synthesis capacity cultures (Schmohl and Horst, 2000; Schmohl *et al.*, 2000). The relative cal-

lose formation (digitonin-induction = 100 %) showed a higher relation to root inhibition (r^2 = 0.85***) than Al-induced callose formation itself (r^2 = 0.49*). The deviation in the ranking of Al sensitivity assessed by both procedures was small. Consequently, the ranking of the genotypes remained overall unaffected by using either Al-induced callose formation or relative callose formation. The genotypes with low Al-induced callose formation also showed low relative callose formation whereas high Al-induced callose formation was confined to high relative callose formation. This might demonstrate that Al-induced callose formation depended only to a minor extend on genotype-specific callose synthesising potential (Fig. 3.9). Hence, it can be concluded that differential callose formation potential was not responsible for the weak relationship between Al-induced callose formation and inhibition of root elongation (Fig. 3.7).

However, it would need more fine tuning in the concentration of digitonin to assure similar stress intensity induced by digitonin and Al. It might be possible that the digitonin concentration used corresponded to a magnitude of stress confined to higher Al levels resulting in higher callose formation. In contrast to Al-induced callose formation, digitonin-induced callose did not disappear. Furthermore, root elongation was severely inhibited by digitonin application, even more than by Al, suggesting a higher stress intensity (Fig. 3.9). On the one hand, potential cell death might explain why for digitonin-treated roots amounts of callose were stable and not reduced as in the case of Al treatment. On the other hand, the low root elongation by digitonin assured callose accumulation within the same region over the 48 h, in contrast to Al, where roots grew about 2 cm. As for callose formation only the first 5 mm were used growth effects might account for lower callose findings in case of Al.

The assignment of the lines in adaptation to acid soils was not reflected by Al-induced callose formation nor root growth inhibition or regrowth. However, using the techniques in nutrient solution similar ratings in Al resistance of the mize genotypes were achieved using either technique. Regrowth after exposure to Al Aniol 1990, Aniol *et al*. 1980) deals with the ability of the cells to recover from Al toxicity, while measurement of root elongation is based on the ability of roots to maintain growth under Al stress. Therefore, the lack of significance in correlation between the two results might not be overestimated. The lack of linkage between the field

assessment and results from nutrient solution culture might be explained by the two different systems used. Nutrient solution techniques focus on seedling stage whereas field assessment is made on later stages of growth. Furthermore, in nutrient solution Al toxicity was the main effect, whereas in the field other factors may have a more important impact on plant development (Blum, 1988). Poor growth on acid soils can be also associated with various factors and their combinations, as low pH, Ca, Mg and P deficiencies (Clark, 1984; Howeler, 1991). Aluminium resistance of soybean seedlings was also not expressed in adult soybean plants in the field (Sartain and Kamprath, 1978; Hanson and Kamprath, 1979). However, short-term studies and assays are useful techniques to examine the effects of soil acidity on plant growth (Edmeades *et al.*, 1995). Edmeades *et al.* (1995) suggested that some of the longer-term effects of soil acidity on plant growth need to be recognised.

Root staining could characterise maize seedlings successfully in their Al resistance, saving time and preventing seedlings from stress if compared to root-length measurements with a ruler. Measurements of root lengths for slowly growing inbred lines might be best after 48 h. When Al-induced callose formation is used to characterise inbred lines an Al exposure of 12 h is sufficient to assess cultivar-specific differences which is only half of the time used in screening experiments conducted before (Llugany *et al.*, 1994; Horst *et al.*, 1997).
Degradation of callose formation was a predominant factor reducing callose in root tips of intact plants. A differential potential in callose formation was present, but should not be overestimated. The results presented for the time course of Al-induced callose formation emphasise the importance to find the best duration of Al treatment to differentiate between species and varieties within one species.

4 Predicting adaptation of maize (*Zea mays* L.) to acid soils by Al-induced callose formation in nutrient solution on a single plant level

Abstract

Maize cultivars and inbred lines were screened for Al resistance in nutrient solution using Al-induced callose formation prior to their transfer into the field. Plants exposed to 25 µM Al were transplanted to an acid Al-toxic site, those not treated with Al to a non-acid site. The shoot height was recorded every two weeks. The ear height, above-ground dry matter and grain yield were determined at maturity. The cultivars were taller and had higher grain yield than inbred lines. Plant growth as well as grain yield were significantly reduced on the acid Al-toxic site as compared to the non-toxic site. Grain yield of cultivars as well as of lines showed significant negative correlations with anthesis silking interval (ASI) ($r_{cultivars}$ = -0.77**; r_{lines} = -0,68***) and positive correlations with plant height ($r_{cultivars}$ = 0.83***; r_{lines} = 0.63***) and ear height ($r_{cultivars}$ = 0.69***; r_{lines} = 0.70*) in the acid soil environment.

For the cultivars, Al-induced callose formation could be significantly negatively correlated to relative yield ($r_{cultivars}$ = -0.79*) and relative above-ground dry matter ($r_{cultivars}$ = -0.84**) at maturity. However, for inbred lines no such correlation could be established. This contrasting results could be explained by a significant influence of the kernel weight on plant establishment and growth at later stages existing only for lines. Kernel weight and plant height at 2 weeks after sowing (WAS) prior to transplanting were significantly positively correlated (r = 0.35**, r = 0.32***) on both sites. Furthermore, plant height from 4 WAS onwards was positively correlated with grain yield on the acid site. A regression analysis clearly demonstrated that plant height of inbred lines prior to transplanting determined grain yield at maturity on both sites. The lack of correlation between Al-induced callose formation in nutrient solution and performance on acid soils of inbred lines might be explained by other factors than Al resistance determining growth and yield.

Introduction

Field screening for acid soil tolerance would seem to be the most desirable approach, because it best approximates the intended cropping environment (Garland Campbell and Carter, 1990). In practice, however, reliable ranking of genotypes in the field might be difficult because soil-heterogeneity interaction with environmental factors mask the expression of adaptation to soil acidity (Goldman *et al.*, 1989). For these reasons, investigators have relied on greenhouse studies to detect genotypic differences in soil-acidity tolerance (Armiger *et al.*, 1968). Although greenhouse experiments could approximate field conditions, they require a considerable amount of both time and space to evaluate relatively few genotypes. Since in many acid soils Al toxicity is the main growth-limiting factor, short-term solution culture methods to assess Al resistance have become a popular substitute (Hanson and Kamprath, 1979; Sapra *et al.*, 1982).

Different approaches were used for assessing Al resistance in nutrient solution. They all have in common that they focused on the primary site of Al-toxicity effects, the root and root apex (Foy, 1988; Bennet and Breen, 1991; Ryan *et al.*, 1993). Measurement of root elongation in Al-toxic solution compared to growth in non-toxic environment (Howeler and Cadavid, 1976; Alva *et al.*, 1986b; Joost *et al.*, 1986; Cosic *et al.*, 1994) and staining of Al-treated roots are common procedures (Moore *et al.*, 1976; Polle *et al.*, 1978; Kalvovoulos and Misopolinos, 1983; Wheeler *et al.*, 1992b; Ma *et al.*, 1997; Cançado *et al.*, 1999). Aniol and Gustafson (1984) and Aniol (1990) used a modified approach based on short-term exposure (pulse) followed by staining and post-cultivation of the seedlings in Al-free solution. Recovery of root elongation from Al injury characterised the cultivars in Al resistance. More recently, callose formation in root tips was described as an even more sensitive technique in assessing Al sensitivity (Wissemeier *et al.* 1987, Schreiner *et al.*, 1994; Horst *et al.*, 1997).

However, it often has been proved difficult to establish correlations between results obtained in nutrient solutions and soil studies (Garland Campbell and Carter, 1990; Edmeades *et al.*, 1995). Furthermore, the correlation between results from nutrient solution and field experiments in acid soil were not expected to be high (Magnavaca *et al.*, 1996; Bahia Filho *et al.*, 1997). Short-term screening could be useful in evaluating large numbers of plants, but the ultimate test would be the growth of plants to maturity under field conditions (Foy *et al.*, 1992; Edmeades *et*

al., 1995). It would answer whether the observed differences in Al resistance during germination and early seedling growth are representative for the Al resistance of genotypes during the whole growth cycle (Horst, 1985). For maize, however, only few studies involving nutrient solution and field experiments have been done (Kasim *et al.*, 1990). In order to validate the laboratory techniques in developing maize genotypes for adaptation to acid soils, results obtained in nutrient solution need to be verified under field conditions. Therefore, a technique was established allowing the assessment of Al resistance of maize seedlings in nutrient solution and their transfer to the field in order to exclude the high variance resulting from comparison of different plants in different environments.

The experiments aimed at answering whether (i) Al resistance could be determined in nutrient solution non-destructively on a single plant level and the individual plants thereafter be transferred into the field, (ii) Al-induced callose formation of cultivars and inbred lines was related to plant performance on an acid Al-toxic soil.

Materials and methods

I. Experiments at CIMMYT/ CIAT, Cali, Colombia.

A study in nutrient-solution culture was combined with the subsequent transfer of the plants to the field. The nutrient-solution experiment was carried out in a glasshouse. Thereafter, seedlings were transferred to an Al-toxic and non-toxic site as described in other studies (Joost *et al.*, 1986). Because of the inherent variation among genotypes in growth and yield potential, a non-stress control is required for a reliable separation of genotypes in terms of growth and yield (Blum, 1988).

Plant material

110 inbred lines derived from SA-4 and SA-5 populations with acid soil tolerance (Pandey *et al.*, 1995) from the CIMMYT breeding programme were used. Furthermore, 9 cultivars were included as standards. These standards consisted of two crosses of the above mentioned inbred lines (66 x 68, 21 x 28), 6 cultivars which have been used in breeding programmes for adaptation to acid soils and physiological studies concerning Al toxicity and resistance (Sikuani, C525M, BR201F, BR201M, ATP-Y, Lixis) and a Brazilian cultivar (Guacuani). The term genotype is

used in the following as a general heading to address inbred lines, cultivars and crosses, respectively.

Nutrient solution culture

The experiments in nutrient solution were conducted in an air-conditioned glass-house with a maximum air temperature of 35 °C during the day. The plants for transfer to the non-acid site Palmira soil were sown on 11. June and for the acid site S. de Quilichao on 6. July 1998. For each location, 12 plants per genotype were sown and split into three replicates of four plants each. One replicate was planted each 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. The paper beakers were placed in a rag on top of plastic boxes containing 20 l of nutrient solution of low ionic strength of the following composition [µM]: KNO_3 400, $CaSO_4$ 250, NH_4NO_3 200, $MgSO_4$ 100, KH_2PO_4 10, Fe-EDDHA 20, H_3BO_3 8, $MnSO_4$ 1, $ZnSO_4$ 0.2, $CuSO_4$ 0.2, $(NH_4)_6Mo_7O_{24}$ 0.1.

The sealing wax layer prevented the contact between substrate and nutrient solution. The nutrient solution was renewed every 2 days. The seedlings were germinated for 4 days. When the roots of all plants penetrated the wax layer into the nutrient solution pH was lowered incrementally to 4.3. Eight days after sowing, the nutrient solution was changed and concentrations of 0 µM or 25 µM Al were added. During the experiment the pH was controlled and adjusted using 0.05 M HCl and 0.05 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 (1.5 ml volume) containing ethanol (96 %) until analysis of callose. Callose was determined as described by Kauss (1989) and Horst *et al.* (1997).

Transfer into the field

Following the evaluation in nutrient solution seedlings were transplanted (Fig. 4.1). The seedlings without Al supply were transplanted into a non-acid soil at Palmira (CIAT headquarters 3°30’N, 76°19’W, 965 m asl). The soil type was classified as a fine-silty mixed, isohyperthermic Aquic Hapludoll. Those seedlings treated with Al

were transferred to an acid Al-toxic soil in S. de Quilichao (Quilichao station, 3°06'N, 76°31'W, 990 m asl). The soil type was classified as a fine, kaolinitic iso-hyperthermic Oxic Dystopept. The soil characteristics (analysed by the department of soil science at CIAT) of both sites are given in Tab. 4.1.

Tab. 4.1: Soil characteristics of the non-acid (Palmira) and acid site (S. de Quilichao).

Location	Organic matter [%]	P [ppm]	pH	Al	Ca	Mg	K	Al-saturation [%]
				[meq/ 100 g soil]				
Palmira	2.7	62.1	7.4	n.d.	22.0	9.8	0.8	-
S. de Quilichao	3.9	9.8	4.7	2.3	1.5	0.6	0.2	50

n.d. = not detected

The seedlings were planted at a spacing of 16 cm within and 75 cm between rows. Solid fertiliser was applied twice directly to the plant at 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 in Palmira only.

At both sites plants showed red leaves 10 days after transplanting. The symptoms disappeared after 5 days in Palmira. In S. de Quilichao more plants were affected, due to the lower P content of the soil, and symptoms persisted 2 weeks longer. Therefore, P was supplied once as foliar spraying (0.2 % P, 20 l per site) after transplanting on both sites.

Only in S. de Quilichao plants were shaded for the first 10 days after transplanting. The plants were irrigated regularly. In Palmira irrigation was carried out by furrow irrigation, in S. de Quilichao a water canon was used. Plant protection was similar at both sites. Lorsban (0.25 %, Chlorpyrifos, DowElanco; Indianapolis, In., USA) was applied twice as arial spray, 10 and 30 days after planting. A third application was sprayed directly on the cobs 10 days after the end of female flowering, when the stigma had dried. Hostathion (Triazophos, AgrEvo, Berlin, Germany) was applied in granules repeatedly after first appearance of spodoptera larvae (*Heliothis zea*). Furthermore, when applying Lorsban to the cob, cob leaves had to be opened at the top to allow contact of Lorsban with the penetrated larvae.

Observations

Single plants were evaluated. The height of each plant was determined prior to transplanting at 2 weeks after sowing (WAS) and thereafter at 4, 6, 8, 10 and 16 weeks after sowing of seeds either into paper beakers or directly into the soil. Plant height was defined by the distance between ground level and last fully developed leaf node. The number of days to 50 % silking and 50 % anthesis were determined for each plant. Around this developmental stage plants were checked every second day. Anthesis silking interval (ASI) was calculated as days to 50 % silking minus days to 50 % anthesis. The ear height of each plant was determined as the distance between ground level and the node of the cob. The number of ears were counted even when no grains were formed after anthesis.

At harvest, plants were cut at the soil surface for dry matter evaluation. They were dried in an air-forced oven at 60 °C for 5 days. The plants were separated into vegetative (stem and leaves) and generative organs (bracts, cobs, grain). The dry weights of individual plant parts were determined as well as total above-ground dry matter (DM). The grain was dried to constant weight in a forced air oven and shelled. The grain yield was estimated of all cobs with at least one kernel. The grain yield loss due to spodoptera infestation was visually estimated in percentage of the whole cob (0 - 75 %) and its grain yield adjusted by this factor.

II. Nutrient solution studies in Germany

In a subsequent experiment Al-induced callose formation (25 µM Al) and digitonin-induced callose formation (10 µM digitonin) were analysed for a subset of 20 from the 110 inbred lines. The cultivars ATP-Y and Lixis were used as standards. Growth conditions and analysis of monomeric Al in the nutrient solution and Al-induced callose formation in the root tips were analysed as described in the previous chapters.

Statistical analysis

A randomised block design was chosen for the two locations. Location and genotype were fixed variables, block was chosen as a random variable. A correlation analysis was performed (Proc Corr), and analysis of variance using Proc GLM (SAS 6.10, SAS Institute Inc., Cary, NC, USA) followed by comparison of means using Tukey-test or T-test at $P < 0.05$.

Fig. 4.1: Steps of the transplanting method of maize cultivars.
A. Maize seedlings sown in paper beakers placed into a rag fixed on top of a plastic box.
B. Transverse section of a pot containing a seedling at the time of the Al treatment.
C. Transplanting of individual seedlings into the field.
D. Transplanted plants at the acid Al-toxic site, S. de Quilichao, at tasseling stage.

Results

Monomeric Al was detected at 17.5 ± 4 µM Al at a supply of 25 µM Al to the nutrient solution, pH was constant within a range of 4.2 ± 0.2 units. Transplanting was successful at both sites. The rate of plants reaching maturity was above 95 %.

Evaluation of the transplanting method

According to the analysis of variance (Tab. 4.2) all factors and their interactions highly significantly contributed to the variation of plant height with the exception of the interaction location*transplanting. The factors WAS, location (Loc), followed by genotype had the greatest influence on variation. Significant interactions existed between genotype and transplanting, showing that transplanting (Transp) effects were genotype-dependent. Therefore, for each genotype studied the comparison of means was carried out separately between the two planting methods for the sites (Fig. 4.2, 4.3):

Tab. 4.2: Analysis of variance for plant height for transplanted/sown maize cultivars at two sites in Colombia. *, **, *** denote probability levels < 0.05, 0.01, and 0.001, n.s. > 0.05.

Source of variation	df	MSQ	
WAS	5	100864752	***
Loc	1	91857195	***
Genotype	11	6909926	***
Transplanting	1	2570448	***
Loc x WAS	5	9049351	***
Loc x Transp	1	290870	
Loc x Transp x WAS	10	1436766	***
Loc x Genotype	11	406772	***
Transp x Genotype	11	513264	***
Loc x Transp x Genotype	11	187177	***
Model	67	13527952	***
Error	3799	83913	

The growth curve followed the same pattern at both sites. Linear growth occurred until 4 WAS, followed by an exponential phase until 10 WAS. Maximum plant height was reached at 10 WAS. Significant differences related to transplanting effects were present at both sites, most pronounced at the non-acid site in Palmira. From emergence until 4 WAS transplanted plants were taller than directly sown

plants. Thereafter, this relation was reversed. Direct sowing led to taller plants at all following dates. The maximum difference between the two procedures of planting was found at 8 WAS. Later differences became smaller and were merely visible at 10 WAS, approx. 10 cm in height, though they were still significant at the non-acid site. The acid soil environment reduced plant height following from 6 WAS onward by 30 – 50 % regardless of the planting method.

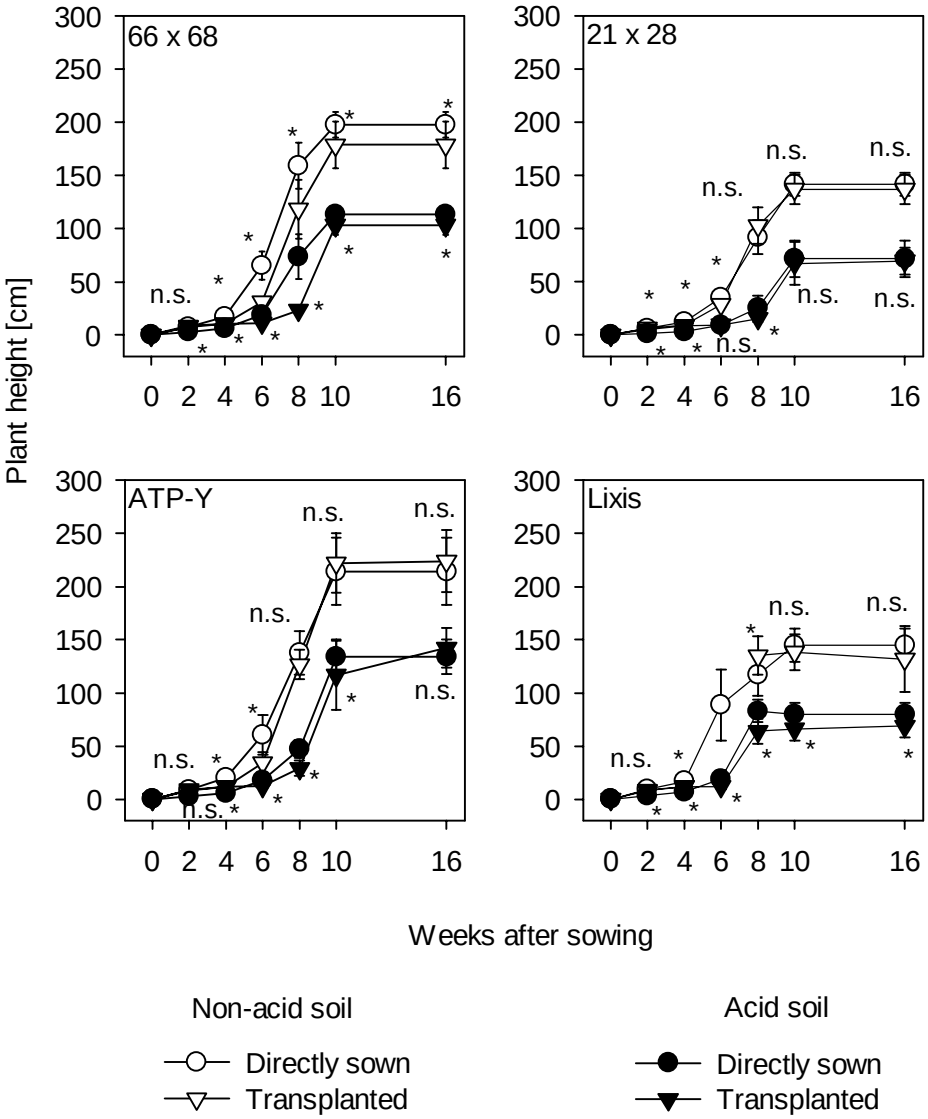

Fig. 4.2: Development of plant height of transplanted and directly sown maize cultivars differing in adaptation to acid soils on an acid and non-acid site. Comparisons of means ± SD (n = 12) were conducted for each date and location separately. According to T-test * indicates significant differences between the means at $P < 0.05$.

