



RESEARCH PAPER

Root cell patterning: a primary target for aluminium toxicity in maize

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Received 7 October 2004; Accepted 23 December 2004

Abstract

The short-term influence (5–180 min) of 50 μ M Al on cell division was investigated in root tips of two *Zea mays* L. varieties differing in Al-resistance. The incorporation of bromodeoxyuridine into S-phase nuclei was visualized by immunofluorescence staining using confocal laser fluorescence microscopy. In Al-sensitive plants 5 min Al exposure was enough to inhibit cell division in the proximal meristem (250–800 μ m from the tip). After 10 or 30 min with Al only, a few S-phase nuclei were found in the cortical initials. By contrast, cell division was stimulated in the distal elongation zone (2.5–3.1 mm). After 180 min the protrusion of an incipient lateral root was observed in this zone. These observations suggest a fast change in cell patterning rather than a general cariotoxic effect after exposure to Al for a short time. No such changes were found in Al-resistant maize. This is the first report showing such fast Al-induced alterations in the number and the position of dividing cells in root tips. The observation that similar changes were induced by a local supply of naphthylphthalamic acid to the distal transition zone suggests that inhibition of auxin transport plays a role in the Al-induced alteration of root cell patterning.

Key words: Aluminium toxicity, auxin, cell division, naphthylphthalamic acid, root development, *Zea mays* L.

Introduction

Aluminium toxicity is among the most important abiotic stress factors limiting crop productivity on acid tropical soils. It is well known that roots are the primary target of phytotoxic

Al. Ryan *et al.* (1993) found the root apex to be the most Al-sensitive zone and Sivaguru and Horst (1998) identified the distal transition zone (DTZ) as the specific site of Al sensitivity. The mechanisms of Al-induced inhibition of root growth, however, are still not clearly established (Kochian, 1995; Barceló *et al.*, 1996; Matsumoto, 2000; Yamamoto *et al.*, 2001; Barceló and Poschenrieder, 2002; Kochian *et al.*, 2004). Pioneer work by Clarkson (1965) revealed Al-induced alterations of root development and he pointed to cell division as a primary site for Al-induced root growth inhibition. Further studies reporting Al-induced inhibition of cell division in root tips and the observation that Al binds to nucleic acids supported the view of Al-induced inhibition of root cell proliferation as a primary target for Al-toxicity (Matsumoto *et al.*, 1976; Morimura *et al.*, 1978).

A major criticism to this view arose in the mid-1990s when short-term investigations pointed to the inhibition of root cell elongation by apoplastic Al as a primary mechanism of Al-induced inhibition of root growth (Horst, 1995). The main arguments favouring this view were based on the observation that Al can induce callose formation and the inhibition of root elongation within 30–60 min (Llugany *et al.*, 1995; Wiese-meier and Horst, 1995), while Al-induced inhibition of root cell division had been observed after hours or even days of exposure. Moreover, Al entrance into the symplast was thought to be a slow process, while the fast inhibition of root elongation observed in several investigations was reversible by washing roots with citrate, probably because citrate binds apoplastic phytotoxic Al in a non-toxic form (Ownby and Popham, 1989). The more recent finding that Al causes inhibition of basipetal auxin transport in maize roots was also interpreted as a mechanism of Al-induced inhibition of root cell elongation (Kollmeier *et al.*, 2000).

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Abbreviations: BrdU, bromodeoxyuridine; DEZ, distal elongation zone; DTZ, distal transition zone; NPA, naphthylphthalamic acid.

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There is now considerable evidence showing that Al may enter the symplasm quite rapidly (Lazof *et al.*, 1996; Vázquez *et al.*, 1999; Silva *et al.*, 2000). Anyway, neither a fast toxic action of Al in the apoplast nor the quickness of the Al-induced inhibition of root growth precludes the possibility of a fast Al-induced inhibition of root cell division. Moreover, Al not only causes a reduction in the length of the main root, but also changes the entire root architecture. To what extent Al-induced alterations in the developmental features of roots are related to primary Al-toxicity effects is not established.

Taking advantage of the improved techniques for visualizing alterations in cell proliferation by immunodetection of S-phase (the DNA-synthesis phase) nuclei labelled with bromodeoxyuridine (BrdU) using laser confocal microscopy (Dolbeare, 1996; Hepler and Gunning, 1998; de Castro *et al.*, 2000), this study investigates the short-term influence of Al on cell division in root tips of maize plants differing in Al resistance. The aim was to re-evaluate the possible implication of Al-induced inhibition of cell division in the early stages of the Al-toxicity syndrome.