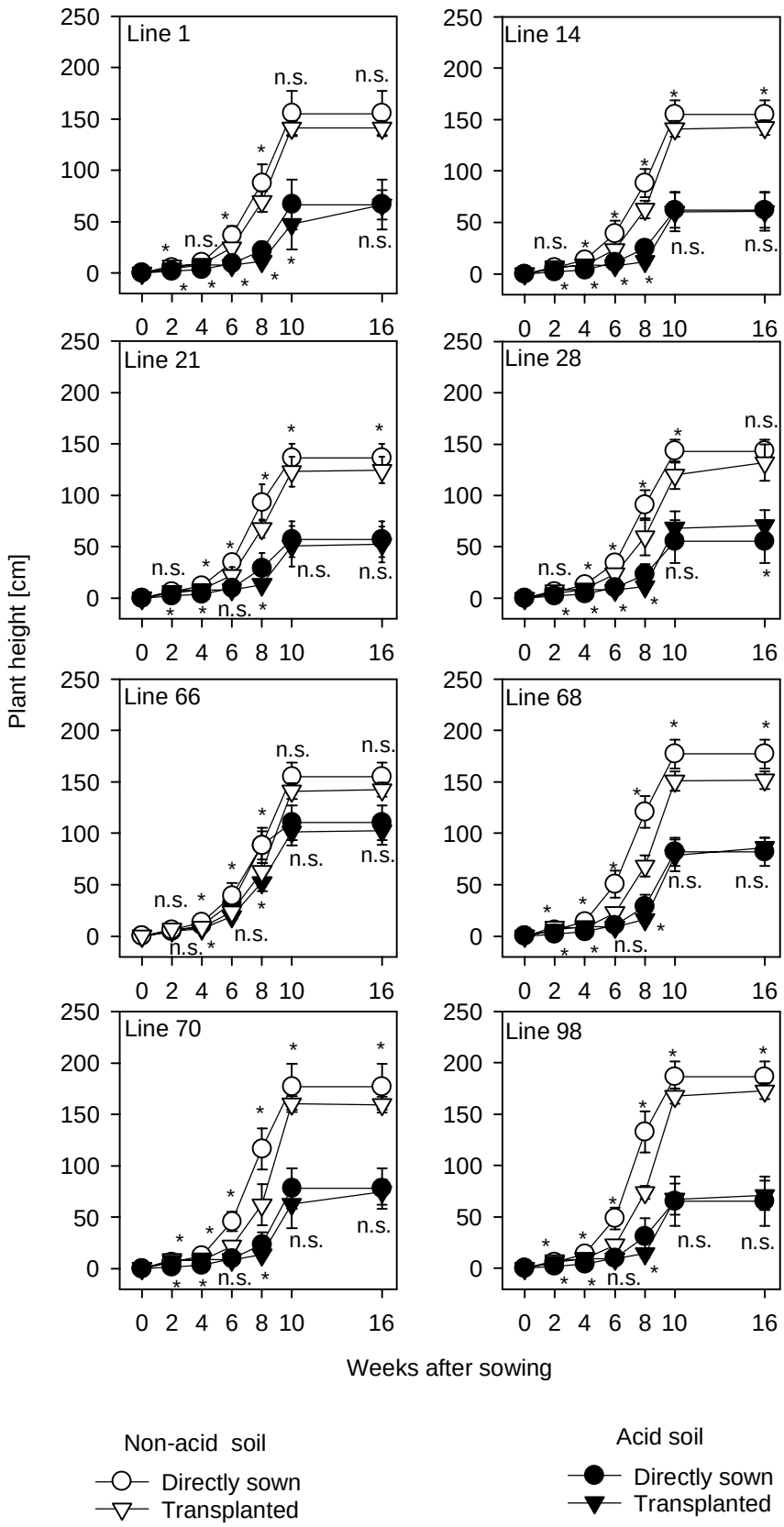

Fig. 4.3: Development of plant height of transplanted and directly sown maize inbred lines differing in adaptation to acid soils on an acid and non-acid site. Comparisons of means ± SD (n = 12) were conducted for each date and location separately. According to T-test * indicates significant differences between the means at $P < 0.05$.

The straw dry matter of transplanted plants was reduced as a consequence of lower plant height (Fig. 4.4). However, no consistent influence on grain yield and above-ground dry matter was found (Fig. 4.5, 4.6).

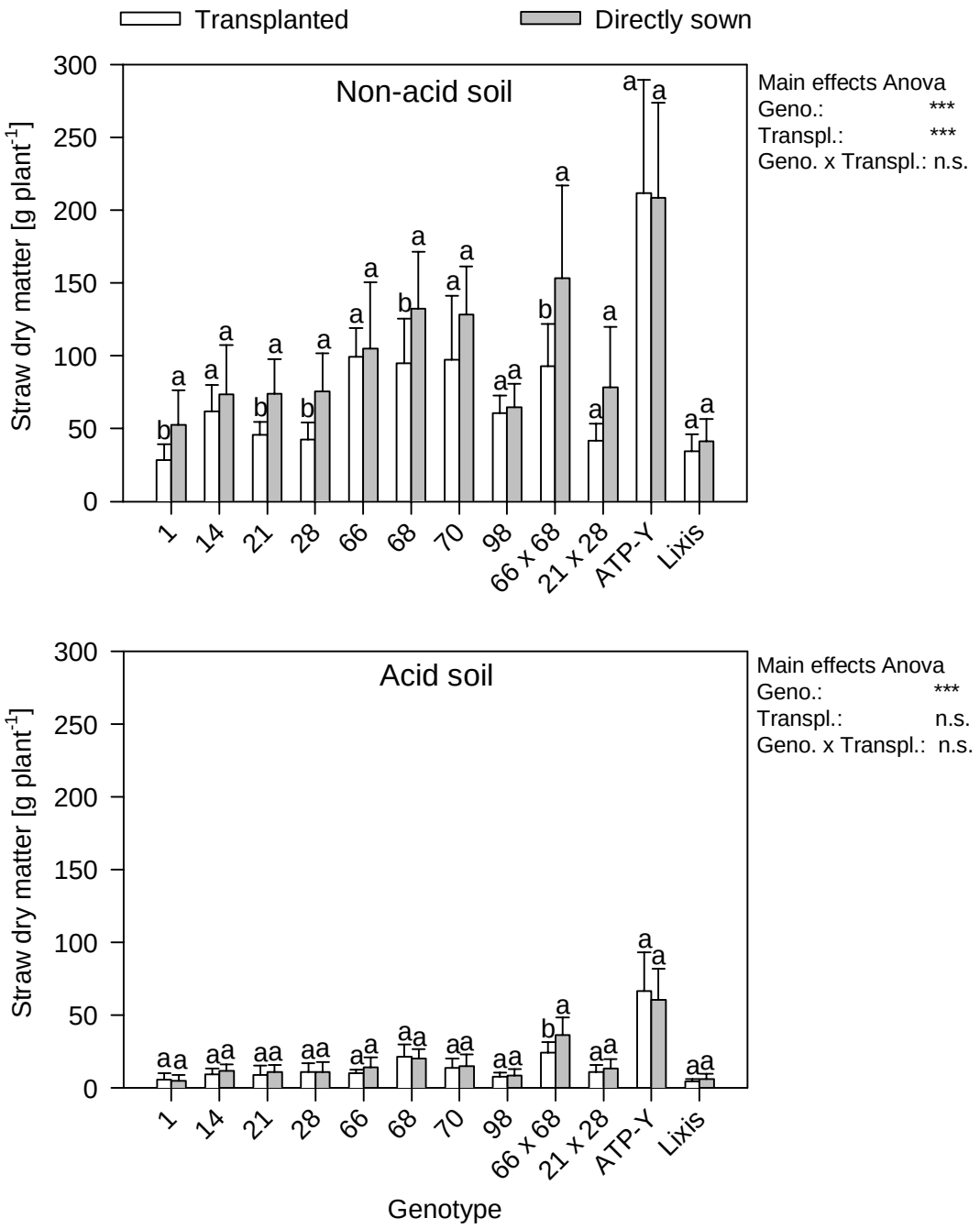

Fig. 4.4: Straw dry matter of individual plants, sown or transplanted to a non-acid or acid Al-toxic site. Bars represent means ± SD for each cultivar with n = 12. Means with similar letters are not significantly different according to Tukey-test at $P < 0.05$. *, **, *** denote probability levels < 0.05, 0.01, and 0.001, n.s. > 0.05.

In general, grain yields of sown and transplanted plants were similar in most cases (Fig. 4.5). When sown on the non-acid site (Fig. 4.5), two lines (68, 98) and one cross (21 x 28) showed lower grain yields when directly sown into the soil compared to transplanted plants. Grain yield of the other genotypes showed no significant differences in grain yield, regardless if transplanted or directly sown into the soil. Lines could be separated into a high yielding (> 50 g plant^{-1}: lines 66, 68, 70, 98) and low yielding group (< 30 g plant^{-1}: lines 1, 14, 21, 28). Among the cultivars the Al-resistant cultivar ATP-Y and the cross 66 x 68 produced higher yields than the Al-sensitive cultivars Lixis and cross 21 x 28. Yield gains through heterotic effects were only achieved for cross 66 x 68 but not for 21 x 28: Grain yield of the cross 66 x 68 was higher than that of the parental inbred lines.

The comparison of transplanting versus sowing led to similar results on the Al-toxic site (Fig. 4.5). Differences between these two methods could be seen only for cultivars ATP-Y and Lixis. ATP-Y yielded about 50 % less when directly sown compared to transplanted plants. In contrast, the Al-sensitive cultivar Lixis yielded higher, when sown directly into the soil.

Among the lines, line 68 yielded highest with 30 g plant^{-1}. The cross 66 x 68 and cultivar ATP-Y showed highest yields among the genotypes, confirming their superior adaptation to soil acidity. The grain yields of these cultivars were nearly three times higher than the yields of the sensitive genotypes Lixis and 21 x 28.

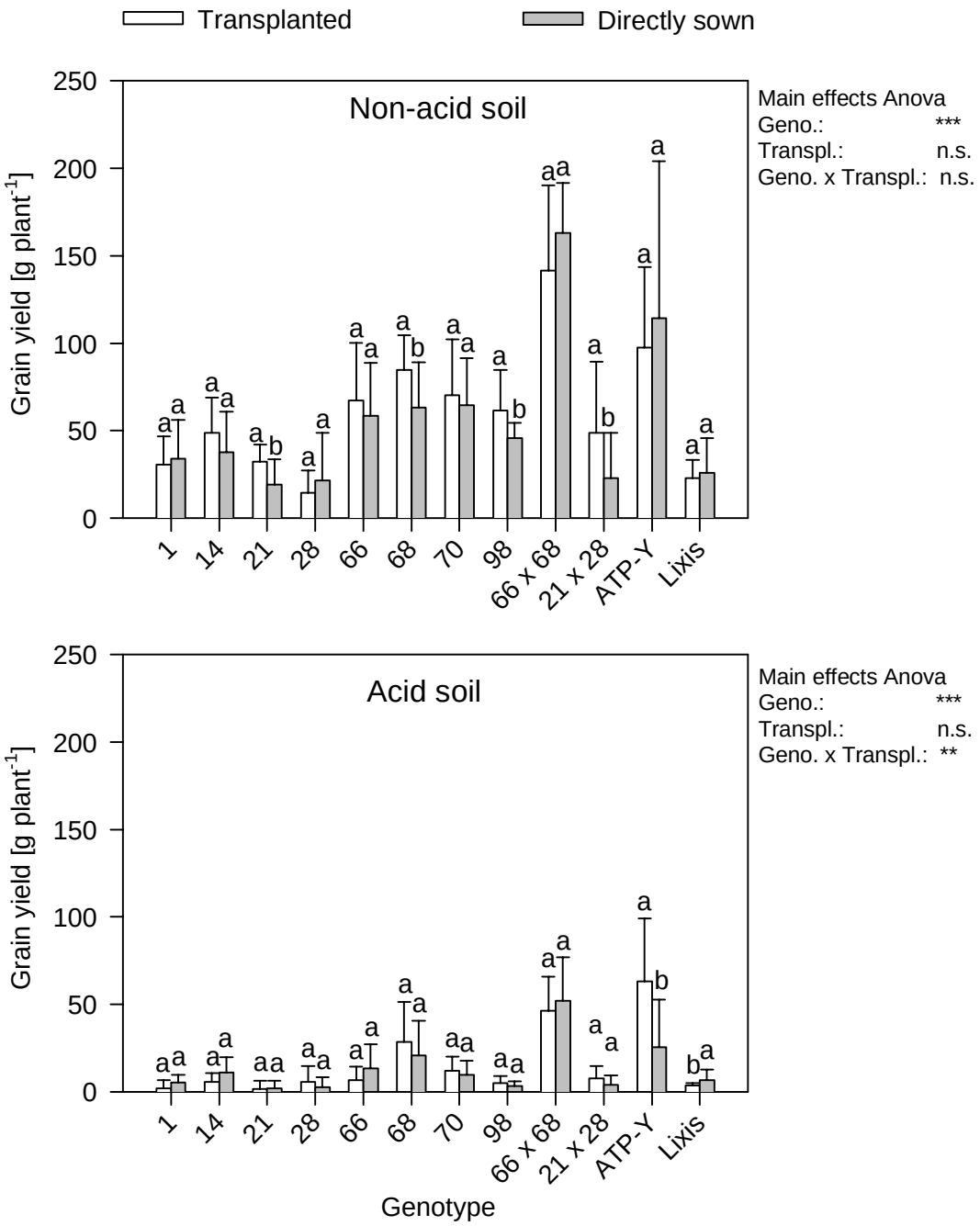

Fig. 4.5: Grain yield of individual plants, sown or transplanted to a non-acid and acid Al-toxic site. Bars represent means ± SD for each cultivar with n = 12. Means with similar letters are not significantly different according to Tukey-test at $P < 0.05$. *, **, *** denote probability levels < 0.05, 0.01, and 0.001, n.s. > 0.05.

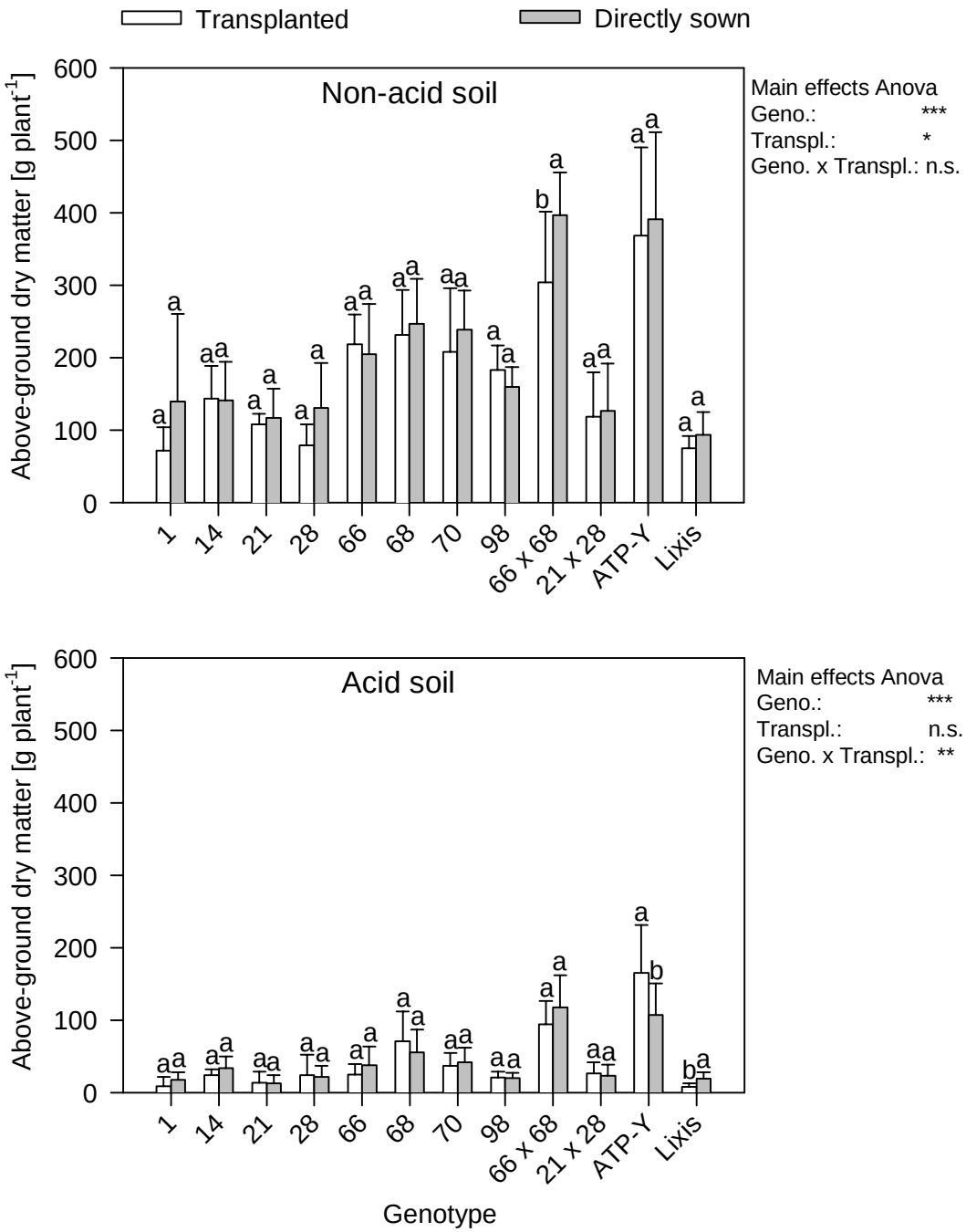

Fig. 4.6: Above-ground dry matter of individual plants, sown or transplanted to a non-acid and acid Al-toxic site. Bars represent means ± SD for each cultivar, n = 12. Means with similar letters are not significantly different according to Tukey-test at $P < 0.05$. *, **, *** denote probability levels < 0.05, 0.01, and 0.001, n.s. > 0.05.

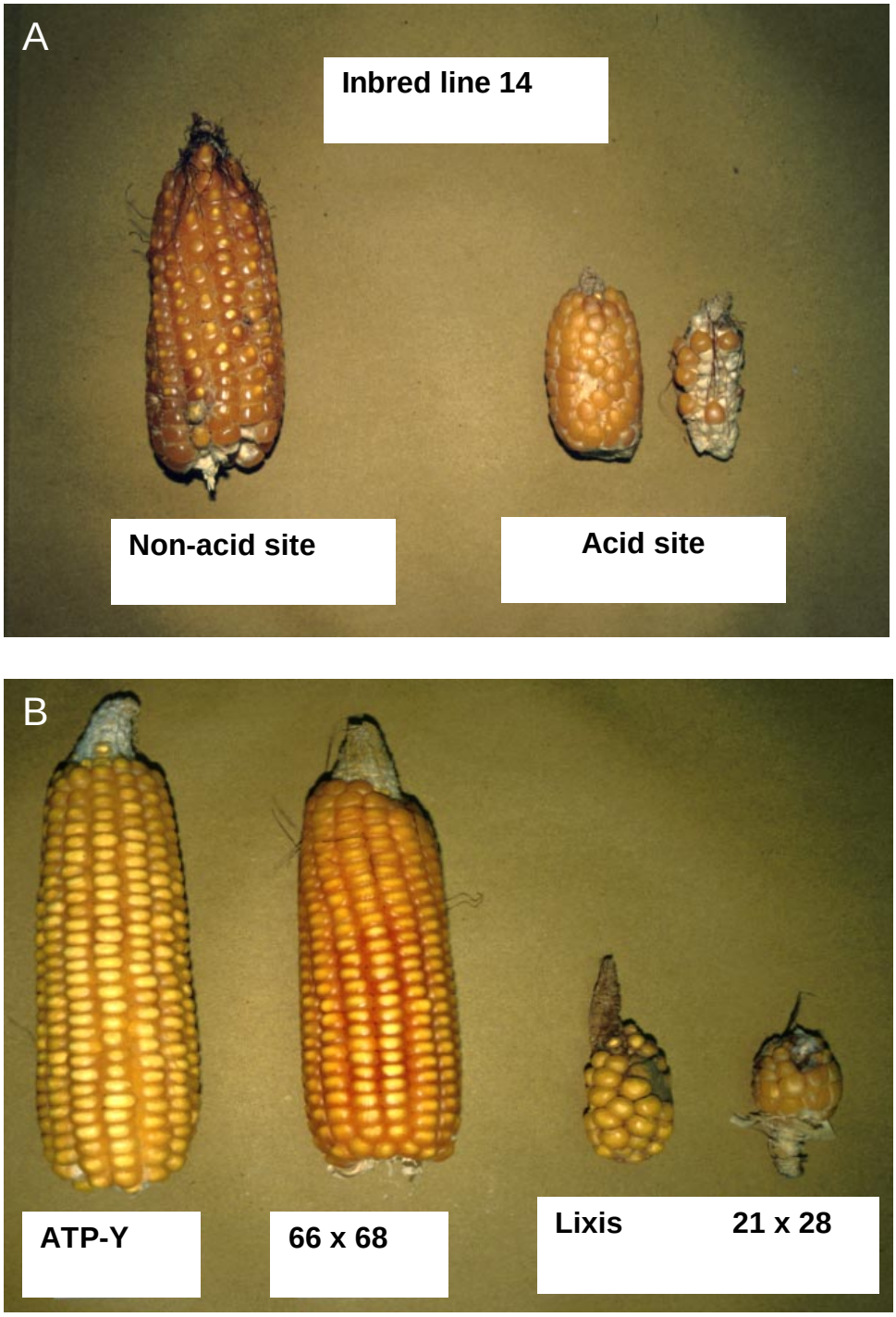

Fig. 4.7: (A) Effects of soil acidity on grain yield of an inbred line and (B) variation in grain yield on an acid soil of cultivars and crosses differing in Al resistance and adaptation to an acid soil.

Significant positively relationships were found for the grain yield and above-ground dry matter on both sites. The linear relationship was near to a 1:1 relationship, indicating that whole plant performance was not consistently affected by transplanting. Genotypes showing higher yields when transplanted than when sown were found and vice versa (Fig. 4.8).

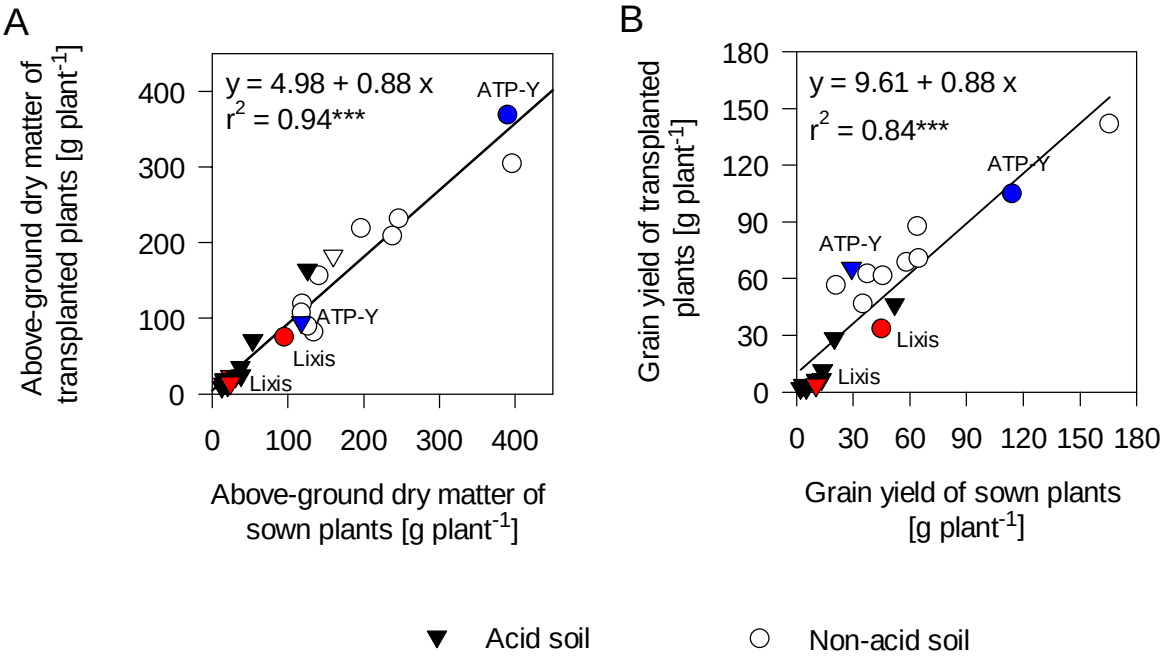

Fig. 4.8: Relationships between above-ground dry matter (A) and grain yield (B) of transplanted and directly sown plants. Regressions were calculated over the two sites. *, **, *** denote probability levels < 0.05, 0.01, and 0.001, n.s. > 0.05.

Calculating the linear regressions separately for the two locations did not yield different results (Tab. 4.3).

Tab. 4.3: Linear regressions between transplanted and sown plants for grain yield and above-ground dry matter for each of the two sites. *, **, *** denote probability levels < 0.05, 0.01, and 0.001, n.s. > 0.05.

Site	Grain yield	Above-ground dry matter
Non-acid soil	y = 10.99 + 0.85 x; r^2 =0.90***	y = 29.77 + 0.7x; r^2 = 0.86***
Acid soil	y = -4.49 + 1.12 x; r^2 = 0.90***	y = -3.48 + 1.18 x; r^2 = 0.90***

Between the two sites significant differences in agronomic traits of the maize genotypes existed (Tab. 4.4). Cultivars showed a higher vigour under both conditions than lines, presumably due to inbred depression of the lines. Impact of soil acidity was stronger pronounced for the inbred lines: Plant height, grain yields as well as above-ground dry matter were significantly lower than for the cultivars. Even more, soil acidity led to a greater reduction of the recorded traits. Therefore, cultivars and lines were analysed separately.

Tab. 4.4: Means ± SD of agronomic traits of transplanted cultivars (n = 9) and inbred lines (n = 110) on an acid Al-toxic and non-acid soil. Similar letters indicate no differences between means according to Tukey-test at $P < 0.05$.

Agronomic trait	Cultivars		Lines	
	Non-acid soil	Acid soil	Non-acid soil	Acid soil
Kernel weight [g (25 kernels)$^{-1}$] at sowing	6.3 ± 1.18		5.2 ± 0.99	
Plant height [cm]				
4 WAS	13.5 ± 4.9 a	8.7 ± 2.7 b	8.8 ± 2.3 a	8.2 ± 2.3 a
6 WAS	45.4 ± 25.3 a	14.2 ± 5.3 b	23.6 ± 80 a	8.7 ± 2.6 b
8 WAS	126.8 ± 27.5 a	45.8 ± 25.7 b	68.4 ± 202 a	15.7 ± 7.9 b
10 WAS	187.8 ± 36.0 a	102.5 ± 28.1 b	143.1 ± 26.9 a	60.9 ± 24.5 b
16 WAS	188.4 ± 38.2 a	110.7 ± 30.1 b	145.4 ± 26.7 a	66.5 ± 22.4 b
Flowering				
50% Silking [d]	61.8 ± 4.8 a	72.2 ± 8.7 b	63.3 ± 3.8 a	78.9 ± 5.5 b
50% Anthesis [d]	61.8 ± 4.4 a	70.2 ± 8.6 b	62.9 ± 3.9 a	74.1 ± 4.6 b
ASI [d]	0 ± 1.8 a	2.3 ± 2.7 b	0.4 ± 3.7 a	5.0 ± 3.9 b
Harvest				
Ear height [cm]	100.3 ± 30.4 a	40.2 ± 20.4 b	60.9 ± 15.7 a	16.8 ± 10.4 b
Ears per plant	0.89 ± 0.2 a	0.88 ± 0.1 a	0.97 ± 0.1 a	0.75 ± 0.23 b
Cob leaves [g]	28.8 ± 19.3 a	10.1 ± 8.8 b	24.9 ± 14.8 a	5.0 ± 3.9 b
Cob [g]	21.7 ± 13.3 a	9.1 ± 6.9 b	15.4 ± 7.8 a	3.8 ± 4.2 b
Above-ground dry matter [g]	301.5 ± 141.3 a	95.6 ± 60.5 b	164.2 ± 65.4 a	30.2 ± 23.2 b
Grain yield [g]	110.3 ± 71.5 a	39.8 ± 30.3 b	55.6 ± 28.0 a	9.7 ± 11.2 b

Soil acidity significantly reduced plant height (Tab. 4.4) beginning from 4 WAS for hybrids and later for inbred lines at 6 WAS. Reduction of growth was highest at 6–8 WAS (64 % for cultivars, 77 % for inbred lines) than at maturity showing a retardation in shoot development on the acid site. Consequently, the onset of flowering on the acid site occurred 10 to 15 days later than on the non-acid site with a higher degree of variation. The interval between 50 % male and 50 % female flowering was elongated as shown by the anthesis silking interval (ASI). Ear height, dry matter of cob leaves, cob dry matter and above-ground dry matter were reduced by 60 - 70 % for cultivars and 76 - 82 % for inbred lines. In contrast, ears per plant showed a 23 % decrease only for inbred lines on the acid site but not for the cultivars. The cultivars were not affected. Even the Al-sensitive cultivar Lixis produced at least one kernel on each cob.

Significant correlations existed between the recorded traits for cultivars (Tab. 4.5). Generally, a high plant height as well as high above-ground dry matter were indicators of high grain yields, less expressed on the non-acid soil. The grain yield was more strongly associated with plant height on the acid (r = 0.79***) than the non-acid soil (r = 0.60; Tab. 4.5). The ear height was positively correlated with grain yield only on the acid site (r = 0.70*). The anthesis silking interval (ASI) significantly correlated with ear and plant height as well as above-ground dry matter indicating its determination by the cultivar-specific ability to cope with the acid soil stress. In contrast, such association was not found on the non-acid site.

Tab. 4.5: Pearson correlation coefficient of 9 cultivars means for phenotypic traits. Above diagonal for acid soil, below for non-acid soil. †, *, **, *** denote probability levels < 0.10, 0.05, 0.01, and 0.001, n.s. > 0.10.

	Plant height	Ear height	Above-ground dry matter	Grain yield	ASI	50 % Anthesis
Plant height	--	0.96 ***	0.76 *	0.83 **	-0.66 †	0.62 †
Ear height	0.71 *	--	0.81 **	0.70 *	-0.70 †	0.67 *
Above-ground dry matter	0.79 *	n.s.	--	0.96 ***	-0.67 †	0.69 *
Grain yield	0.60 †	n.s.	0.89 ***	--	-0.77 *	n.s.
ASI	n.s.	n.s.	n.s.	0.73 †	--	n.s.
50 % Anthesis	0.71 †	0.71 †	0.83 *	n.s.	n.s.	--

Similar observations were made for the inbred lines (Tab. 4.6). The negative association between ASI and plant height, above-ground dry matter and grain yield were highly significant on the acid soil only. Furthermore, on both sites plant height positively correlated with grain yield and ear height (Tab. 4.5).
In contrast to the cultivars, for the inbred lines on the acid (r = 0.69***) and non-acid site (r = 0.45***) grain yield was significantly correlated with ear height.

Tab. 4.6: Pearson correlation coefficient for means of phenotypic traits of 110 inbred lines. Above diagonal for acid Al-toxic soil, below for non-acid soil. †, *, **, *** denote probability levels < 0.10 ,0.05, 0.01, and 0.001, n.s. > 0.10.

	Plant height	Ear height	Above-ground dry matter	Grain yield	ASI	50 % Anthesis
Plant height	--	0.84 ***	0.70 ***	0.63 ***	-0.55 ***	0.39 ***
Ear height	0.81 ***	--	0.75 ***	0.69 ***	-0.75 ***	0.43 ***
Above-ground dry matter	0.56 ***	0.66 ***	--	0.95 ***	-0.70 ***	0.26 ***
Grain yield	0.53 ***	0.45 ***	0.83 ***	--	-0.68 ***	0.17 †
ASI	n.s.	n.s.	n.s.	n.s.	--	-0.31***
50 % Anthesis	0.37 ***	0.58 ***	0.32 ***	n.s.	-0.25 **	--

Differences between the cultivars and the inbred lines were apparent when the grain yields of the two environments were compared (Fig. 4.9B). While for the cultivars (Fig. 4.9A) no relationship between grain yield on the acid and non-acid environment was found, a highly positive correlation could be established for inbred lines (r = 0.42***). Those lines which performed well in the non-acid environment also showed higher yield in the acid environment. The symptoms of P-deficiency occurring after transplanting on the acid site had no major effect on long-term plant growth and the grain yield of the lines. The symptoms disappeared during later stages of growth. Furthermore, grain yield of those lines showing symptoms were found within the range of 0 - 30 g plant^{-1}, similarly to lines without symptoms.

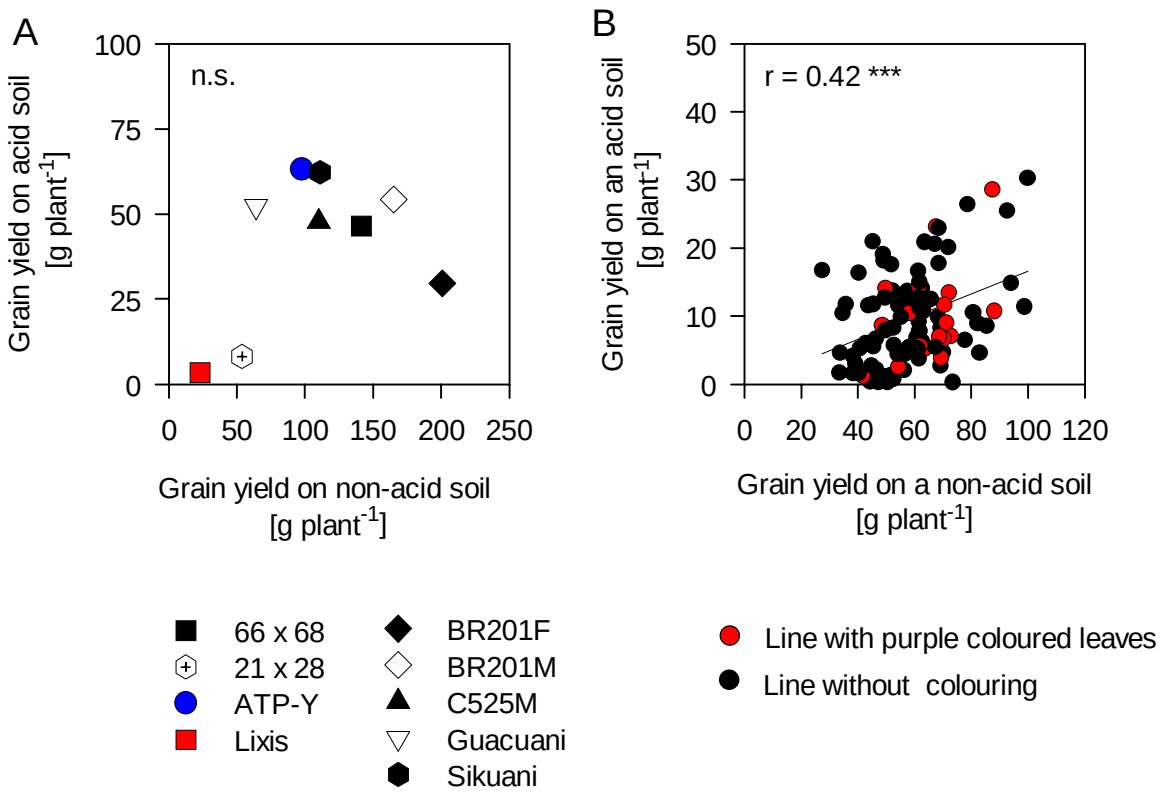

Fig. 4.9: Relationships between grain yields of maize genotypes on an acid and non-acid site for (A) transplanted cultivars (n = 9) and (B) inbred lines (n = 110). *, **, *** denote probability levels < 0.05, 0.01, and 0.001, n.s. > 0.05.

Relationships between Al-induced callose formation and agronomic traits

Aluminium-induced callose formation has been used as an indicator of Al toxicity in nutrient solution. Therefore, a relationship between the response to Al in nutrient solution to at least one of the agronomic parameters (Tab. 4.4) would be expected. Since absolute performance of the cultivars and inbred lines on acid soil might be due to factors apart from adaptation to soil acidity (Garland Campbell and Carter, 1990), agronomic parameters were related to Al-induced callose formation on a relative basis using the non-acid site as reference (100 %). Though a cultivar-specific variation in Al-induced callose formation and in plant height existed, no significant correlation could be established between both parameters at 6, 8, 10 and 16 WAS (Fig. 4.10). The cross 21 x 28 was not included into the analysis because of severely curled roots in nutrient solution accompanied by a low level of callose synthesis. At 6 WAS, differences in plant height were rather small between

the cultivars. The Al-sensitive cultivar Lixis did not show a lower plant height than the Al-resistant cultivar ATP-Y. This pattern changed at 8 WAS. Cultivar Lixis reached its maximum height before all other cultivars, leading to a positive correlation of Al-induced callose synthesis and plant height.

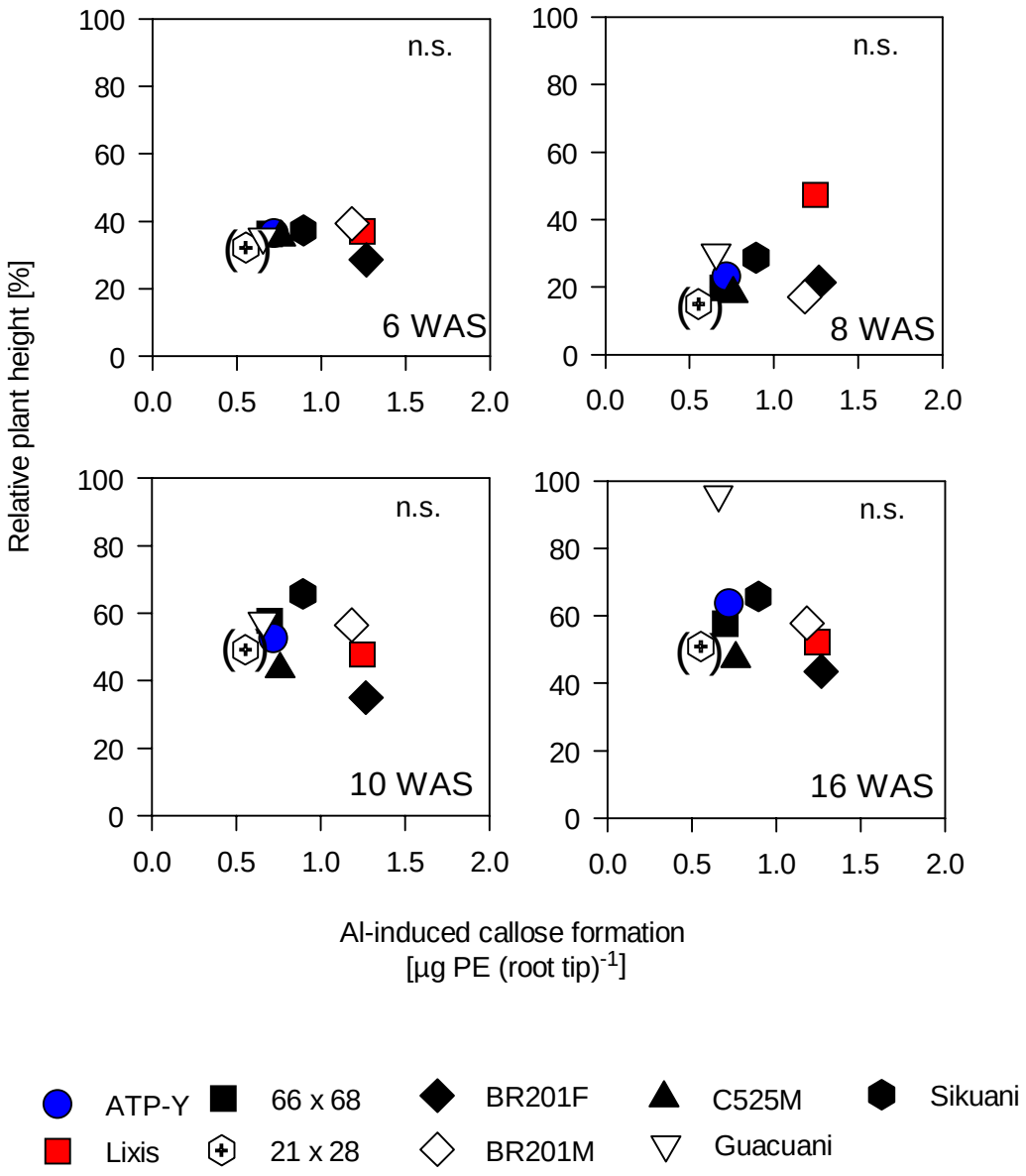

Fig. 4.10: Relationship between Al-induced callose formation in root tips of intact maize cultivars after 12 h in nutrient solution (25 µM Al, pH = 4.3) and relative plant height at 6, 8, 10, 16 WAS after transplanting to a non-acid site (100 %) and acid site. *, **, *** denote probability levels < 0.05, 0.01, and 0.001, n.s. > 0.05. Cross 21 X 28 excluded from correlation analysis.

For the inbred lines similar results were found (Fig. 4.11). The level of Al-induced callose contents ranging from 0.5 to 1.5 µg PE root tip^{-1} were similar for lines and cultivars. The relationship between Al-induced callose formation in nutrient solution and relative plant height in the field at the Al-toxic site was not significantly correlated to the plant stages except at 8 WAS when a significant positive correlation was found. When Al-induced callose formation was related to parameters representing later stages of growth significant correlations could be established (Fig. 4.12).

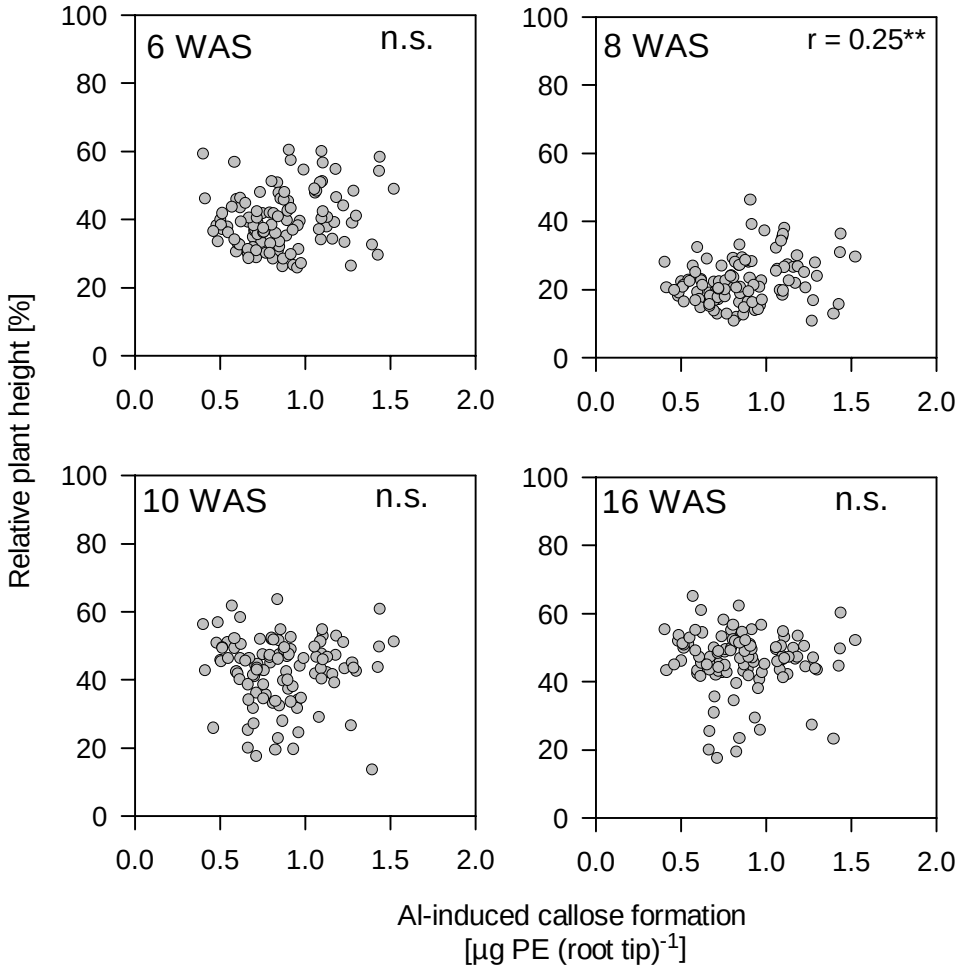

Fig. 4.11: Relationship between Al-induced callose formation in root tips of intact maize inbred lines after 12 h in nutrient solution (25 µM Al, pH = 4.3) and relative plant height at 6, 8, 10, 16 WAS after transplanting to a non-acid site (100 %) and acid site. *, **, *** denote probability levels < 0.05, 0.01, and 0.001, n.s. > 0.05.