Materials and methods

Plant material and growth conditions

Seeds of the simple-hybrid maize (*Zea mays* L.) varieties HS 16×36 (Al-sensitive) and CAT-AL 237/67×SLP 181/71 (Al-resistant; called Cateto for abbreviation), kindly provided by EMBRAPA (Sete Lagoas, Brazil) were germinated on moist filter paper (0.5 mM CaSO₄) for 4 d at 27 °C. Uniform seedlings were individually transferred to plastic culture flasks filled with 0.7 l continuously aerated, low-ionic-strength nutrient solution of the following composition in µM: 200 CaSO₄·2H₂O, 100 MgSO₄·7H₂O, 400 KNO₃, 300 NH₄NO₃, 10 Fe-EDTA, 8 H₃BO₃, 5 NaH₂PO₄·H₂O, 5 MnSO₄·H₂O, 0.38 ZnSO₄·7H₂O, 0.16 CuSO₄·5H₂O, 0.06 (NH₄)₆Mo₇O₂₄·H₂O (pH 4.3). Control plants received this solution throughout the experiment. For Al treatments, the nutrient solution was supplemented with 50 µM Al in the form of AlCl₃·6H₂O. According to GEOCHEM speciation (Parker *et al.*, 1995) the Al³⁺ activity in this solution was 17 µM. Plants were grown in an environmentally controlled growth chamber under the following conditions: 16/8 h photoperiod at 300 µmol photons m⁻² s⁻¹, day/night temperature 27/20 °C, and 70% RH.

Plants used in the experiments with the auxin transport inhibitor NPA were germinated as described above. A ring (1 mm thick) of 1.2% agarose (Bio-Rad Laboratories, Inc., Hercules, USA) containing nutrient solution without Al and 10 µM naphthylphthalamic acid (NPA) was applied for 30 min to the root tips covering the root perimeter at 1.0–2 mm from the apex.

Root growth measurement and haematoxylin staining

To avoid handling of the plants which may affect root growth rates, the root elongation rates of control and Al-treated plants were determined on digital pictures of the culture flasks containing the individual plant roots. Photographs were taken with a digital camera (Finepix S602Zoom; Fuji Photo Film CO, LTD, Tokyo, Japan) at different times: 30 min before Al supply and at 15, 30, 45, 60, 120, 180, and 240 min after the start of the treatment. Comparative measurements of the length of the roots at different times were made on the pictures with Image Pro Plus version 3.0 (Media Cybernetics,

Inc., Silver Spring, MD, USA). Results were expressed as relative root elongation taking as a reference the root length in the first photograph (–30 min time sample). The growth experiment was performed three times without significant differences between the experiments. The results are from one run showing mean values from three plants per variety, time sample, and treatment.

Haematoxylin staining of root tips exposed to the control or the 50 µM Al-containing nutrient solutions for 20 h (Polle *et al.*, 1978; Corrales *et al.*, 1997) and visual alterations of root growth and root morphology after 5 d of Al treatment were used as additional indicators for varietal differences in Al resistance.

For bromodeoxyuridine labelling of S-phase nuclei plants grown in control or Al-containing nutrient solution were taken out of the culture flasks at 5, 10, 30, or 180 min after the start of the treatment. Roots were immediately washed for 10 min with either distilled water or 0.1 mM citrate and then transferred to the labelling solution (see below). Comparative runs using citrate-desorbed and non-desorbed roots did not reveal any influence of the citrate wash on labelling index of either control or Al-treated plants. If not otherwise stated the results are from non-desorbed roots.

Bromodeoxyuridine labelling of S-phase nuclei

Roots grown as described above were transferred to the labelling solution containing 5-bromo-2'-deoxyuridine (Sigma-Aldrich, Inc. St Louis, MO, USA) 1:500 in control nutrient solution without Al. After 120 min labelling, 5 mm root tips were excised and processed for confocal laser fluorescence microscopy as described below.

Sample preparation for immunofluorescence localization of S-phase nuclei

Excised 5 mm root tips were longitudinally split and the halves were fixed with 4% paraformaldehyde (w/v) and 0.2% glutaraldehyde (v/v) in PBS (140 mM NaCl and 20 mM phosphate, pH 7.4) for 40 min under partial vacuum.

After fixation the samples were washed with PBS and used for immunostaining of BrdU. To facilitate antibody penetration, sections were treated with pectolyase (0.5% for samples from HS 16×36 and 1% for Cateto) from *Aspergillus japonicus* (Sigma-Aldrich, Inc. St Louis, MO, USA) for digestion of cell walls for 20 min. The specimens were permeabilized for 20 min in 0.1% Triton X-100 in PBS. To assure good penetration of fluorescent dyes and antibodies, adequate concentrations and required times for sample treatments with pectolyase and Triton X-100 had been determined in preliminary investigations with control and Al-treated plants from both varieties using propidium iodide, mouse monoclonal anti- α -tubulin clone DM 1A (Sigma-Aldrich Inc., St Louis, MO, USA) and secondary antibody-anti-mouse Ig with Alexa 488 (Molecular Probes, Inc., Eugene, OR, USA) as test substances.