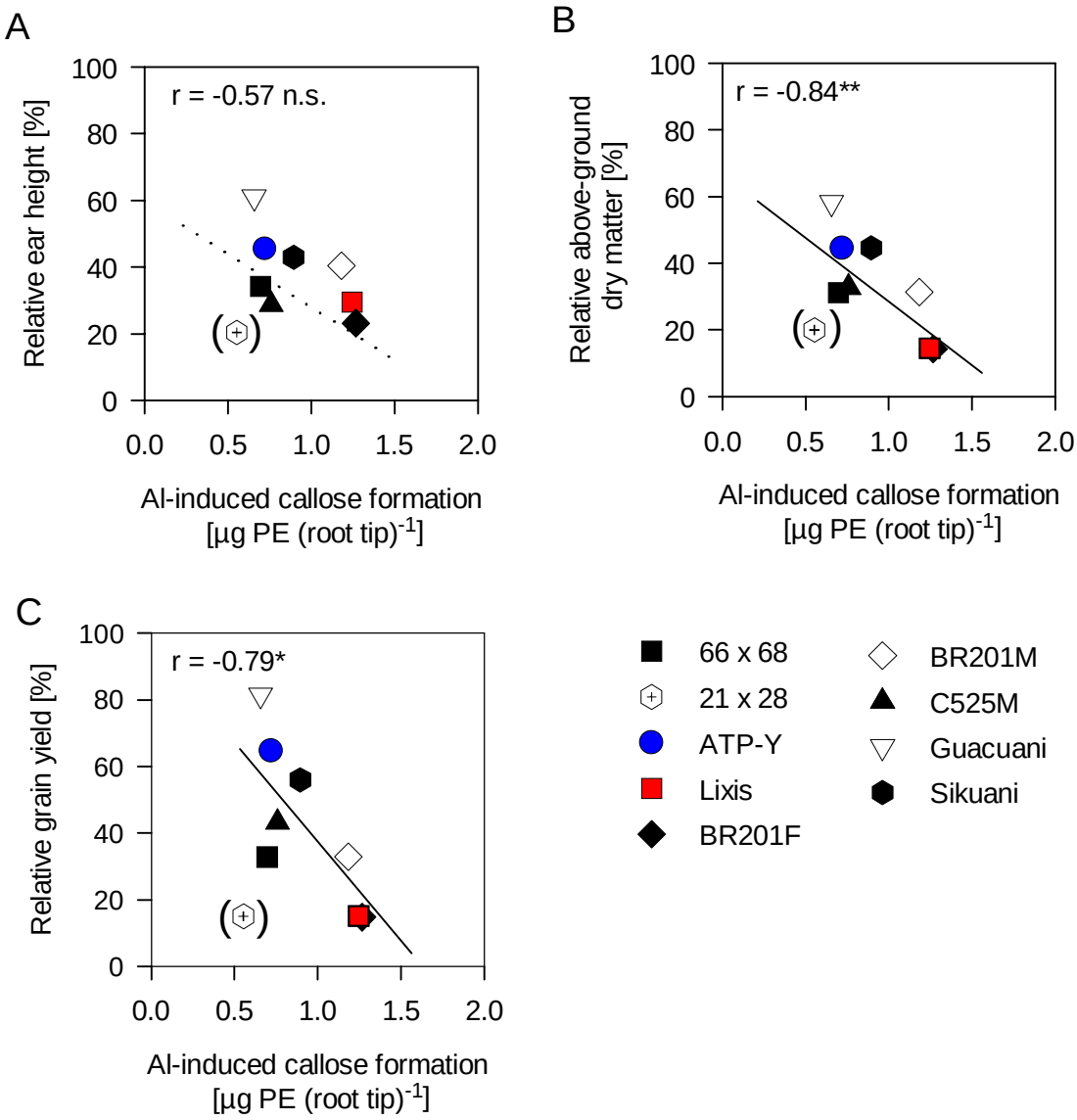

Fig. 4.12: Relationship between Al-induced callose formation in root tips of intact plants of maize cultivars in nutrient solution (25 µM Al, pH = 4.3) and relative ear height for (A), relative above-ground dry matter (B) and relative grain yield (C) after transplanting to a non-acid (100 %) and an acid site. *, **, *** denote probability levels < 0.05, 0.01, and 0.00.1, n.s. > 0.05. Cross 21 X 28 excluded from correlation analysis.

Figure 4.12 shows the relationship between Al-induced callose formation and relative ear height (Fig. 4.12A), relative above-ground dry matter (Fig. 4.12B) as well as relative grain yield on an Al-toxic soil for the cultivars (Fig. 4.12C). All three parameters were negatively associated with Al-induced callose formation in nutrient

solution. Relative grain yield as well as relative above-ground dry matter were significantly negatively correlated with Al-induced callose formation (r = -0.84**, r = -0.79*). The cultivar Lixis known as Al-sensitive showed the highest callose formation accompanied by the lowest relative ear height, relative above-ground dry matter and relative yield, thus reflecting its Al sensitivity. Together with the cultivar BR201F, an Al-sensitive variety, it had the highest influence on the negative correlations. Not only for the means but also for the single values findings were significantly correlated (rel. ear height: r = -0.24*; rel. above-ground dry matter: r = -0.51***; rel. grain yield: r = -0.48***).

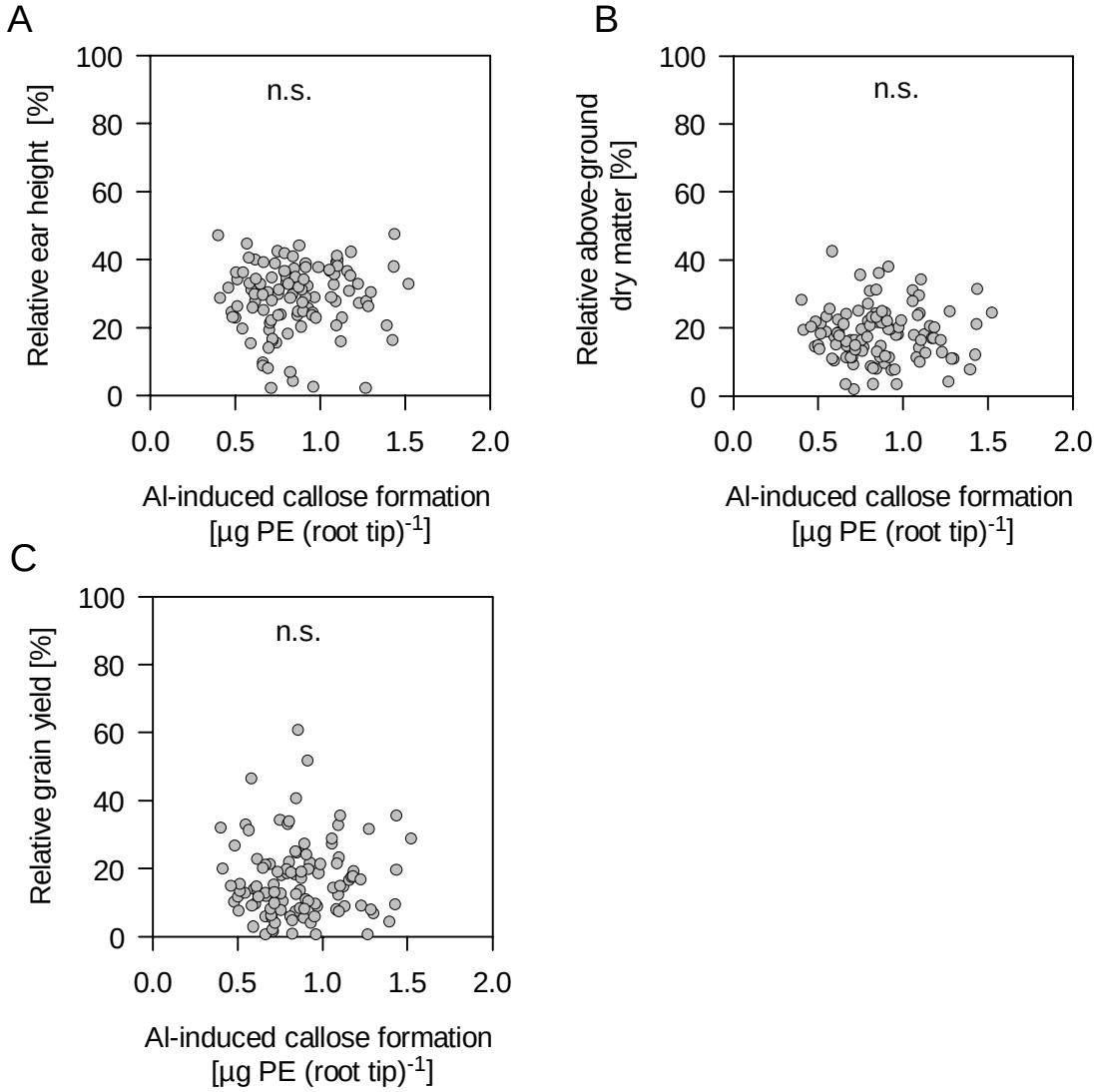

Fig. 4.13: Relationship between Al-induced callose formation in root tips of intact plants of maize inbred lines in nutrient solution (25 µM Al, pH = 4.3) and relative ear height (A), relative above-ground dry matter (B) and relative grain yield (C) after transplanting to a non-acid (100 %) and an acid site. *, **, *** denote probability levels < 0.05, 0.01, and 0.001, n.s. > 0.05.

In contrast to the results for cultivars, no relationship was found for the inbred lines between Al-induced callose formation and relative ear height (Fig. 4.13A), relative above-ground dry matter (Fig. 4.13B) or relative grain yield (Fig. 4.13C). The contradictory results for inbred lines and cultivars could have been due to inherent impaired capacity for callose formation of the inbred lines. Therefore, in a subsequent experiment a subset of 20 lines was treated with digitonin to determine the callose synthesising capacity. A treatment with 25 µM Al was included for the comparison with callose formation in the previous trial and for calculating relative callose formation (digitonin as reference, 100 %). Genotypical Al-induced callose formation in both experiments, conducted in Colombia and Hannover, were highly significantly correlated ($y = -0.104 + 1.156\ x$, $r^2 = 0.84$***). Also, absolute levels of Al-induced callose formation were similar regardless of the location. As before in Colombia, Al-induced callose formation assessed under controlled environmental conditions in Hannover was not corrrelated to relative grain yield. In contrast, Al-induced callose formation of the standard cultivars ATP-Y and Lixis was in agreement with their performance in the field. Relative values of Al-induced callose formation did not give a better relationship to relative grain yield on the field sites (Fig. 4.14B).

As indicated by the significant correlation between grain yields on both sites (Fig. 4.9) plant vigour seemed to be a limiting factor for inbred lines. A re-evaluation of seed kernel weight resulted in a significant variation in kernel weight of 95 inbred lines ranging from 1.53 to 7.26 g 25 kernels^{-1} with a mean of 5.2 ± 0.99 g. For cultivars it ranged from 4.7 to 8.98 g with an average of 6.3 ± 1.18 g. This kernel weight was significantly correlated to plant height at 2 WAS, just before transplanting to the two sites (Tab. 4.7) for the inbred lines only. Additionally, a significant positive correlation between grain yield and plant height at early seedling stage was found.

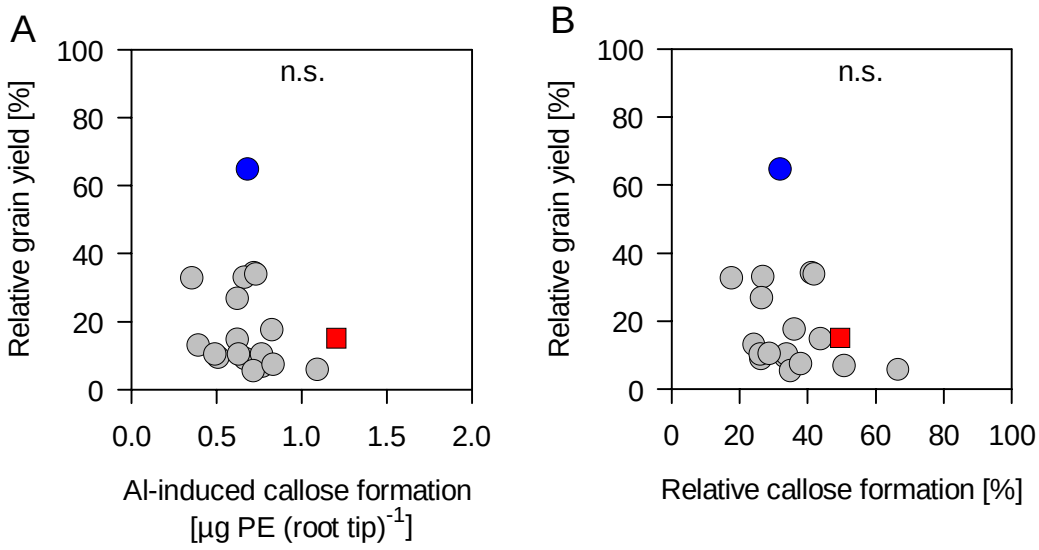

Fig. 4.14: Relationship between relative grain yield (acid site in % of non-acid site) and (A) Al-induced callose formation and (B) relative callose formation (digitonin-induced callose formation = 100 %) as determined in a second experiment (Hannover). *, **, *** denote probability levels < 0.05, 0.01, and 0.001, n.s. > 0.05.

Tab. 4.7: Correlation coefficients (Pearson) calculated among traits measured on 110 maize inbred lines grown on an Al-toxic and non-toxic site in Colombia. *, **, *** denote probability levels < 0.05, 0.01, and 0.001, n.s. > 0.05.

Plant height (WAS)	Grain yield		Above-ground dry matter		Ear height		ASI		Kernel weight	
	Site		Site		Site		Site		Site	
	acid	non-acid	acid	non-acid	acid	non-acid	acid	non-acid	acid	non-acid
2	n.s.	0.32 ***	0.21 *	0.32 ***	n.s.	0.36 ***	n.s.	0.28 **	0.35 ***	0.32 **
4	0.32 ***	0.22 *	0.37 ***	0.25 ***	0.45 ***	0.41 ***	-0.30 ***	n.s.	0.26 ***	0.33 **
6	0.53 **	n.s.	0.60 ***	0.29 **	0.63 ***	0.41 ***	-0.54 ***	n.s.	n.s.	0.24 *
8	0.58 ***	0.31 **	0.65 ***	0.42 ***	0.66 ***	0.55 ***	-0.61 ***	n.s.	n.s.	n.s.
10	0.62 ***	0.52 ***	0.65 ***	0.57 ***	0.77 ***	0.81 ***	-0.57 ***	n.s.	n.s.	n.s.
16	0.63 ***	0.53 ***	0.70 ***	0.56 ***	0.84 ***	0.80 ***	-0.55 ***	n.s.	n.s.	n.s.

Using plant height at 2 WAS (before transplanting) and location as factors in a regression model for grain yield a significant influence of plant height was found for the inbred lines.

A positive linear regression between plant height and grain yield at 2 WAS could be established (Fig. 4.15A). Grain yield could be explained by the variation of plant height even before the seedling was transplanted. For the cultivars such relationships were not found (Fig. 4.15B).

A 2-factorial regression model showed a significant influence of plant height at 2 WAS on grain yield regardless of the site for inbred lines only (Tab. 4.8) .

Tab. 4.8: Linear regressions describing the influence of location and plant height (2 WAS) prior to transplanting upon grain yield at maturity. *, **, *** denote probability levels < 0.05, 0.01, and 0.001, n.s. > 0.05. ° at 2 WAS.

	Regression
Inbred lines	Grain yield = 90.03 + 0.18 height° – 45.08 location, r^2 = 0.25***
Cultivars	Grain yield = 188.01 – 0.27 height° – 63.92 location, n.s.

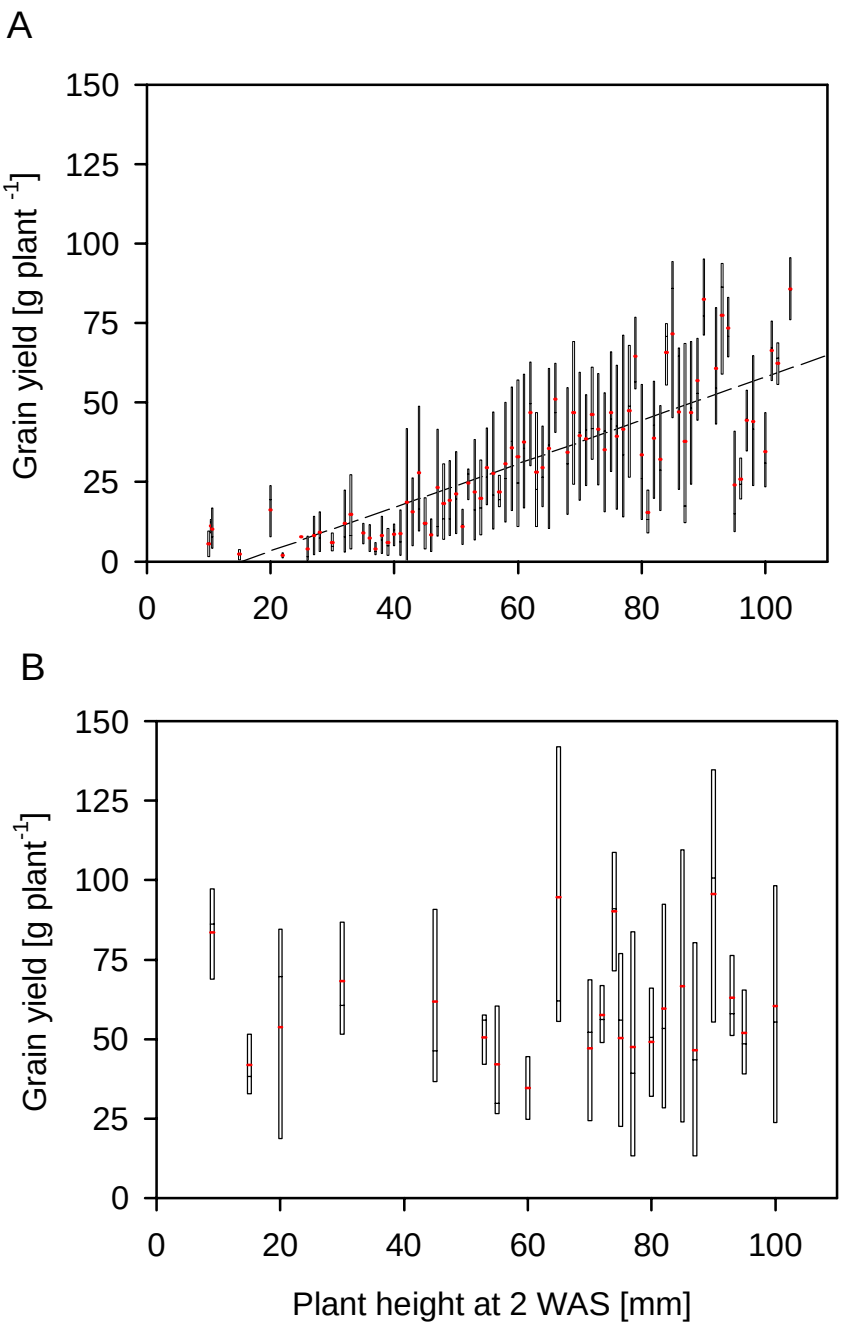

Fig. 4.15: Effect of plant height prior to transplanting on grain yield of inbred lines (A) and cultivars (B). Box plots show grain yield for the plant height at 2 WAS, expressed by lower 0.25 quartil, median and upper 0.75 quartil. Red line refers to the mean.

Discussion

Methodological aspects

The transplanting of maize has been practised in traditional cultivation of maize or under specific climatic conditions to adapt the growth cycle to short growing seasons (de la Rosa, 1846; de Leon, personal communication). It was carried out on a large scale, without examining effects on single plant performance.

In the present study transplanting affected plant height, especially at 8 WAS, on the non-acid soil. An explanation might be found in the loss of those roots during the transplanting that were growing within the nutrient solution. Consequently, shoot growth depended on a smaller root volume. The appearance of P-deficiency symptoms for a few days might be also related to a low root/ shoot ratio. The above-ground growth was retarded. However, at maturity differences between the two planting systems were small. In contrast to plant height, above-ground dry matter and grain yield were not consistently influenced by transplanting (Fig. 4.5, 4.6). Similar means were found for the observed cultivars for both parameters. Hence, in this study, soil and genotype had a much higher influence on plant development, which could be seen in the stronger reduction in plant height, above-ground dry matter and grain yield due to soil acidity.

The adaptation of maize to acid soils might be based on expression of vigour at the young seedling stage allowing the development of an homogeneous and efficient crop canopy (Welcker, personal communication). However, in wheat differences in Al resistance were more clearly expressed in established than in young seedlings (de Lima and Copeland, 1990). Seedling growth within the heterotrophic phase is determined mostly by the endosperm resources. After approx. 3 weeks when endosperm reserves are exhausted, seedlings depend on their ability to generate assimilates to produce a canopy (Pevilla *et al.*, 1999). Therefore, effects of soil acidity on the shoot are likely to be detected thereafter, e.g., as in this study at 6 WAS. Furthermore, whole plant performance, as indicated by grain yield and above-ground dry matter did not indicate that effects of soil acidity on plant development were reduced through transplanting.

The results presented demonstrate that individual plants could be successfully transplanted from nutrient solution into the field after sampling for Al-induced callose formation. Considering the small overall effect on plant performance and grain yield (Fig. 4.4, 4.5) transplanting allowed an evaluation of results achieved in nutrient solution culture and its relationship to plant performance on a particular soil.

Effect of soil acidity on plant growth

The agronomic traits reflected a high stress intensity at the Al-toxic site as described for maize and other cereals (Flores *et al.*, 1988; Pandey *et al.*, 1994; Clark *et al.*, 1997). Cultivars and inbred lines responded similarly to soil acidity. Reduction in plant height, shoot dry matter, delay of silking, lower ear heights, as well as reduced yields with an increased level of soil acidity stress have been reported (Granados *et al.*, 1993; Duque-Vargas *et al.*, 1994; Pandey *et al.*, 1994; Clark *et al.*, 1997; Salazar *et al.*, 1997).

The grain yield showed a significant positive correlation with plant height at maturity (r_{lines} = 0.63***, $r_{cultivars}$ = 0.83***; Tab. 4.5, 4.6). Only for inbred lines a significant correlation between plant height and grain yield could be observed at earlier stages of growth. Following 6 WAS close relationships between plant height and yield as well as other yield determining factors (ASI, ear height) and overall plant performance (above-ground dry matter) were found (Tab.4.2). Therefore, plant height at early stages might be an adequate index to screen inbred lines for acid soil tolerance as proposed by Foy *et al.* (1992) and Zeigler *et al.* (1995). Field trials might be shortened by the use of this trait if grain yield is the main target provided that not other factors as kernel weight, plant vigour or inbreeding depression (see below) are growth-limiting. Furthermore, grain yield of lines and cultivars showed highly significant negative correlations with ASI (r_{lines} = -0,68***, $r_{cultivars}$ = -0.77**), and positive correlation with ear height (r_{lines} = 0.70*, $r_{cultivars}$ = 0.69***) in the acid soil environment. These relationships were consistent with those reported by other authors (Granados *et al.*, 1993; Duque-Vargas *et al.*, 1994; Salazar *et al.*, 1997). Apart from soil acidity an increase of ASI was also reported under low N supply and water stress (Edmeades *et al.*, 1992; Bänzinger *et al.,* 1997). The negative phenotypic correlation between days to silking and ear height could be a response to stress caused by soil acidity which delayed silking and reduced plant and ear height (Borrero *et al.*, 1995). The delay of flowering and thus lengthening of the life

cycle as well as a higher ASI might have contributed to a reduced plant fertility: Grain yield in the acid soil environment showed a highly significant negative correlation with ASI (r_{lines} = -0.77**; $r_{cultivars}$ = -0.68***). The potential use of ASI as a trait for identification of stress-tolerant genotypes could be confirmed.

An increased number of ears per plant has been reported to contribute to higher maize yields in stress environments (Hallauer, 1972) and a lower number of ears per plant is a characteristic of abiotic stress (Pandey *et al.*, 1994; Chapman and Edmeades, 1999). However, in our study the number of ears per plant was significantly reduced only for the inbred lines, but not for cultivars (Tab. 4.4). Differences in the definitions of the trait 'ear' might have accounted for these findings. In this study an ear was counted when at least one kernel was produced per cob. Other definitions and criteria might exist.

Aluminium-induced callose formation of maize cultivars and inbred lines as a marker for adaptation to an acid soil

Cultivars

Aluminium-induced callose formation differed significantly between cultivars. In agreement with other reports, Al-induced callose formation classified cultivars Lixis, BR201F as Al-sensitive and ATP-Y, C525M, BR201M as Al-resistant (Horst *et al.*, 1997; Kollmeier *et al.*, 2000). Aluminium primarily affects the root (Foy, 1988; Bennet and Breen, 1991; Ryan *et al.*, 1993). On the acid soil, shoot development, reported to be a reliable (though indirect) estimate of tolerance in terms of root effects on plant growth (Rajaram *et al.*, 1991; Foy *et al.*, 1993), showed cultivar-specific differences on the acid soil (Fig. 4.2, 4.3). Significant relationships between root parameters in nutrient solution and shoot growth were reported (Foy and da Silva, 1991; Baier *et al.*, 1995). For example, the Al-sensitive cultivar Lixis produced only 25 % of the shoot dry matter of the Al-resistant cultivar ATP-Y (Fig. 4.4). However, Al-induced callose formation was not correlated with relative plant height after transplanting to the field.

Effects on shoot development might be expressed only at later stages of growth as a result of altered water and nutrient uptake (Blamey *et al.*, 1993; Moustakas and Ouzounidou, 1995). Consequently, Al injury was most clearly expressed between

6 and 10 WAS when shoot elongation was at its maximum rate. This might indicate that Al-induced callose formation specifically referred to Al-induced injury and not to the current state of the shoot/ root system. This suggestion is confirmed by the significant correlations between Al-induced callose formation and relative grain yield ($r_{cultivars}$ = -0.79*), and even more by relative above-ground dry matter ($r_{cultivars}$ = -0.84**) at maturity (Fig. 4.12B, C).

Aluminium-induced callose formation turned out to be a sensitive indicator for adaptation to an acid soil. The correlations found here were even stronger than those reported for root length assessed in nutrient solution and grain yield on acid soils (Kasim *et al.*, 1990; Magnavaca and Bahia Filho, 1993).

Still, the predictive value of the method might be limited by plant inherent factors. Low Al-induced callose formation of the cross 21 x 28 was in contrast to its poor performance on acid soil in this and previous studies (de Leon, personal communication). The low callose formation was accompanied by the occurrence of curled roots in nutrient solution, which might have been a sign of disturbed root growth. Since the primary site of Al toxicity is located within the first 3 mm of the root tip (Foy, 1988; Bennet and Breen, 1991; Ryan *et al.*, 1993; Sivaguru and Horst, 1998; Sivaguru *et al.*, 1999), effects of Al toxicity might have been masked by a decreased capacity for callose formation. Larsen *et al.* (1996) reported that induced mutagenesis with ethyl-methylsulfonate resulted in *Arabidospsis* mutants with high inhibition of root elongation but low callose formation. However, digitonin-induced callose formation of the parental lines (21, 28) and the other inbred lines did not show that callose synthesising capacity was impaired (chapter III).

Inbred lines

In contrast to the cultivars, Al-induced callose formation of inbred lines did neither correlate with relative grain yield or above-ground dry matter (Fig. 4.13). This might have been due to inbreeding effects leading to a loss of vitality and fertility (Nass and Crane, 1970; Falconer, 1984). In this study, inbred lines generally had a lower plant height, yield and above-ground dry matter than the subset of hybrids on the non-acid environment (Tab. 4.4). Within only one cycle of selfing a 30 % decrease in grain yield caused by deleterious genes in open pollinating maize populations was found (Lima and Miranda Filho, 1984). The inbred line, derived from SA-4 and SA-7 populations representing a small genetic base (Pandey *et al.*,

1994), passed 5 cycles of selfing leading to inbreeding depression, also affecting the kernel weight of the used seeds. In soybean increased seed weight was associated with tolerance to acid Al-toxic soils (Hanson, 1991; Hanson and Kamprath, 1979). In maize, seed size effects on plant development have been discussed (Hicks *et al.*, 1976; Graven and Carter, 1990; Pommel, 1990; Nafziger, 1992; Bockstaller and Girardin, 1994). A lower seed weight of inbred lines might have decreases plant vigour. It appeared that a low seed weight of inbred lines (1.53 to 7.26 g (25 kernel)$^{-1}$), limited the available endosperm resources in the heterotrophic phase of seedling growth (Cooper and MacDonald, 1970). Consequently, it could have determined seedling vigour up to the 4 or 8 leaf stage as reported by Pommel (1990) and Bockstaller and Girardin (1994). The reaching of physiological stages remained unaffected (Pommel, 1990; Bockstaller and Girardin, 1994). Differences in shoot dry weight are also expressed to a lesser extent in plant height (Bockstaller and Girardin, 1994) explaining the small value of the correlation coefficient of kernel weight and plant height within this study (Tab. 4.7). A high kernel weight was therefore of advantage for the performance of inbred lines before stem elongation. The influence of seed size on plant dry weight was reported to disappear before silking (Bockstaller and Girardin, 1994). However, the linear regression clearly shows that plant height at 2 WAS had a significant effect also on grain yield (Tab. 4.8).

Regarding a low fitness of inbred lines, those lines with a relatively good vigour under non-stress conditions were also performing better under stress. This was demonstrated by the significant correlation (r_{lines} = 0.42***) between the grain yields achieved in the non-acid environment and the acid environment (Fig. 4.9) which was much stronger than reported for maize (r_{lines} = 0.16**, Borrero *et al.*, 1995) using a SA-4 population on the same acid site.

Overall, results indicate that starting conditions as present in the seed were important for the growth of inbred lines and affected plant performance regardless of the environmental conditions. As callose formation is an indicator of stress in general, alteration of Al-induced callose formation might have resulted from inbreeding-induced reduction of vitality. Therefore, prior to its use in screening of inbred lines Al-induced callose formation needs to be proven being independent from inbreeding depression. Further work might also include comparisons on more field sites

and the study of heritability of Al-induced callose formation in maize as it has been shown for relative root length in maize (Lima *et al.*, 1992).

As kernel weight influenced grain yield, those lines producing small grains might be discarded or at least kernel weight should be included as an additional variable in field experiments. If grain yield was limited by the seed size of the planted kernels, the effectiveness of yield and yield-related parameters to assess adaptation to acid soils would have to be questioned in the case of inbred lines.

For inbred lines parameters as plant height might be a useful trait for the estimation of grain yield. The findings presented indicate that it would be desirable to evaluate inbred lines in hydroponics because of the reduced effect of seedling vigour on the plants assessment (Bahia Filho *et al.*, 1997). The results demonstrate furthermore, that a non-acid control is needed to evaluate if inherent growth limiting parameters exist, which might be seen only on the non-acid site or in contrast to the acid site.

The results show that maize seedlings can be transplanted from nutrient solution into the soil for evaluation of plant performance. Transplanting might be used for validating screening methods targeting at the root. Evaluation of callose formation during 12 h in nutrient solution was effective as a preliminary screen for maize cultivars. This system should be especially useful in studies of inheritance of Al tolerance where seed supply is small and it is desirable to save individual plants after screening for transplanting into pots or the field for further observations and/ or seed multiplication.

GENERAL DISCUSSION

A general agreement between soil and Al-toxic solution culture rankings in the area of soil acidity is suggested on the one hand (Howeler and Cadavid, 1976; Konzak *et al.*, 1976; Foy *et al.*, 1989; Ruiz-Torres *et al.*, 1992; Wheeler *et al.,* 1992c). On the other hand, however, it was often difficult to demonstrate a relationship between nutrient solution and field studies (Sartain and Kamprath, 1978; Sapra *et al.*, 1982; Campbell *et al.*, 1990, 1991). The lack of relationships was related to the composition of the solution (Blamey *et al.*, 1991; Edmeades *et al.*, 1995) interfering with Al toxicity, inadequate soil properties (Mugwira *et al.*, 1981; Ruiz-Torres *et al.*, 1992; Mugwira and Haque, 1993), insufficient numbers of replications within the greenhouse trial used as reference (Garland Campbell and Carter, 1990), or the use of inadequate plant parameters (Campbell *et al.*, 1988). Hairiah *et al.* (1992) suggested another reason for the contradictory results between nutrient solution and field observations: the phenomenon of Al avoidance. Al avoidance in that case described promoted growth of those roots not exposed to Al, accompanied by a strongly enhanced growth inhibition of roots in contact with Al, regardless of the cultivars' Al resistance. Therefore, the use of homogeneous Al supply in nutrient solution in conventional screening experiments might have been misleading. However, the study presented here clearly demonstrates that at least in maize there was no evidence for an Al avoidance reaction for the Al-resistant cultivar. A compensatory growth was only observed in the Al-sensitive cultivar and should be considered a general plant inherent reaction toward soil heterogeneity as reported in the past (Scott Russell, 1977; Fitter, 1994; Robinson, 1994, 1996; Marschner, 1996). Parts of the root system growing in favourable environment show an enhanced growth. In contrast, the remaining root system tends to grow less. The ranking of the cultivars in Al resistance was similar at homogeneous as well as heterogeneous Al supply. The observed compensatory growth might explain why some Al-sensitive cultivars perform better on an acid field site than in nutrient solution.

In the light of the present findings screening under homogeneous Al supply is not limited. Screening in nutrient solution offers a time-saving and simplified approach

as long as the recommendations for conducting these kind of studies are considered (Pavan and Bingham, 1982; Blamey *et al.*, 1991; Edmeades *et al.*, 1995).

Nevertheless, oversimplification of the screening system should be avoided. The use of excised root tips has been reported in studies of Al toxicity (Huett and Menary, 1979; Macklon and Sim, 1981; Malavolta *et al.*, 1981; Samuels *et al.*, 1997), but turned out to be inappropriate for studying callose formation in maize. This does not question the approach of using Al-induced callose formation as it was shown to work in protoplasts, suspension culture and roots of intact plants (Staß and Horst, 1995; Wagatsuma *et al.*, 1995; Horst *et al.*, 1997). However, root tip tissue seemed to be more fragile and less independent in metabolism from other plant parts and organs. However, it remained amasing that working with excised root tips of wheat differences in Al resistance between cultivars could be reported. The findings demonstrate the lack of experimental evidence collected so far about the regulation of callose formation (Delmer, 1987; Kauss, 1987b), especially in the case of Al toxicity. Use of Al-induced callose formation after any duration of Al treatment was inappropriate. Time course of callose formation was of major importance. Callose formation has been reported as early as 30-90 min (Wissemeier *et al.*, 1992; Zhang *et al.*, 1994; Wissemeier and Horst, 1995) after exposure to Al in nutrient solution. In maize, 12 h of Al exposure seem to be necessary to differentiate maize cultivars and inbred lines. Results achieved by Al treatments without including the time-specific curvature as conducted by Wissemeier *et al.* (1998) and Gunsé *et al.* (2000) might be misleading as shown in chapter III.

In contrast to time-course, a differential potential in callose formation might be of minor influence. A cultivar-specific potential in callose formation, as well as an assumed decomposition of callose after 12 h of Al treatment might explain, why in previous studies in some maize cultivars Al-induced callose formation was not related to root length-inhibition (Llugany *et al.*, 1994; Horst *et al.*, 1997). Digitonin might be used as an additional control to examine the potential of callose formation if Al-induced callose formation is low in specific cultivars. As digitonin also inhibited root elongation, more fine tuning of the concentration applied is needed for intact plants. Only when an inhibition of root elongation could be achieved in a range that is proportional to the effect of the Al applied, amounts of callose formation under both conditions might be comparable. Furthermore, Al-induced callose

formation might only be used for assessing maize cultivars stabile in growth and yield. As inbred lines suffered from inbreeding depression as well as of potential mutagenesis, callose formation might have been impaired as in the case of mutagenised *Arabidopsis* (Larsen *et al*., 1996). Consequently, assessment of Al resistance by Al-induced callose formation was erroneous.

The high correlation between Al-induced callose formation in nutrient solution and grain yield and plant dry matter contrast the expected low correlations between results from nutrient solution and field experiments in acid soil (Magnavaca *et al*., 1996; Bahia Filho *et al*., 1997). Significant negative relationships 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). Characterisation of cultivars in Al resistance with subsequent transplanting proved that induction of Al-induced callose formation after 12 h of Al exposure was sensitive enough to separate cultivars in their adaptation to an acid soil. This is even more important since a linkage between seedling stage and maturity could be demonstrated. The presented work could fulfil most of the characteristics of an ideal screening system (Scott and Fisher, 1993): A high correlation between the screening results and performance in the target environment was found.
110 lines, each represented by 12 individuals could be handled by a single person, transplanting of individuals and proven differentiation match the postulation of an ability to handle large populations, ability to differentiate between candidate genotypes, and non-destructiveness to the plant. Reproducibility of the results achieved in nutrient solution and field was shown for *Triticale* by Oettler *et al*. (1997) and might therefore hold true also for maize.

ZUSAMMENFASSUNG

Weltweit ist Al-Toxizität in Zusammenhang mit Bodenversauerung eine der größten wachstumshemmenden Faktoren. Dennoch wird auf ca. 8 bis 26 Millionen Hektar sauren Bodens Mais angebaut. Die züchterische Anpassung von Mais an diese Bedingungen stellt eine dauerhaft umweltverträgliche, relativ kostengünstige und nachhaltige Form dar, um die Erträge auf solchen Standorten zu erhöhen. Schnelle und effektive Selektionsverfahren könnten den Fortschritt in der Züchtung erhöhen. Die Nutzung von Nährlösungskulturen stellt dabei ein wirksames Instrument in der Unterstützung der Züchtung dar. Al-induzierte Callosebildung kann als Maß für die Schädigung der Wurzel und somit auch als Parameter in der Selektion verwendet werden. Allerdings können die in Nährlösungskultur erzielten Ergebnisse nicht ohne weiteres auf die komplexeren Bedingungen im Feld übertragen werden. Eine Überprüfung bzw. Kombination von Feld und Nährlösungsversuchen ist daher unabdingbar. In der vorliegenden Arbeit wurde geprüft,

(i) ob die Aussagen über Al-Resistenz unter üblichen Verwendung einer einheitlichen Al-Applikation an das gesamte Wurzelsystem nur eingeschränkte Aussagen liefert.
(ii) ob ein Screening von Mais durch Verwendung von abgeschnittenen Wurzelspitzen anstelle der intakten Pflanze vereinfacht werden kann.
(iii) ob Maisinzuchtlinien mittels Al-induzierter Callosebildung und Hemmung des Wurzellängenwachstums hinsichtlich ihrer Al-Resistenz eingestuft werden können.
(iv) ob mittels Al-induzierter Callosebildung eine Aussage über die Anpassung von Mais an saure Böden gemacht werden kann.

(i) Mittels eines ‚Split-root'-Systems wurden Teile des Wurzelsystems von 9 Tage alten Maispflanzen eines Al-resistenten (BR201M) und Al-sensitiven (Lixis) Genotyps einem unterschiedlichen Al (0, 10 µM) und P-Angebot (1, 25 µM) in der Nährlösung ausgesetzt. Im Gegensatz zu hohem P-Angebot führte ein gleichmäßig niedriges P-Angebot in der Nährlösung einerseits zu einer Erhöhung des Wurzelwachstums und andererseits zu einer Verringerung der Spross-P-Gehalte. Die

durch Al ausgelöste Hemmung des Wurzellängenwachstums war für die Al-sensitive Sorte Lixis deutlicher ausgeprägt als für die Al-resistente Sorte BR201M. Al-behandelte Wurzelspitzen wiesen höhere Gehalte an Al und Al-induzierter Callose auf als solche ohne Al-Behandlung. War nur ein Teil des Wurzelsystems Al ausgesetzt, so trat lediglich bei der Al-sensitiven Sorte Lixis kompensatorisches Wurzelwachstum auf der Al-freien Seite auf. Hingegen zeigte die Al-resistente Sorte BR201M keine solche Reaktion. Ebenfalls konnte entgegen der Erwartung keine sogenannte Al-Vermeidungsreaktion gefunden werden, bei der einem höherem Wachstum auf der Al-freien Seite zusätzlich eine deutliche Wachstumsreduktion auf der Al-behandelten Seite gegenüberstehen sollte. Die Einschätzung der Sorten als Al-resistent bzw. sensitiv wurde durch die Behandlung nur einer oder beider Seiten des Wurzelsystem mit Al nicht beeinträchtigt.

(ii) Abgeschnittene Wurzeln und Wurzeln intakter Pflanzen von 10 verschieden **Maissorten** wurden einem Al-Angebot von 0 und 25 µM Al in Nährlösung ausgesetzt. Wurden intakte Pflanzen verwendet, so konnten die Sorten hinsichtlich ihrer Al-Resistenz deutlich besser durch die Bildung von Al-induzierter Callose als durch die Hemmung des Wurzellängenwachstums charakterisiert werden. Es wurden signifikante Korrelationen zwischen Al-induzierte Callose und der Hemmung des Wurzellängenwachstums ($r^2 = 0.80$***) und des Al-Gehalts ($r^2 = 0.64$**) gefunden. Im Gegensatz dazu wurden bei Verwendung inkubierter Wurzelspitzen keine signifikanten Beziehungen gefunden. Ebenso bestand kein Zusammenhang zwischen den Parametern intakter und inkubierter Wurzelspitzen. Diese Ergebnisse stehen im Widerspruch zur Literatur, in der an Protoplasten und Suspensionskulturen sortenspezifische Unterschiede in der Al-resistenz beschrieben wurden. Die Gabe von Glucose erhöhte zwar die Callosebildung in abgeschnittenen Wurzelspitzen, ebenso wie das Längenwachstum und die Vitalität, Verbesserungen in der Charakterisierung der Genotypen in der Al-Resistenz ergaben sich jedoch nicht. Aus den vorgestellten Ergebnisse geht hervor, daß die Funktion von Al-Resistenzmechanismen in abgeschnittenen Wurzelspitzen eingeschränkt ist. Unter den geschilderten Bedingungen erscheint die Nutzung von abgeschnittener Wurzelspitzen als ein vereinfachtes System für das Screening ungeeignet zu sein.

(iii) **Maisinzuchtlinien** wurden in ihrer Al-Resistenz in Nährlösung (0, 25 µM Al) untersucht. Dazu wurden die nach 48 h gebildete Al-induzierte Callosebildung und das Wurzellängenwachstum, ermittelt durch Färbung der Wurzeln bei Versuchsbeginn mit Neutralrot, bestimmt. Die beste Auftrennung der Linien erfolgte durch den Parameter Hemmung des Wurzellängenwachstum und entgegen den Erwartungen nicht durch die Al-induzierte Callosebildung. Eine mögliche Erklärung des daraus resultierenden schlechten Zusammenhangs beider Parameter lag in einer zeitabhängigen Callosebildung und möglichen -degradation: Ein Maximum der Callosebildung war im Bereich von 6 - 12 h Al-Behandlung zu finden. Danach nahm der Callosegehalt i.d.R. genotypisch-spezifisch ab, so daß nach 24 h nur noch geringfügige Mengen Callose in den Wurzelspitzen zu finden waren. Bei einer Inzuchtlinie war hingegen kein Abfall sondern ein gradueller Anstieg der Callosegehalte bis zum Versuchsende bei 48 h festzustellen. Signifikante Korrelationen zwischen der Hemmung des Wurzelwachstums und Al-induzierter Callosebildung wurden bei 12 (r^2=0.49*) und 24 h (r^2=0.55**) Al-Behandlung gefunden, nicht jedoch bei 48 h. Ein unterschiedliches Potential in der Callosesynthese spielte nur eine untergeordnete Rolle. Die Ergebnisse belegen die Notwendigkeit vor der Verwendung von Callose als Marker für eine Al-spezifische Schädigung den zeitlichen Einfluß auf die Callosegehalte zu überprüfen.

(v) In Kolumbien wurden **Maisinzuchtlinien** und **Maissorten** in Nährlösung (0, 25 µM Al) hinsichtlich ihrer Callosebildung untersucht und anschließend an einen sauren, Al-toxischen bzw. nicht sauren Boden verpflanzt. Auf dem sauren Boden war eine signifikante Reduktion des Pflanzenwachstums und des Kornertrags im Vergleich zum nicht sauren Boden festzustellen. Zwischen dem Kornertrag und den Parametern Zeitintervall von männlicher und weiblicher Blüte (r_{Linien} = -0,68***, r_{Sorten} = -0.77**), Pflanzenhöhe (r_{Linien} = 0.63***, r_{Sorten} = 0.83***), Insertionshöhe des Kolbens (r_{Linien} = 0.70*, r_{Sorten} = 0.69***) konnten sowohl für die Linien als auch die Sorten signifikante Beziehungen ermittelt werden. Allerdings wurden signifikante Beziehungen zwischen Al-induzierter Callose in Nährlösung und dem relativen Kornertrag (r_{Sorten} = -0.79*) und der relativen oberirdischen Pflanzenmasse (r_{Sorten} = -0.84**) nur für die Sorten gefunden, nicht jedoch für die Linien. Dieses widersprüchliche Ergebnis konnte darauf zurückgeführt werden, daß nur bei den Linien dem Korngewicht und daraus folgend der Pflanzenhöhe vor dem

Verpflanzen eine ertragsbestimmende Bedeutung zufiel. Werden Feldversuche durchgeführt, kann die Pflanzenhöhe der Inzuchtlinien, da mit dem Kornertrag bereits nach kurzer Zeit signifikant korreliert, ein zusätzliches, frühzeitiges Maß für deren Anpassung an saure Böden sein. Liegen jedoch keine pflanzeninherenten limitierenden Faktoren vor, wie es bei den Sorten anzunehmen ist, kann mittels Al-induzierter Callosebildung eine schnelle Aussage über deren Al-Resistenz und Anpassung an saure Böden getroffen werden. Die gezeigte Möglichkeit des Verpflanzen von Mais nach dem Abschluß des Nährlösungsversuchs kann in Züchtungsprogrammen von Bedeutung sein, wo nur geringe Mengen an Saatgut vorhanden und individuelle Pflanzen für Vermehrungszwecken oder Ertragsuntersuchungen erforderlich sind.

Schlagwörter: Aluminium, Screening, *Zea mays*

APPENDIX

Tab. 1.2 Mineral element contents in root tips of cultivar BR201M exposed to a spatially distributed Al (0, 10 µM) and P (1, 25 µM) and supply in a split-root system.

Distribution of Al, P		Element content [µg (root tip)$^{-1}$]		
P	Al	Ca	Mg	K
	-	0.39 ± 0.49	0.42 ± 0.17	5.03 ± 1.03
	-	0.36 ± 0.16	0.48 ± 0.18	5.32 ± 2.77
1 µM P	-	0.57 ± 0.08	0.41 ± 0.08	7.46 ± 3.99
1 µM P	+	0.43 ± 0.24	0.51 ± 0.22	5.05 ± 0.72
	+	0.50 ± 0.24	0.58 ± 0.08	6.80 ± 1.35
	+	0.46 ± 0.20	0.50 ± 0.16	6.67 ± 1.64
	-	0.75 ± 0.31	0.62 ± 0.17	4.98 ± 0.76
	-	0.33 ± 0.19	0.54 ± 0.19	5.70 ± 0.68
25 µM P	-	0.38 ± 0.29	0.53 ± 0.35	6.43 ± 1.65
25 µM P	+	0.48 ± 0.31	0.49 ± 0.19	5.52 ± 1.55
	+	0.39 ± 0.39	0.46 ± 0.25	5.77 ± 3.05
	+	0.50 ± 0.30	0.41 ± 0.25	6.88 ± 0.86
	-	0.40 ± 0.18	0.37 ± 0.16	4.85 ± 2.28
	-	0.60 ± 0.19	0.43 ± 0.11	4.22 ± 1.00
1 µM P	-	0.27 ± 0.03	0.73 ± 0.21	4.70 ± 0.58
25 µM P	+	0.26 ± 0.20	0.36 ± 0.10	4.65 ± 1.66
	+	0.48 ± 0.26	0.25 ± 0.17	9.19 ± 1.84
	-	0.53 ± 0.14	0.60 ± 0.29	6.18 ± 2.31
	+	0.73 ± 0.79	0.30 ± 0.12	5.78 ± 1.85
	+	0.45 ± 0.21	0.66 ± 0.19	7.39 ± 1.38

Tab. 1.3 Mineral element contents in root tips of the Al-sensitive cultivar Lixis exposed to a spatially distributed Al (0, 10 µM) and P (1, 25 µM) supply in a split-root system.

Distribution of Al, P		Element content [µg (root tip)$^{-1}$]		
P	Al	Ca	Mg	K
	-	0.65 ± 0.50	0.27 ± 0.09	4.2 ± 1.35
	-	0.36 ± 0.17	0.23 ± 0.05	6.3 ± 3.28
1 µM P	-	0.94 ± 0.50	0.40 ± 0.14	9.3 ± 1.54
1 µM P	+	0.65 ± 0.45	0.33 ± 0.06	8.5 ± 1.32
	+	0.48 ± 0.16	0.29 ± 0.07	8.8 ± 2.17
	+	0.38 ± 0.18	0.26 ± 0.11	8.0 ± 2.32
	-	0.74 ± 0.25	0.37 ± 0.08	8.9 ± 3.19
	-	0.70 ± 0.25	0.39 ± 0.03	9.9 ± 5.04
25 µM P	-	0.60 ± 0.27	0.28 ± 0.05	5.9 ± 3.06
25 µM P	+	1.02 ± 0.94	0.35 ± 0.14	9.3 ± 1.44
	+	1.04 ± 0.20	0.52 ± 0.04	9.0 ± 2.65
	+	0.65 ± 0.14	0.38 ± 0.12	7.4 ± 0.82
	-	0.90 ± 0.71	0.31 ± 0.06	4.6 ± 2.01
	-	0.69 ± 0.27	0.28 ± 0.12	8.0 ± 3.16
1 µM P	-	0.76 ± 0.32	0.31 ± 0.05	8.3 ± 1.77
25 µM P	+	0.74 ± 0.75	0.46 ± 0.11	7.3 ± 3.12
	+	0.39 ± 0.04	0.26 ± 0.01	9.6 ± 3.62
	-	0.84 ± 0.73	0.31 ± 0.07	8.9 ± 3.54
	+	0.65 ± 0.35	0.28 ± 0.09	11.1 ± 1.02
	+	0.71 ± 0.56	0.37 ± 0.13	16.1 ± 7.33

Tab. 3.4: Dye residues of neutral red led to a visible colouring of the nutrient solution. This contamination was determined to be in the range of 0.2 - 0.32 µM neutral red. As a consequence, intercept of the Al calibration curve was significantly increased only when neutral red concentration was above 0.32 µM. The slope of the regression curve remained similar.

	Calibration curve for the detection of monomeric Al [0 - 40µM Al]	
NR concentration	Intercept	Slope
0 [µM]	0.0647 c	0.0206 a
0.32 [µM]	0.0869 bc	0.0203 a
1.6 [µM]	0.1446 a	0.0198 a

Tab. 4.9: Correlation coefficients (Pearson) calculated among traits measured on 110 maize inbred lines grown on an Al-toxic and non-toxic site in Colombia. *, **, *** denote probability levels < 0.05, 0.01, and 0.001, n.s. > 0.05.

Plant height (WAS)	Grain yield		Above-ground dry matter		Ear height		ASI		Kernel weight	
	Site		Site		Site		Site		Site	
	acid	non-acid	acid	non-acid	acid	non-acid	acid	non-acid	acid	non-acid
2	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
4	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
6	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.45 *
8	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
10	0.85 **	n.s.	0.76 *	0.78 *	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
16	0.85 **	060	0.89 *	0.79*	0.96 ***	0.71 *	n.s.	n.s.	n.s.	n.s.

REFERENCES

Adalsteinsson, S. (1994). Compensatory root growth in winter wheat: Effects of copper exposure on root geometry and nutrient distribution. *J. Plant Nutr.* **17**, 1501-1512.

Adams, F. (1984). Aluminum toxicity and calcium deficiency in acid subsoil horizons of two coastal plains soil series. *Soil Sci. Soc. Am. J.* **48**, 1305-1309.

Ahlrichs, J. L., Karr, M. C., Baligar, V. C., and Wright, R. J. (1990). Rapid bioassay of aluminum toxicity in soil. *Plant Soil* **122**, 279-285.

Altman, F. P. (1976). Tetrazolium Salts and Formazans. *Progr. Histochem. Cytochem.* **9**, 1-52.

Alva, A. K., Edwards, D. G., Asher, C. J., and Blamey, F. P. C. (1986a). Effects of phosphorus/ aluminum molar ratio and calcium concentration on plant response to aluminium toxicity. *Soil Sci. Soc. Am. J.* **50**, 133-137.

Alva, A. K., Edwards, D. G., Asher, C. J., and Blamey, F. P. C. (1986b). Relationships between root length of soybean and calculated activities of aluminum monomers in nutrient solution. *Soil Sci. Soc. Am. J.* **50**, 959-962.

Anderson, R. L., and Ray, P. M. (1978). Labeling of the plasma membrane of pea cells by a surface-localized glucan synthase. *Plant Physiol.* **61**, 723-730.

Anghioni, I., and Barber, S. A. (1980). Phosphorus influx and growth characteristics of corn roots as influenced by phosphorus supply. *Agron J.* **72**, 685-688.

Anghioni, I., and Barber, S. A. (1988). Corn root growth and nitrogen uptake as affected by ammonium placement. *Agron. J.* **80**, 799-802.

Aniol, A. (1984). Induction of Al tolerance in wheat seedlings by low doses of aluminum in nutrient solution. *Plant Physiol.* **75**, 551-555.

Aniol, A. (1990). Genetics of tolerance to aluminum in wheat (*Triticum aestivum* L. Thell). *Plant Soil*, 223-227.

Aniol, A., Hill, R. D., Larter, E. N. (1980). Aluminum tolerance of spring rye inbred lines. *Crop Sci.* **20**, 205-208.

Aniol, A., and Gustafson, J. P. (1984). Chromosome location of genes controlling aluminum tolerance in wheat, rye and triticale. *Can. J. Gen. Cyt.* **26**, 701-705.

Armiger, W. H., Foy, C. D., Fleming, A. L., and Caldwell, B. E. (1968). Differential tolerance of soybean varieties to an acid soil high in exchangeable aluminum. *Agron. J.* **60**, 67-70.

Bänziger, M., Bertrán, F. J., and Lafitte, R. H. (1997). Efficiency of high nitrogen selection environments for improving maize for low-nitrogen target environments. *Crop Sci.* **37**, 1103-1109.

Bahia Filho, A. F. C., Magnavaca, R., Schaffert, R. E., and Alves, V. M. C. (1997). Identification, utilization, and economic impact of maize germplasm tolerant to low levels of phosphorus and toxic levels of exchangeable aluminum in Brazilian soils. *In* "Plant-soil interactions at low pH: Sustainable agriculture and forestry production" (A. C. Moniz, A. M. C. Furlani, R. E. Schaffert, N. K. Fageria, C. A. Rosolem and H. Cantarella, eds.), pp. 59-70. Brazilian Soil Science Society.

Baier, A. C., Somers, D. J., and Gustafson, J. P. (1995). Aluminium tolerance in wheat: correlating hydroponic evaluations with field and soil performances. *Plant Breeding* **114**, 291-296.

Baligar, V. C., and Fageria, N. K. (1997). Nutrient use efficiency in acid soils: nutrient management and plant use efficiency. *In* "Plant-soil interactions at low pH: Sustainable agriculture and forestry production" (A. C. Moniz, A. M. C. Furlani, R. E. Schaffert, N. K. Fageria, C. A. Rosolem and H. Cantarella, eds.), pp. 75-95. Brazilian Soil Science Society.

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

Baligar, V. C., Wright, R. J., Ritchey, K. D., Ahlrichs, J. L., and Woolum, B. K. (1991). Soil and solution property effects on root growth of aluminium tolerant and intolerant wheat species. *In* "Plant-soil interactions at low pH" (R. J. Wright, V. C. Baligar and R. P. Murrmann, eds.), pp. 245-252. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Basu, U., Basu, A., and Taylor, G. J. (1994). Differential exudation of polypeptides by roots of aluminum-resitant and aluminum-sensitive cultivars of *Triticum aestivum* L. in response to aluminum stress. *Plant Physiol.* **106**, 151-158.

Basu, U., McDonald-Stephenson, J. L., Archambault, D. J., Good, A. G., Briggs, K. G., Taing-Aung, and Taylor, G. J. (1997). Genetic and physiological analysis of doubled-haploid, aluminium-resistant lines of wheat provide evidence for the involvement of a 23 kD, root exudate polypeptide in mediating resistance. *In* "Plant nutrition - for sustainable food production and environment" (T. Ando, K. Fujita, T. Mae, H. Matsumoto, S. Mori and J. Sekiya, eds.), pp. 433-438. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Bennet, R. J., and Breen, C. M. (1991). The aluminium signal: New dimensions to mechanisms of aluminium tolerance. *Plant Soil* **134**, 153-166.

Bennet, R. J., Breen, C. M., and Fey, M. V. (1984). Aluminium uptake sites in the primary root of *Zea mays* L. *S. Afr. J. Plant Soil* **2**, 1-7.

Bennet, R. J., Breen, C. M., and Fey, M. V. (1986). Aluminium toxicity and induced nutrient disorders involving the uptake and transport of P, K, Ca and Mg in *Zea mays* L. *S. Afr. Tydskr. Plant Grond* **3**, 11-17.

Blamey, F. P. C., Edmeades, D. C., Asher, C. J., Edwards, D. G., and Wheeler, D. M. (1991). Evaluation of solution culture techniques for studying aluminum toxicity in plants. *In* "Plant-soil interactions at low pH" (R. J. Wright, V. C. Baligar and R. P. Murrmann, eds.), pp. 905-912. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Blamey, F. P. C., Edwards, D. G., and Asher, C. J. (1983). Effects of aluminum, OH:Al and P:Al molar ratios, and ionic strength on soybean root elongation in solution culture. *Soil Sci.* **136**, 197-207.

Blamey, F. P. C., Robinson, N. J., and Asher, C. J. (1992). Interspecific differences in aluminum tolerance in relation to root cation-exchange capacity. *Plant Soil* **146**, 77-82.

Blamey, F. P. C., Asher, C. J., Edwards, D. G., and Kerven, G. L. (1993). *In vitro* evidence of aluminum effects on solution movement through root cell walls. *J. Plant Nutr.* ***16***, 555-562.

Blancaflor, E. B., Jones, D. L., and Gilroy, S. (1998). Alterations in the cytoskeleton accompany aluminum-induced growth inhibition and morphological changes in primary roots of maize. *Plant Physiol.* **118**, 159-172.

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

Bockstaller, C., and Girardin, P. (1994). Effects of seed size on maize growth from emergence to silking. *Maydica* **39**, 213-218.

Borkert, C. M., and Barber, S. A. (1983). Effect of supplying P to a portion of the soybean root system on root growth and P uptake kinetics. *J. Plant Nutr.* **6**, 895-910.

Borrero, J. C., Pandey, S., Ceballlos, H., Magnavaca, R., and Bahia Filho, A. F. C. (1995). Genetic variances for tolerance to soil acidity in a tropical maize population. *Maydica* **40**, 283-288.

Brouder, S. M., and Cassman, K. G. (1994). Cotton root and shoot responses to localized supply of nitrate, phosphate and potassium: Split-pot studies with nutrient solution and vermiculitic soil. *Plant Soil* **161**, 179-193.

Brouquisse, R., James, F., Raymond, P., and Pradet, A. (1991). Study of glucose starvation in excised maize root tips. *Plant Physiol.* **96**, 619-626.

Brouwer, R. (1981). Co-ordination of growth phenomena within a root system of intact maize plants. *Plant Soil* **63**, 65-72.

Bryce, J. H., and Reesap, T. (1985). Effects of sucrose on the rate or respiration of the roots of *Pisum sativum*. *J. Plant Physiol.* **120**, 363-367.

Budíková, S. (1999). Structural changes and aluminium distribution in maize root tissue. *Biol. Plant.* **42**, 259-266.

Bushamuka, V. N., and Zobel, R. W. (1998). Maize and soybean tap, basal, and lateral root responses to a stratified acid alumium-toxic soil. *Crop Sci.* **38**, 416-421.

Campbell, T. A., Elgin Jr., J. H., Foy, C. D., and McMurtrey III, J. E. (1988). Selection in alfalfa for tolerance to toxic levels of aluminum. *Can. J. Plant Sci.* **68**, 743-753.

Campbell, T. A., Nuernberg, N. J., and Foy, C. D. (1990). Differential response of red clover germplasm to aluminum stress. *J. Plant Nutr.* **13**, 1463-1474.

Campbell, T. A., Nuernberg, N. J., and Foy, C. D. (1991). Differential response of *Sericea lespedeza* to aluminium stress. *J. Plant Nutr.* **14**, 1057-1065.

Cançado, G. M. A., Loguercio, L. L., Martins, P. R., Parentoni, S. N., Paiva, E., Borém, A., and Lopes, M. A. (1999). Hematoxylin staining as a phenotypic index for aluminum tolerance selection in tropical maize (*Zea mays* L.). *Theor. Appl. Genet.* **99**, 747-754.

Carvalho, M. C. S., and van Raij, B. (1997). Calcium sulphate, phosphogypsum and calcium carbonate in the amelioration of acid subsoils for root growth. *Plant Soil* **192**, 37-48.

Ceballos, H., Pandey, S., Knapp, E. B., and Duque, J. (1995). Progress from selection for tolerance to soil acidity in five tropical maize populations. *In* "Plant-soil interactions at low pH: Principles and management" (R. A. Date, N. J. Grunden, G. E. Rayment and M. E. Probert, eds.), pp. 419-424. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Chaillou, S., Rideout, J. W., Raper, C. D., and Morot-Gaudry, J.-F. (1994). Responses of soybean to ammonium and nitrate supplied in combination to the whole root system or separately in a split-root system. *Physiol. Plant.* **90**, 259-268.

Chapman, S. C., and Edmeades, G. O. (1999). Selection improves drought tolerance in tropical maize populations: II. Direct and correlated responses among secondary traits. *Crop Sci.* **39**, 1315-1324.

Chastain, C. J., and Hanson, J. B. (1982). Control of proton efflux from corn root tissue by an injury-sensing mechanism. *Plant Sci. Letters* **24**, 97-104.

Clark, R. B. (1984). Physiological aspects of calcium, magnesium, and molybdenum deficiencies in plants. *In* "Soil acidity and liming" (F. Adams, ed.), Vol. 12, pp. 99-170. ASA-CSSA-SSSA, Madison, Wisconsin, USA.

Clark, R. B., and Duncan, R. R. (1991). Improvement of plant mineral nutrition through breeding. *Field Crops Res.* **27**, 219-240.

Clark, R. B., Zeto, S. K., Baligar, V. C., and Ritchey, K. D. (1997). Growth traits and mineral concentrations of maize hybrids grown on unlimed and limed acid soil. *J. Plant Nutr.* **20**, 1773-1795.

Clarkson, D. T. (1965). The effect of aluminum and some other trivalent metal cations on cell division in the root apices of *Allium cepa*. *Ann. Bot. (London)* **29**, 309-315.

Comin, J. J., Barloy, J., Bourrie, G., and Trolard, F. (1999). Differential effects of monomeric and polymeric aluminium on the root growth and on the biomass production of root and shoot of corn in solution culture. *Eur. J. Agron.* **11**, 115-122.

Cooper, C. S., and MacDonald, P. W. (1970). Energetics of early seedling growth in corn (*Zea mays* L.). *Crop Sci.* **10**, 136-139.

Cosic, T., Poljak, M., Custic, M., and Rengel, Z. (1994). Aluminium tolerance of durum wheat germplasm. *Euphytica* **78**, 239-243.

Crossett, R. N., Campbell, D. J., and Stewart, H. E. (1975). Compensatory growth in cereal root systems. *Plant Soil* **42**, 673-683.

Currier, H. B., and Webster, D. H. (1964). Callose formation and subsequent disappearance: Studies in ultrasound stimulation. *Plant Physiol.* **39**, 843-847.

Datema, R., Schwarz, R. T., Rivas, L. A., and Lezica, R. P. (1983). Inhibition of ß-1,4-glucan biosynthesis by deoxyglucose. *Plant Physiol.* **71**, 76-81.

de Jager, A. (1982). Effects of localized supply of H_2PO_4, NO_3, SO_4, Ca and K on the production and distribution of dry matter in young maize plants. *Neth. J. agric. Sci.* **30**, 193-203.

de Jager, A. (1984). Effects of a localized supply of H_2PO_4, NO_3, Ca and K on the concentration of that nutrient in the plant and the rate of uptake by roots in young maize plants in solution culture. *Neth. J. agric. Sci.* **32**, 43-56.

de la Rosa, L. (1846). “Memoria sobre el cultivo del maiz. Reprint 1989”, Grupo editorial Miguel Ángel Porrúa, Mexico.

de Lima, M. L., and Copeland, L. (1990). The effect of aluminum on the germination of wheat seeds. *J. Plant Nutr.* **13**, 1489-1497.

Degenhardt, J., Larsen, P. B., Howell, S. H., and Kochian, L. V. (1998). Aluminum resistance in the *Arabidopsis* mutant alr-104ls caused by an aluminum-induced increase in rhizosphere pH. *Plant Physiol.* **17**, 19-27.

Delhaize, E., Craig, S., Beaton, C. D., Bennet, R. J., Jagadish, V. C., and Randall, P. J. (1993a). Aluminum tolerance in wheat (*Triticum aestivum* L.) I. Uptake and distribution of aluminum in root apices. *Plant Physiol.* **103**, 685-693.

Delhaize, E., Ryan, P. R., and Randall, P. J. (1993b). Aluminum tolerance in wheat (*Triticum aestivum* L.). II. Aluminum-stimulated excretion of malic acid from root apices. *Plant Physiol.* **103**, 695-702.

Delmer, D. P. (1987). Cellulose biosynthesis. *Annu. Rev. Physiol.* **38**, 259-290.

Dieuaide-Noubhani, M., Canioni, P., and Raymond, P. (1997). Sugar starvation-induced changes of carbon metabolism in excised maize root tips. *Plant Physiol.* **115**, 1505-1513.

Douglas, L. A., and Field, J. F. B. (1975). Determination of phosphate and trace amounts of orthophosphate solution. *Anal. Letters* **8**, 195-203.

Drew, M. C. (1975). Comparison of the effects of a localized supply of phosphate, nitrate, ammonium and potassium on the growth of the seminal root system, and the shoot, in barley. *New Phytol.* **75**, 479-490.

Drew, M. C., and Saker, L. R. (1975). Nutrient supply and the growth of the seminal root system in barley II. Localized, compensatory increases in lateral root growth and rates of nitrate uptake when nitrate supply is restricted to only one part of the root system. *J. Exp. Bot.* **26**, 79-90.

Drew, M. C., and Saker, L. R. (1978). Nutrient supply and the growth of the seminal root system in barley. III Compensatory increases in growth of lateral roots and in rates of phosphate uptake in response to a localized supply of phosphate. *J. Exp. Bot.* **29**, 435-451.

Drew, M. C., Saker, L. R., and Ashley, T. W. (1973). Nutrient supply and the growth of the seminal root system in barley. I. The effect of nitrate concentration on the growth of axes and laterals. *J. Exp. Bot.* **24**, 1189-1202.

Duncan, R. R., Clark, R. B., and Furlani, P. R. (1983). Laboratory and field evaluations of sorghum for response to aluminum and acid soils. *Agron. J.* **75**, 1023-1026.

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.

Edmeades, G. O., Bolaños, J. and Lafitte, H. R. (1992). Progress in selecting for drought tolerance in maize. Proc. Annu. Corn and Sorghum Ind. Res. Conf. 47th , Chicago pp. 93-111. ASTA, Washington DC, USA.

Edmeades, D. C., Blamey, F. P. C., and Farina, M. P. W. (1995). Techniques for assessing plant responses to acid soils. *In* "Plant-soil interactions at low pH: Principles and management" (R. A. Date, N. J. Grundon, G. E. Rayment and M. E. Probert, eds.), pp. 221-233. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Eshel, A., and Waisel, Y. (1996). Multiform and multifunction of various constitutents of one root system. *In* "Plant Roots. The hidden half" (Y. Waisel, A. Eshel and U. Kafkaki, eds.), pp. 175-192. Marcel Dekker, New York.

Eswaran, H., Reich, P., and Beinroth, F. (1997). Global distribution of soils with acidity. *In* "Plant-soil interactions at low pH: Sustainable agriculture and forestry production" (A. C. Moniz, A. M. C. Furlani, R. E. Schaffert, N. K. Fageria, C. A. Rosolem and H. Cantarella, eds.), pp. 159-164. Brazilian Soil Science Society.

Etherton, B., Tabor, C. A., and Herries, E. (1983). Aluminum effects on membrane potentials of plant cells. *Plant Physiol.* **72** (suppl).

Fageria, N. K., and Baligar, V. C. (1993). Screening crop genotypes for mineral stresses. *In* "A workshop on adaptation of plants to soil stresses", pp. 142-159. INTSORMIL Publication No. 94-2, University of Nebraska, Lincoln, Nebraska, USA.

Falconer, D.S. (1984). "Einführung in die quantitative Genetik", Eugen Ulmer GmbH & Co.

Farina, M. P. W. (1997). Management of subsoil acidity in environments outside the humid tropics. *In* "Plant-soil interactions at low pH: Sustainable agriculture and forestry production" (A. C. Moniz, A. M. C. Furlani, R. E. Schaffert, N. K. Fageria, C. A. Rosolem and H. Cantarella, eds.), pp. 176-190. Brazilian Soil Science Society.

Feldman, L. J. (1984). Regulation of root development. *Annu. Rev. Plant Physiol.* **35**, 223-242.

Fincher, G. B., and Stone, B. A. (1981). Metabolism of noncellulosic polysaccharides. *In* "Encyclopedia of Plant Physiology, New Series Vol. 13B" (W. Tanner and F. A. Loewus, eds.), Vol. 133, pp. 68-132. Springer Verlag, Berlin.

Fitter, A. H. (1996). Characteristics and functions of root systems. *In* "Plant Roots. The hidden half" (Y. Waisel, A. Eshel and U. Kafkaki, eds.), pp. 1-20. Marcel Dekker, New York.

Fitter, A. H. (1994). Architecture and biomass allocation as components of the plastic response of root systems to soil heterogeneity. *In* "Exploitation of environmental heterogeneity by plants" (M. M. Caldwell and R. W. Pearcy, eds.), pp. 305-322. Academic Press.

Flores, C. I., Clark, R. B., and Gourley, L. M. (1988). Growth and yield traits of sorghum grown on acid soil at varied aluminum saturations. *Plant Soil* **106**, 49-57.

Fox, R. L., Hue, N. V., Jones, R. C., and Yost, R. S. (1991). Plant -soil interactions associated with acid, weathered soils. *In* "Plant-soil interactions at low pH" (R. J. Wright, V. C. Baligar and R. P. Murrmann, eds.), pp. 197-204. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Fox, R. L., Yost, R. S., Saidy, N. A., and Kang, B. T. (1985). Nutritional complexities with pH variables in humid tropical soils. *Soil Sci. Soc. Am. J.* **49**, 1475-1480.

Foy, C. D. (1974). Effects of aluminum on plant growth. *In* "The plant root and its environment" (E. W. Carson, ed.), pp. 601-642. University Press of Virginia, Charlottsville, USA.

Foy, C. D. (1976). General principles involved in screening plants for aluminum and manganese tolerance. *In* "Plant adaptation to mineral stress in problem soils" (M. J. Wright and A. S. Ferrari, eds.), pp. 256-267. Cornell University Agricultural Experimental Station, National Agricultural Library, Beltsville, Maryland, USA.

Foy, C. D. (1984). Hydrogen, aluminum, and manganese toxicity in acid soil. *In* "Soil acidity and liming" (F. Adams, ed.), Vol. 12, pp. 55-98. ASA-CSSA-SSSA, Madison, WI 53711, USA.

Foy, C. D. (1988). Plant adaptation to acid, aluminum toxic soils. *Commun. Soil Sci. Plant Anal.* **19**, 959-987.

Foy, C. D., Carter Jr., T. E., Duke, J. A., and Devine, T. E. (1993). Correlation of shoot and root growth and its role in selecting for aluminum tolerance in soybean. *J. Plant Nutr.* **16**, 305-325.

Foy, C. D., Chaney, R. L., and White, M. C. (1978). The physiology of metal toxicity in plants. *Annu. Rev. Plant Physiol.* **29**, 511-566.

Foy, C. D., and da Silva, A. R. (1991). Tolerances of wheat germplasm to acid subsoil. *J. Plant Nutr.* **14**, 1277-1295.

Foy, C. D., Dewald, C. L., and Berg, W. A. (1989). Differential tolerances of two old world bluestem genotypes to excess aluminum in acid soil and nutrient solution. *J. Plant Nutr.* **12**, 1231-1254.

Foy, C. D., Duke, J. A., and Devine, T. E. (1992). Tolerance of soybean germplasm to an acid Tatum subsoil. *J. Plant Nutr.* **15**, 527-547.

Foy, C. D., Fleming, A. L., Burns, G. R., and Armiger, W. H. (1967). Characterization of differential aluminum tolerance among varieties of wheat and barley. *Soil Sci. Soc. Am. Proc.* **31**, 513-521.

Fransen, B., Blijjenberg, J., and de Kroon, H. (1999). Root morphological and physiological plasticity of perennial grass species and the exploitation of spatial and temporal heterogeneous nutrient patches. *Plant Soil* **211**, 179-189.

Furlani, P. R., and Clark, R. B. (1981). Screening sorghum for aluminum tolerance in nutrient solution. *Agron. J.* **73**, 587-594.

Furlani, P. R., and Furlani, A. M. C. (1991).Tolerância a aluminio e eficiência a fósforo em milho e arroz: características independentes. Bragantia. Campinas 50, 331-340.

Garland Campbell, K. A., and Carter Jr., T. E. (1990). Aluminum tolerance in soybean: I. Genotypic correlation and repeatability of solution and greenhouse screening methods. *Crop Sci.* **30**, 1049-1054.

Gericke, S., and Kurmies, B. (1952). Die kolorimetrische P_2O_5-Bestimmung mit Ammonium-Vanadat-Molybdat und ihre Anwendung in der Pflanzenanalyse. *Z. Pflanzenernähr. Düng. Bodenkd.* **59**, 235-247.

Gersani, M., and Sachs, T. (1992). Development correlations between roots in heterogeneous environment. *Plant Cell Environ.* **15**, 463-469.

Godbold, D. L., and Kettner, C. (1991). Use of root elongation studies to determine aluminum and lead toxicity in *Picea abies* seedlings. *J. Plant Physiol.* **138**, 95-99.

Goldman, I. L., Carter Jr., T. E., and Patterson, R. P. (1989). Differential genotypic response to drought stress and subsoil aluminum in soybean. *Crop Sci.* **29**, 330-333.

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.

Granato, T. C., and Raper Jr., C. D. (1989). Proliferation of maize (*Zea mays* L.) roots in response to localized supply of nitrate. *J. Exp. Bot.* **40**, 263-275.

Graven, L. M., and Carter, P. R. (1990). Seed size/ shape and tillage system effect on corn growth and grain yield. *J. Prod. Agric.* **3**, 445-452.

Gunsé, B., Poschenrieder, C., and Barceló, J. (2000). The role of ethylene metabolism in the short-term responses to aluminium by roots of two maize cultivars different in Al-resistance. *Environ. Exp. Bot.* **43**, 73-81.

Hairiah, K., Stulen, I., and Kuiper, P. J. C. (1990). Aluminum tolerance of the velvet beans *Mucuna pruriens* var. *utilis* and *Mucuna deeringiana*. I. Effects of aluminium on growth and mineral composition. *In* “Plant nutrition- Pysiology and applications” (M. L. van Beusichem, ed.), pp. 365-374. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Hairiah, K., Stulen, I., van Nordwijk, M., and Kuiper, P. J. C. (1994). Al avoidance and Al tolerance of *Mucuna pruriens* var. *utilis*: Effects of a heterogenous root environment and the nitrogen form in the root environment. *Plant Soil* **167**, 67-72.

Hairiah, K., van Nordwijk, M., and Setijono, S. (1991). Tolerance to acid soil conditions of the velvet beans *Mucuna pruriens* var. *utilis* and *M. deeringia*. I. Root development. *Plant Soil* **134**, 95-105.

Hairiah, K., van Noordwijk, M., Stulen, I., Meijboom, F. W., and Kuiper, P. J. C. (1993). Phosphate nutrition effects on aluminium avoidance of *Mucuna pruriens* var. *utilis*. *Environ. Exp. Bot.* **33**, 75-83.

Hairiah, K., van Nordwijk, M., Stulen, I., and Kuiper, P. J. C. (1992). Aluminium avoidance by *Mucuna pruriens*. *Physiol. Plant.* **86**, 17-24.

Hanson, W. D. (1991). Root characteristics associated with divergent selection for seedling aluminum tolerance in soybean. *Crop Sci.* **31**, 125-129.

Hallauer, A. R. (1972). Third phase in yield evaluation of synthetic varieties of maize. *Crop Sci.* **12**, 16-18.

Hanson, W. D., and Kamprath, E. J. (1979). Selection for aluminium tolerance in soybeans based on seedling-root growth. *Agron. J.* **71**, 581-586.

Haug, A. (1984). Molecular aspects of aluminum toxicity. *CRC Crit. Rev. Plant Sci.* **1**, 345-373.

Hecht-Buchholz, C., Brady, D. J., Asher, C. J., and Edwards, D. G. (1990). Effects of low activities of aluminium on soybean (*Glycine max*) II. Root cell structure and root hair development. *In* “Plant nutrition- Physiology and applications” (M. L. van Beusichem, ed.), pp. 335-343. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Hecht-Buchholz, C., and Foy, C. D. (1981). Effect of aluminium toxicity on root morphology of barley. *Plant Soil* **63**, 93-95.

Henry, R. J., and Stone, B. A. (1982). Factors influencing ß-glucan synthesis by particulate enzymes from suspension-cultured *Lolium multiflorum* endosperm cells. *Plant Physiol.* **69**, 632-636.

Hicks, D. R., Peterson, R. H., Lueschen, W. E., and Ford, J. H. (1976). Seed grade effects on corn performance. *Agron. J.* **68**, 819-820.

Horst, W. J. (1985). Quick screening of cowpea (*Vigna unguiculata*) genotypes for aluminium in an aluminium-treated acid soil. *Z. Pflanzenernähr. Bodenkd.* **148**, 335-348.

Horst, W. J. (1987). Aluminium tolerance and calcium efficiency of cowpea genotypes. *J. Plant Nutr.* **10**, 1121-1129.

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

Horst, W. J., Asher, C. J., Cakmak, I., Szulkiewicz, P., and Wissemeier, A. H. (1992). Short-term responses of soybean roots to aluminium. *J. Plant Physiol.* **140**, 174-178.

Horst, W. J., and Klotz, F. (1990). Screening soybean for aluminium tolerance and adaptation to acid soils. *In* “Genetic aspects of plant mineral nutrition” (N. El Bassam, M. Dambroth and B. C. Loughman, eds.), pp. 355-360. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Horst, W. J., Klotz, F., and Szulkiewicz, P. (1990). Mechanical impedance increases aluminium tolerance of soybean (*Glycine max*) roots. *In* “Plant nutrition - Physiology and applications” (M. L. van Beusichem, ed.), pp. 351-355. Kluwer Academic Publishers, Dordrecht, The Netherlands.

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

Horst, W. J., Wagner, A., and Marschner, H. (1982). Mucilage protects meristems from aluminium injury. *Z. Pflanzenphysiol.* **105**, 435-444.

Horst, W. J., Wagner, A., and Marschner, H. (1983). Effect of aluminium on root growth, cell-division rate and mineral element contents in roots of *Vigna unguiculata* genotypes. *Z. Pflanzenphysiol.* **109**, 95-103.

Howeler, R. H. (1991). Identifying plants adaptable to low pH conditions. *In* “Plant-soil interactions at low pH” (R. J. Wright, V. C. Baligar and R. P. Murrmann, eds.), pp. 885-904. Kluwer Academic Pubishers, Dordrecht, The Netherlands.

Howeler, R. H., and Cadavid, L. F. (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.

Huang, J. W., Grunes, D. L., and Kochian, L. V. (1992). Aluminium effects on the kinetics of calcium uptake into cells of the wheat root apex. Quantification of calcium fluxes using a calcium-selective vibrating microelectrode. *Planta* **188**, 414-421.

Huett, D. O., and Menary, R. C. (1979). Aluminum uptake by excised roots of cabbage, lettuce and kikuyu grass. *Aust. J. Plant Physiol.* **6**, 643-653.

Hush, J. M. and Overall, R. L. (1989). Steady ionic currents around pea (*Pisum sativum* L.) root tips: the effects of tissue wounding. *Biol. Bull.* **176** (S.), 56-64

Jaffe, M. J., Huberman, M., Johnson, J., and Telewski, F. W. (1985). Thigmomorphogenesis: The induction of callose formation and ethylene evolution by mechanical perturbation in bean stems. *Physiol. Plant.* **64**, 271-279.

Jensen, C. O., Sacks, W., and Baldauski, F. A. (1951). The reduction of triphenyltetrazolium chloride by dehydrogenases of corn embryos. *Science* **113**, 65-66.

Jeschke, W. D., Holobradá, M., and Hartung, W. (1997). Growth of *Zea mays* L. plants with their seminal roots only. Effects on plant development, xylem transport, mineral nutrition and the flow and distribution of abscisic acid (ABA) as a possible shoot to root signal. *J. Exp. Bot.* **48**, 1229-1239.

Johnson, J. W., and Wilkinson, R. E. (1992). Wheat growth responses of cultivars to H^+ concentration. *Plant Soil* **146**, 55-59.

Johnson, M. G., and McBride, M. B. (1991). Solubility of aluminium and silicon in acidified spodosols: Evidence for soluble aluminosilicate. *In* “Plant-soil interactions at low pH” (R. J. Wright, V. C. Baligar and R. P. Murrmann, eds.), pp. 15-24. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Jounneau, J. P., Lapous, D., and Guern, J. (1991). In plant protoplasts, the spontaneous expression of defense reaction and the responsiveness to exogenous elicitors are under auxin control. *Plant Physiol.* **96**, 459-466.

Joost, R. E., Hoveland, C. S., Donnelly, E. D., and Fales, S. L. (1986). Screening *Sericea lespedeza* for aluminum tolerance. *Crop Sci.* **26**, 1250-1251.

Jorns, A. C., Hecht-Buchhoz, C., and Wissemeier, A. H. (1991). Aluminum-induced callose formation in root tips of Norway spruce (*Picea abies* (L.) Karst.). *Z. Pflanzenernähr. Bodenkd.* **154**, 349-353.

Jungk, A., Asher, C. J., Edwards, D. G., and Meyer, D. (1990). Influence of phosphate status on phosphate uptake kinetics of maize (*Zea mays*) and soybean (*Glycine max*). *Plant Soil* **124**, 175-182.

Kalvovoulos, J. M., and Misopolinos, N. D. (1983). Aluminum detection on corn roots by the qualizarin method. *Plant Soil* **74**, 131-132.

Kamprath, E. J. (1984). Crop response to lime on soils in the tropics. *In* "Soil acidity and liming" (F. Adams, ed.), Vol. 12, pp. 349-368. ASA-CSSA-SSSA, Madison, WI 53711, USA.

Kasim, F., Haag, W. L., and Wassom, C. E. (1990). Genotypic response of corn to aluminum stress. II. Field performance of corn varieties in acid soils and its relationship with performance at seedling stage. *Ind. J. Crop Sci.* **5**, 53-65.

Kauss, H. (1987a). Callose-Synthese: Regulation durch induzierten Ca^{2+}-Einstrom in Pflanzenzellen. *Naturwissenschaften* **74**, 275-281.

Kauss, H. (1987b). Some aspects of calcium-dependent regulation in plant metabolism. *Annu. Rev. Plant Physiol.* **38**, 47-72.

Kauss, H. (1989). Fluorometric measurement of callose and other 1,3-ß-glucans. *In* "Modern methods of plant analysis. Plant Fibers. New series" (H. F. Linskens and J. F. Jackson, eds.), Vol. 10, pp. 127-137. Springer.

Kauss, H. (1996). Callose synthesis. *In* "Membranes: Specialized functions in plants" (M. Smallwood, J. P. Knox and D. J. Bowles, eds.), pp. 77-92. BIOS Scientific Publishers Ltd., Oxford, UK.

Kauss, H., and Jeblick, W. (1991). Induced Ca^{2+} uptake and callose synthesis in suspension-cultured cells of *Catharanthus roseus* are decreased by the protein phosphatase inhibitor orkadaic acid. *Physiol. Plant.* **81**, 309-312.

Kauss, H., Waldmann, T., Jeblick, W., and Takemoto, J. Y. (1991). The phytotoxin syringomycin elicits Ca^{2+}- dependent callose synthesis in suspension-cultured cells of *Catharanthus roseus*. *Physiol. Plant.* **81**, 134-138.

Keltjens, W. G. (1997). Plant adaptation and tolerance to acid soils; its possible Al avoidance- A review. *In* "Plant -soil interactions at low pH: Sustainable agriculture and forestry production" (A. C. Moniz, A. M. C. Furlani, R. E. Schaffert, N. K. Fageria, C. A. Rosolem and H. Cantarella, eds.), pp. 109-117. Brazilian Soil Science Society.

Kerley, S. J., Leach, J. E., Swain, J. L., and Huyghe, C. (2000). Investigations into the exploitation of heterogeneous soils by *Lupinus albus* L. and *L. pilosus* Murr. and the effects upon plant growth. *Plant Soil* **222**, 241-253.

Kerven, G. L., Edwards, D. G., Asher, C. J., Hallman, P. S., 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.

Kinraide, B. K., Arnold, R. C., and Baligar, V. C. (1985). A rapid assay for aluminum phytotoxicity at submicromolar concentrations. *Physiol. Plant.* **65**, 245-250.

Kinraide, T. B. (1991). Identity of the rhizotoxic aluminium species. *Plant Soil* **134**, 167-178.

Kinraide, T. B., and Ryan, P. (1991). Cell surface charge may obscure the identity of the rhizotoxic aluminum species. *Curr. Topics Plant Biochem. Physiol.* **10**, 94-106.

Kochian, L. V. (1995). Cellular mechanisms of aluminum toxicity and resistance in plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **46**, 237-260.

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.

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

Konzak, C. F., Polle, E., and Kittrick, J. A. (1976). Screening several crops for aluminum tolerance. *In* “Plant adaptation to mineral stress in problem soils” (M. J. Wright, ed.), pp. 311-327. Cornell University Agricultural Experimental Stationity, National Agricultural Library, Beltsville, Maryland.

Lainé, P., Ourry, A., and Boucaud, J. (1995). Shoot control of nitrate uptake rates by roots of *Brassica napus* L.: Effects of localized nitrate supply. *Planta* **196**, 77-83.

Larcher, W. (1969). Anwendung und Zuverlässigkeit der Tetrazoliummethode zur Feststellung von Schäden in pflanzlichen Geweben. *Mikroskopie* **25**, 207-218.

Larsen, P. B., Kochian, L. V., and Howell, S. H. (1997). Al inhibits both shoot development and root growth in als3, an Al-sensitive *Arabidopsis* mutant. *Plant Physiol.* **114**, 1207-1214.

Larsen, P. B., Tai, C.-Y., Kochian, L. V., and Howell, S. H. (1996). Arabidopsis mutants with increased sensitivity to aluminum. *Plant Physiol.* **110**, 743-751.

Lazof, D. B., Rincon, T. W., Ruffy, T. W., Mackown, C. T., and Carter, T. E. (1994). Aluminum accumulation and associated effects on $^{15}NO_3^-$ influx in roots of two soybean genotypes differing in Al tolerance. *Plant Soil* **164**, 291-297.

Lima, M., Furlani, P. R., and Miranda Filho, J. B. (1992). Divergent selection for aluminum tolerance in a maize (*Zea mays* L.) population. *Maydica* **37**, 123-132.

Lima, M., and Miranda Filho, J. B. (1984). Inbreeding depression in Brazilian populations of maize (*Zea mays* L.). *Maydica* **29**, 213-215.

Lindberg, S., Szynkier, K., and Greger, M. (1991). Aluminium effects on transmembrane potential in cells of fibrous roots of sugar beet. *Physiol. Plant.* **83**, 54-62.

Llugany, M., Massot, N., Wissemeier, A., Poschenrieder, C., Horst, W. J., and Barceló, J. (1994). Aluminium tolerance of maize cultivars as assessed by callose production and root elongation. *Z. Pflanzenernähr. Bodenkd.* **157**, 447-451.

Llugany, M., Poschenrieder, C., and Barceló, J. (1995). Monitoring of aluminium-induced inhibition of root elongation in four maize cultivars differing in tolerance to aluminium and proton toxicity. *Physiol. Plant.* **93**, 265-271.

Ma, J. F., Zheng, S. J., Li, X. F., Takeda, K., and Matsumoto, H. (1997). A rapid hydroponic screening for aluminium tolerance in barley. *Plant Soil* **191**, 133-137.

Ma, J. F. (2000). Role of organic acids in detoxification of aluminum in higher plants. *Plant Cell Physiol.* **41**, 383-390.

Macklon, A. E. S., and Sim, A. (1981). Cortical cell fluxes and transport to the stele in excised root segments of *Allium cepa* L. *Planta* **152**, 381-387.

Magnavaca, R., Gardner, C. O., and Clark, R. B. (1987). Inheritance of aluminum tolerance in maize. *In* "Genetic aspects of plant mineral nutrition" (H. W. Gabelman and B. C. Loughman eds.) Martinus Nijhoff Publishers, Dordrecht/ Boston/Lancaster.

Magnavaca, R., and Bahia Filho, A. F. C. (1993). Success in maize acid soil tolerance. *In* “A workshop on adaptation of plants to soil stresses”, pp. 209-220. Intsormil Publ. No. 94-2, University of Nebraska, Lincoln, Nebraska, USA.

Magnavaca, R., Bahia Filho, A. F. C., and Schaffert, R. E. (1996). Maize adaptation to acid soils. *In* “Fourth International Symposium on plant-soil interactions at low pH”, Belo Horizonte, Brazil.

Malavolta, E., Nogueira, F. D., Oliveira, I. P., Nakayama, L., and Eimori, I. (1981). Aluminum tolerance in sorghum and bean - Methods and results. *J. Plant Nutr.* **3,** 687-694.

Marienfeld, S., Lehmann, H., and Stelzer, R. (1995). Ultrastructural investigations and EDX-analyses of Al-treated oat (*Avena sativa*) roots. *Plant Soil* **171**, 167-173.

Marienfeld, S., Schmohl, N., Klein, M., Schröder, W. H., Kuhn, A. J., and Horst, W. J. (2000). Localisation of aluminium in root tips of *Zea mays* and *Vicia faba*. *J. Plant Physiol.* **156**, 666-671.

Marschner, H. (1991). Mechanisms of adaption of plants to acid soils. *Plant Soil* **134**, 1-20.

Marschner, H. (1996). “Mineral nutrition of higher plants”, 2nd Ed., Academic Press, London.

Marschner, H., Kirkby, E. A., and Cakmak, I. (1996). Effect of nutritional status on shoot-root partitioning of photoassimilates and cycling of mineral nutrients. *J. Exp. Bot.* **47**, 1255-1263.

Massot, N., Llugany, M., Poschenrieder, C., and Barceló, J. (1999). Callose production as indicator of aluminum toxicity in bean cultivars. *J. Plant Nutr.* **22**, 1-10.

McClure, G. W. (1972). Nutrient distribution in root zones. III. Further studies of the effects of phosphorus distribution on corn and wheat. *Can. J. Bot.* **50**, 2275-2282.

McLay, C. D. A., and Ritchie, G. S. P. (1993). Effect of gypsum and lime on wheat growth in pots containing an acidic subsoil. *In* "Plant nutrition- from genetic engineering to field practice" (N. J. Barrow, ed.), pp. 747-750. Kluwer Academic Publishers, Dordrecht, The Netherlands.

McNairn, R. B., and Currier, H. B. (1965). The influence of boron on callose formation in primary leaves of *Phaseolus vulgaris* L. *Phyton* **22**, 153-158.

Menzies, N. W., Kerven, G. L., and Edwards, D. G. (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.

Mertz Jr., S. M., and Higinbotham, N. (1976). Transmembrane electropotential in barley roots as related to cell type, cell location, cutting and aging effects. *Plant Physiol.* **57**, 123-128.

Messiaen, J. M., Nérinckx, F., and van Cutsem, P. V. (1995). Callose synthesis in spirostanol treated carrot cells is not triggered by cytosolic calcium, cytosolic pH or membrane potential charges. *Plant Cell Physiol.* **36**, 1213-1220.

Meyer, A. J., and Weisenseel, M. H. (1997). Wound-induced changes of membrane voltage, endogenous currents, and ion fluxes in primary roots of maize. *Plant Physiol.* **114**, 989-998.

Miranda, L. T., Furlani, R. R., Miranda, L. E. C., and Sawazki, E. (1984). Genetics of environmental resistance and super genes: Latent aluminum tolerance. *Maize Genet. Coop. Newsl.* **58**, 46-48.

Miyasaka, S. C., Kochian, L. V., Shaff, J. E., and Foy, C. D. (1989). Mechanisms for aluminum tolerance in wheat. An investigation of genotypic differences in rhizosphere pH, K^+ and H^+ transport, and root-cell membrane potentials. *Plant Physiol.* **91**, 1188-1196.

Moore, A. E., and Stone, B. A. (1972). Effect of senescence and hormone treatment on the activity of a ß-1,3-glucan hydrolase in *Nicotiana glutinosa* leaves. *Planta* **104**, 93-109.

Moore, D. P., Kronstad, W. E., and Metzger, R. J. (1976). Screening wheat for aluminum tolerance. *In* "Plant adaptation to mineral stress in problem soils", pp. 287-295. Cornell University Agricultural Experimental Station, National Agricultural Library, Beltsville, Maryland.

Morgan, D. R., and Street, H. E. (1959). The carbohydrate nutrition of tomato roots. *Ann. Bot.* **23**, 89-105.

Morimura, S., Takahashi, E., and Marsumoto, H. (1978). Association of aluminium with nuclei and inhibition of cell division in onion (*Allium cepa*) roots. *Z. Pflanzenphysiol.* **88**, 395-401.

Moustakas, M., and Ouzounidou, G. (1995). Aluminum effects on photosynthesis and elemental uptake in an aluminum-tolerant and non-tolerant wheat cultivar. *J. Plant Nutr.* **18**, 669-683.

Mugwira, L. M. V., Sapra, V. T., Patel, S. U., and Chandry, M. A. (1981). Aluminum tolerance of triticale and wheat cultivars developed in different regions. *Agron. J.* **73**, 470-475.

Mugwira, L. M., and Haque, I. (1993). Screening forage and browse legumes germplasm to nutrient stresses: IV. Growth rates of sesbania as affected by aluminum and low phosphorus in soils and nutrient solutions. *J. Plant Nutr.* **16**, 67-83

Murphy, J., and Riley, J. P. (1962). A modified single solution method for the determination of phosphate in natural waters. *Anal. Chim. Acta* **27**, 31-36.

Nable, R. O., and Loneragan, J. F. (1984). Translocation of manganese in subterranean clover (*Trifolium subterraneum* L. cv. Seaton Park). II. Effects of leaf senescence and of restricting supply of manganese to part of a split root system. *Aust. J. Plant Physiol.* **11**, 113-118.

Nafziger, E. D. (1992). Seed size effects on yield of two corn hybrids. *J. Prod. Agric.* **5**, 538-540.

Naidu, R., Haynes, R. J., Gawandar, J. S., Morrsion, R. J., and Fitzpatrick, R. W. (1991). Chemical and mineralogical properties and soil solution composition of acid soils from the South Pacific Islands. *In* “Plant-soil interactions at low pH” (R. J. Wright, V. C. Baligar and R. P. Murrmann, eds.), pp. 43-53. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Nair, V. D., and Prenzel, J. (1978). Calculations of equilibrium concentration of mono- and polynuclear hydroxyaluminium species at different pH and total aluminium concentrations. *Z. Pflanzenernähr. Bodenkd.* **141**, 741-751.

Nass, H. G., and Crane, P. L. (1970). Effect of endosperm mutants on germination and early seedling growth rate in maize (*Zea mays* L.). *Crop Sci.* **10**, 139-140.

Oettler, G., Geiger, R., Wiethölter, S., Gaus, C. S., and Hesemann, C.-U. (1997). Methodische und genetische Untersuchungen zur Aluminiumtoleranz von Triticale. *Vortr. Pflanzenzücht.* **38**, 41-56.

Ownby, J. D., and Popham, H. R. (1989). Citrate reverses the inhibition of wheat root growth caused by aluminum. *J. Plant Physiol.* **135**, 588-591.

Pandey, S., Ceballos, H., and Granados, G. (1995). Registration of four tropical maize populations with acid soil tolerance: SA-4, SA-5, SA-6 and SA-7. *Crop Sci.* **35**, 1230-1231.

Pandey, S., Ceballos, H., Magnavaca, R., Bahía Filho, A. F. C., Duque-Vargas, J., and Vinasco, L. E. (1994). Genetics of tolerance to soil acidity in tropical maize. *Crop Sci.* **34**, 1511-1514.

Pandey, S., and Gardner, C. O. (1992). Recurrent selection for population, variety, and hybrid improvement in tropical maize. *Adv. Agron.* **48**, 1-87.

Papernik, L. A., and Kochian, L. V. (1997). Possible involvement of Al-induced electrical signals in Al tolerance in wheat. *Plant Physiol.* **115**, 657-667.

Parker, D. R., Kinraide, T. B., and Zelazny, L. W. (1988). Aluminum speciation and phytotoxicity in dilute hydroxy-aluminum solutions. *Soil Sci. Soc. Am. J.* **52**, 438-444.

Parker, D. R., Kinraide, T. B., and Zelazny, L. W. (1989). On the phytotoxicity of polynuclear hydroxy-aluminum complexes. *Soil Sci. Soc. of Am. J.* **53**, 789-796.

Pavan, M. A., and Bingham, F. T. (1982). Toxicity of aluminum to coffee seedlings grown in nutrient solution. *Soil Sci. Soc. Am. J.* **46**, 993-997.

Pellet, D. M., Grunes, D. L., and Kochian, L. V. (1995). Organic acid exudation as an aluminum-tolerance mechanism in maize (*Zea mays* L.). *Planta* **196**, 788-795.

Peterson, C. A., and Rauser, W. E. (1979). Callose deposition and photoassimilate export in *Phaseolus vulgaris* to excess cobalt, nickel, zinc. *Plant Physiol.* **63**, 1170-1174.

Pevilla, P., Butrón, A., Malvar, R. A., and Ordás, A. (1999). Relationships among kernel weight, early vigor, and growth of maize. *Crop Sci.* **39**, 654-658.

Polle, E., Konzak, C. F., and Kittrick, J. A. (1978). Visual detection of aluminium tolerance levels in wheat by hematoxylin staining of seedling roots. *Crop Sci.* **18**, 823-827.

Pommel, B. (1990). Influence du poids de las semence et de la profondeur de semis sur la croissance et le développement de la plantule de maïs. *Agronomie* **10**, 699-708.

Poschenrieder, C., Llugany, M., and Barceló, J. (1995). Short-term effects of pH and aluminium on mineral nutrition in maize varieties differing in proton and aluminium tolerance. *J. Plant Nutr.* **18**, 1495-1507.

Quaggio, J. A., Ramos, V. J., Furlani, P. R., and Carelli, M. L. C. (1991). Liming and molybdenum effects on nitrogen uptake and grain yield of corn. *In* “Plant-soil interactions at low pH” (R. J. Wright, V. C. Baligar and R. P. Murrmann, eds.), pp. 327-332. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Raghothama, K. G. (1999). Phosphate acquisition. *Plant. Mol. Biol.* **50**, 665-693.

Rajaram, S., Kohli, M. M., and Lopez-Cesati, J. (1991). Breeding for tolerance to aluminium toxicity in wheat. *In* “Plant-soil interactions at low pH” (R. J. Wright, V. C. Baligar and R. P. Murrmann, eds.), pp. 1019-1208. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Rengel, Z. (1996). Uptake of aluminium by plant cells. *New Phytol.* **134**, 389-406.

Rengel, Z., and Elliott, D. C. (1992). Aluminium inhibits net $^{45}Ca^{2+}$ uptake by *Amaranthus* protoplasts. *Biochem. Physiol. Pflanzen* **188**, 177-186.

Rengel, Z., Piñeros, M., and Tester, M. (1995). Transmembrane calcium fluxes during Al stress. *Plant Soil* **171**, 125-130.

Reuter, C. D., and Robinson, J. B. (1986). “Plant analysis. An interpretation manual”, Inkata Press.

Rhue, R.D., Grogan, C. O., Stockmeyer, E. W., and Everett, H. L. (1978). Genetic control of aluminum tolerance in corn. *Crop Sci.* **18**, 1063-1067.

Richter, G. (1988). “Stoffwechselphysiologie der Pflanzen”, Thieme Verlag.

Rincón, M., and Gonzales, R. A. (1992). Aluminum partitioning in intact roots of aluminum-tolerant and aluminum-sensitive wheat (*Triticum aestivum* L.) cultivars. *Plant Physiol.* **99**, 1021-1028.

Robinson, D. (1994). Transley Review No. 73. The response of plants to non-uniform supplies of nutrients. *New Phytol.* **127**, 635-674.

Robinson, D. (1996). Variation, co-ordination and compensation in root systems in relation to soil variability. *Plant Soil* **187**, 57-66.

Rufty Jr., T. W., MacKown, C. T., Lazof, D. B., and Carter, T. E. (1995). Effects of aluminium on nitrate uptake and assimilation. *Plant Cell Environ.* **18**, 1325-1331.

Ruiz-Torres, N. A., Carver, B. F., and Westerman, R. L. (1992). Agronomic performance in acid soils of wheat lines selected for hematoxylin staining patterns. *Crop Sci.* **32**, 104-107.

Russell, E. W. (1973). “Soil conditions and plant growth”, 10 Ed. Longman.

Ryan, P. R., Delhaize, E., and Randall, P. J. (1995a). Characterisation of Al-stimulated efflux of malate from the apices of Al-tolerant wheat roots. *Planta* **196**, 103-110.

Ryan, P. R., Delhaize, E., and Randall, P. J. (1995b). Malate efflux from root apices and tolerance to aluminium are highly correlated in wheat. *Austral. J. Plant Physiol.* **22**, 531-536.

Ryan, P. R., di Tomaso, J. M., and Kochian, L. V. (1993). Aluminium toxicity in roots: An investigation of spatial sensitivity and the role of the root cap. *J. Exp. Bot.* **44**, 437-446.

Saglio, P. H., and Pradet, A. (1980). Soluble sugars, respiration, and energy charge during aging of excised maize root tips. *Plant Physiol.* **66**, 516-519.

Saglio, P. H., Rayond, P., and Pradet, A. (1980). Metabolic activity and energy charge of excised maize root tips under anoxia. *Plant Physiol.* **66**, 1053-1057.

Salazar, F. S., Pandey, S., Narro, L., Perez, J. C., Ceballos, H., Parentoni, S. N., and Bahia Filho, A. F. C. (1997). Diallel analysis of acid tolerant and intolerant tropical maize populations. *Crop Sci.* **37**, 157-1462.

Samuels, T. D., Küçükakyüz, K., and Rincón-Zachary (1997). Al partitioning and root growth as related to Al sensitivity and Al tolerance in wheat. *Plant Physiol.* **113**, 527-534.

Sanchez, P. A., and Salinas, J. G. (1981). Low input technology for managing Oxisols and Ultisols in tropical America. *Adv. Agron.* **34**, 280-406.

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

Sartain, M., and Kamprath, E. J. (1978). Aluminum tolerance of soybean cultivars based on root elongation in solution culture compared with growth in acid soil. *Agron. J.* **70**, 17-20.

Sasaki, M., Kasai, M., Yamamoto, Y., and Matsumoto, H. (1995). Involvement of plasma membrane potential in the tolerance mechanism of plant roots to aluminium toxicity. *Plant Soil* **171**, 119-124.

Sasaki, M., Kasai, M., Yamamoto, Y., and Matsumoto, H. (1994). Comparison of the early response to aluminum stress between tolerant and sensitive wheat cultivars: Root growth, aluminum content and efflux of K^+. *J. Plant Nutr.* **17**, 1275-1288.

Sattelmacher, B., and Thoms, K. (1995). Morphology and physiology of the seminal root system of young maize (*Zea mays* L.) plants as influenced by a locally restricted nitrate suppy. *Z. Pflanzenernähr. Bodenkd.* **158**, 493-497.

Schaeffer, H. J., and Walton, J. D. (1990). Aluminum ions induce oat protoplasts to produce an extracellular (1,3)-ß-D-glucan. *Plant Physiol.* **94**, 13-19.

Schinkel, H. (1991). Die Verwendung spektrochemischer Puffer in der Flammen -AAS. *GIT Fachz. Lab.* **35**, 25-30.

Schmohl, N., and Horst, W. J. (2000). Cell wall pectin content modulates aluminium sensitivity of *Zea mays* (L.) cells grown in suspension culture. *Plant Cell Environ.* **23**, 735-742.

Schmohl, N., Pilling, J., Fisahn, J., and Horst, W. J. (2000). Pectin methylesterase modulates aluminium sensitivity in *Zea mays* and *Solanum tuberosum*. *Physiol. Plant.* **109**, 419-427.

Schortemeyer, M., and Feil, B. (1996). Root morphology of maize under homogenous or spatially separated supply of ammonium and nitrate at three concentrations ratios. *J. Plant Nutr.* **19**, 1089-1097.

Schreiner, K. A., Hoddinott, J., and Taylor, G. J. (1994). Aluminium induced deposition of (1,3)-ß-glucans (callose) in *Triticum aestivum* L. *Plant Soil* **162**, 273-280.

Schumacher, T. E., Smucker, A. J. M., Eshel, A., and Curry, R. B. (1983). Measurement of short-term root growth by prestaining with neutral red. *Crop Sci.* **23**, 1212-1214.

Scott, B. J., and Fisher, J. A. (1993). Comparing selection strategies for tolerance of acid soils. *In* “A workshop on adaptation of plants to soil stresses”, pp. 103-116. Intsormil Publ. No. 94-2, University of Nebraska, Lincoln, Nebraska, USA.

Scott, B. J., and Robson, A. D. (1991). The distribution of Mg, P and K in the split roots of subterranean clover. *Ann. Bot.* **67**, 251-256.

Scott Russell, R. (1977). “Plant root systems: Their function and interaction with the soil”, McGraw-Hill.

Sivaguru, M., Baluska, F., Volkmann, D., Felle, H. H., and Horst, W. J. (1999). Impacts of aluminum on the cytoskeleton of the maize root apex. Short-term effects on the distal part of the transition zone. *Plant Physiol.* **119**, 1073-1082.

Sivaguru, M., and Horst, W. J. (1998). The distal part of the transition zone is the most aluminum-sensitive apical root zone of *Zea mays* L. *Plant Physiol.* **116**, 155-163.

Spehar, C. R. (1994). Field screening of soya bean (*Glycine max* (L.) Merrill) germplasm for aluminium tolerance by the use of augmented design. *Euphytica* **76**, 203-213.

Stahlberg, R., and Cosgrove, D. J. (1994). Comparison of electric and growth responses to excision in cucumber and pea seedlings. I. Short-distance effects are a result of wounding. *Plant Cell Environ.* **17**, 1143-1151.

Staß, A., and Horst, W. J. (1995). Effect of aluminium on membrane properties of soybean (*Glycine max*) cells in suspension culture. *Plant Soil* **171**, 111-118.

Tabatake, R., and Shimmen, T. (1997). Inhibition of electrogenesis by aluminum in characean cells. *Plant Cell Physiol.* **38**, 1264-1271.

Taylor, G. J. (1991). Current views of the aluminum stress response; the physiological basis of tolerance. *Curr. Topics Plant Biochem. Physiol.* **10**, 57-93.

Taylor, G. J. (1997). Mechanisms of plant adaptation and tolerance to acid soils. *In* "Plant-soil interactions at low pH: Sustainable agriculture and forestry production" (A. C. Moniz, A. M. C. Furlani, R. E. Schaffert, N. K. Fageria, C. A. Rosolem and H. Cantarella, eds.), pp. 107-108. Brazilian Soil Science Society.

Thoms, K., and Sattelmacher, B. (1990). Influence of nitrate placement on morphology of maize (*Zea mays*) root systems. *In* "Plant nutrition - physiology and applications" (M. L. van Beusichem, ed.), pp. 29-32. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Tice, K. R., Parker, D. R., and DeMason, D. A. (1992). Operationally defined apoplastic and symplastic aluminum fractions in root tips of aluminum-intoxicated wheat. *Plant Physiol.* **100**, 309-318.

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

van Raij, B. (1991). Fertility of acid soils. *In* "Plant-soil interactions at low pH" (R. J. Wright, V. C. Baligar and R. P. Murrmann, eds.), pp. 159-167. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Vucinic, Z., and Vuletic, M. (1995). The effect of addition of sucrose on the energy status and the trans-root electrical potential difference of excised maize roots. *Plant Cell Physiol.* **36**, 45-52.

Wagatsuma, T., Ishikawa, S., Obata, H., Tawaraya, K., and Katohda, S. (1995). Plasma membrane of younger and outer cell is the primary site for aluminium toxicity in roots. *Plant Soil* **171**, 105-112.

Wagatsuma, T., Kaneko, M., and Hayasaka, Y. (1987). Destruction process of plant root cells by aluminium. *Soil Sci. Plant Nutr.* **33,** 57-67.

Waldmann, T., Jeblick, W., and Kauss, H. (1988). Induced net Ca^{2+} uptake and callose biosynthesis in suspension-cultured plant cells. *Planta* **173**, 88-95.

Webb, M. J., and Loneragan, J. F. (1990). Zinc translocation to wheat roots and its implications for a phosphorus/ zinc interaction in wheat plants. *J. Plant Nutr.* **13**, 1499-1512.

Wheeler, D. M., Edmeades, D. C., Christie, R. A., Putterill, J., and Gardner, R. (1992a). Effect of aluminum and pH on relative yield and plant chemical concentrations of 7 dicotyledonous species grown in solution at low ionic strength. *J. Plant Nutr.* **15**, 419-433.

Wheeler, D. M., Edmeades, D. C., Smith, D. R., and Wedderburn, M. E. (1992b). Screening perennial rye-grass from New Zealand for aluminium tolerance. *Plant Soil* **146**, 9-19.

Wheeler, D. M., Edmeades, D. C., Christie, R. A., and Gardner, R. (1992c). Comparison of techniques for determining the effect of aluminium on the growth of, and the inheritance of aluminium tolerance in wheat. *Plant Soil* **146**, 1-8.

Wijesinghe, D. K., and Hutchings, M. J. (1997). The effetcs of spatial scale of environmental heterogeneity on the growth of a clonal plant: an experimental study with *Glechhoma hederacea*. *J. Ecol.* **85**, 17-28.

Wissemeier, A. H., Diening, A., Hergenröder, A., Horst, W. J., and 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., Hahn, G., and Marschner, H. (1998). Callose in roots of Norway spruce (*Picea abies* (L.) Karst.) is a sensitive parameter for aluminium supply at a forest site (Höglwald). *Plant Soil* **199**, 53-57.

Wissemeier, A. H., and 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.

Wissemeier, A. H., Klotz, F., and Horst, W. J. (1987). Aluminium induced callose synthesis in roots of soybean (*Glycine max.* L.). *J. Plant Physiol.* **129**, 487-492.

Wissemeier, A. H., Hergenröder, A., Mix-Wagner, G., and Horst, W. J. (1993). Induction of callose by manganese in cell suspension cutlure and leaves of soybean (*Glycine max* L.). *J. Plant Physiol.* **142**, 67-73.

Wissemeier, A. H., and Horst, W. J. (1992). Effect of light intensity on manganese toxicity symptoms and callose formation in cowpea (*Vigna unguiculata* (L.) Walp.). *Plant Soil* **143**.

Wright, R. J., Baligar, V. C., Ritchey, K. D., and Wright, S. F. (1989). Influence of soil solution aluminum on root elongation of wheat seedlings. *Plant Soil* **113**, 294-298.

Xia, J.-H., and Saglio, P. H. (1988). Characterization of the hexose transport system in maize root tips. *Plant Physiol.* **88**, 1015-1020.

Yan, F., Schubert, S., and 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.

Zeigler, R. S., Pandey, S., Miles, J., Gourley, L. M., and Sarkarung, S. (1995). Advances in the selection and breeding of acid-tolerant plants: Rice, maize, sorghum and tropical forages. *In* “Plant-soil interactions at low pH: Principles and management” (R. A. Date, N. J. Grundon, G. E. Rayment and M. E. Probert, eds.), pp. 391-406. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Zekri, M., and Parsons, L. R. (1990). Response of split-root sour orange seedlings to NaCl and polyethylene glycol stresses. *J. Exp. Bot.* **41**, 35-40.

Zhang, G., Archambault, D. J., Slaski, J. J., and Taylor, G. J. (1995). Effects of protein synthesis inhibitors on uptake of aluminium in aluminium-resistant and aluminium-sensitive cultivars of wheat. *J. Plant Physiol.* **147**, 457-462.

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

Zhang, G., and Jessop, R. S. (1998). Analysis of genetic variability of aluminium tolerance response in triticale. *Euphytica* **102**, 177-182.

Zhang, G., and Taylor, G. J. (1989). Kinetics of aluminum uptake by excised roots of aluminum-tolerant and aluminum-sensitive cultivars of *Triticum aestivum* L. *Plant Physiol.* **91**, 1094-1099.

Zhang, G., and Taylor, G. J. (1991). Effects of biological inhibitors on kinetics of aluminum uptake by excised roots and purified cell wall material of aluminum-tolerant and aluminum-sensitive cultivars of *Triticum aestivum* L. *J. Plant Physiol.* **138**, 533-539.

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