After DNA denaturation by 2 N HCl for 20 min and three washes in PBS, all specimens were incubated in 5% (w/v) bovine serum albumin (BSA; Sigma-Aldrich Inc., St Louis, MO, USA) in PBS to block non-specific binding sites. The mouse monoclonal antibody against BrdU (Hoffmann-La Roche, Basel, Switzerland) at a dilution 1:30 in PBS with 0.1% BSA was added to the specimens and incubated overnight at 4 °C in a humidified chamber. After three 5 min washes in PBS, the specimens were incubated with secondary antibody-anti-mouse IgG with Alexa 488 (Molecular Probes, Inc., Eugene, OR, USA) diluted 1:500 in PBS for 60 min at 37 °C. Sections were washed twice (5 min each time) in PBS and were mounted in glycerol medium with *n*-propyl gallate.

Root tip samples were observed with a confocal microscope (TCS SP2 AOBs; Leica microscopy Systems, LTD, Heerbrugg, Switzerland) using the 488 nm excitation line from the argon laser and an emission window between 500–600 nm. Nuclei labelled with BrdU (S-phase) were clearly distinguishable due to green fluorescence.

Micrographs were used to count the labelled S-phase nuclei present in areas of 150–300 cells and the results are shown as a labelling index (percentage of labelled nuclei). Results are means from at least three different roots per time sample, treatment, and variety. Micrographs shown in the paper are representative of the variety and treatment effects observed.

Results

Root elongation and Al accumulation

Under control conditions maize variety HS 16×36 exhibited a higher root elongation rate than variety HS CAT-AL 237/67×SLP 181/71 (called Cateto) (Fig. 1). The varieties clearly differed in their root growth response to Al (Figs 1, 2). Root elongation of the Al-sensitive variety HS 16×36 was reduced by the 50 μM Al treatment after 45 min of exposure (Fig. 1A) while no Al effect on root growth was observed in the Al-resistant variety Cateto even after 4 h of Al treatment (Fig. 1B). Due to the inherently lower root growth rate in Cateto, the relative root elongation rates of either control or Al-treated plants observed over a 4 h exposure period in this variety were not statistically different from those of Al-treated HS 16×36. Elongation of roots relieved from Al stress during the 120 min labelling period was lower in Al-sensitive HS 16×36 (2.0 mm) than in Al-

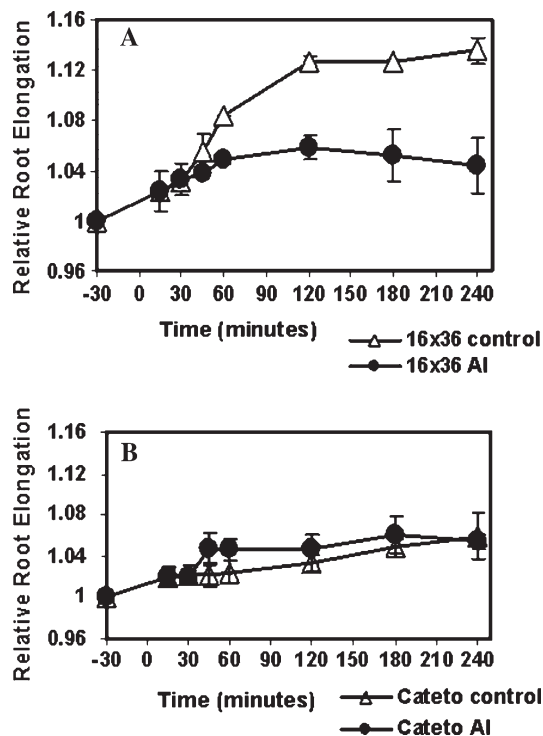


Fig. 1. Relative root elongation of 4-d-old maize seedlings growing in nutrient solution either without Al (control) or supplemented from time 0 min with 50 μM Al (17 μM Al³⁺ activity). (A) Al-sensitive variety HS 16×36; absolute root length at beginning (reference time -30 min) was 9.3±0.13 cm. (B) Al-resistant variety Cateto; absolute length at beginning was 8.31±0.11 cm. Values are means ±SE ($n=3$).

resistant Cateto (5.3 mm). Strong varietal differences in Al resistance was fully evident after longer exposure times. Figure 2A exhibits the enormous differences in Al effects on root growth and development between Cateto and HS 16×36 after 5 d of the Al treatment. Besides the drastically reduced root length, abnormal branching in roots of the Al-sensitive variety HS 16×36 is clearly visible. Haematoxylin staining of root tips (Fig. 2B) revealed higher Al accumulation in root tips of the Al-sensitive HS 16×36 than in those of the Al-resistant Cateto.

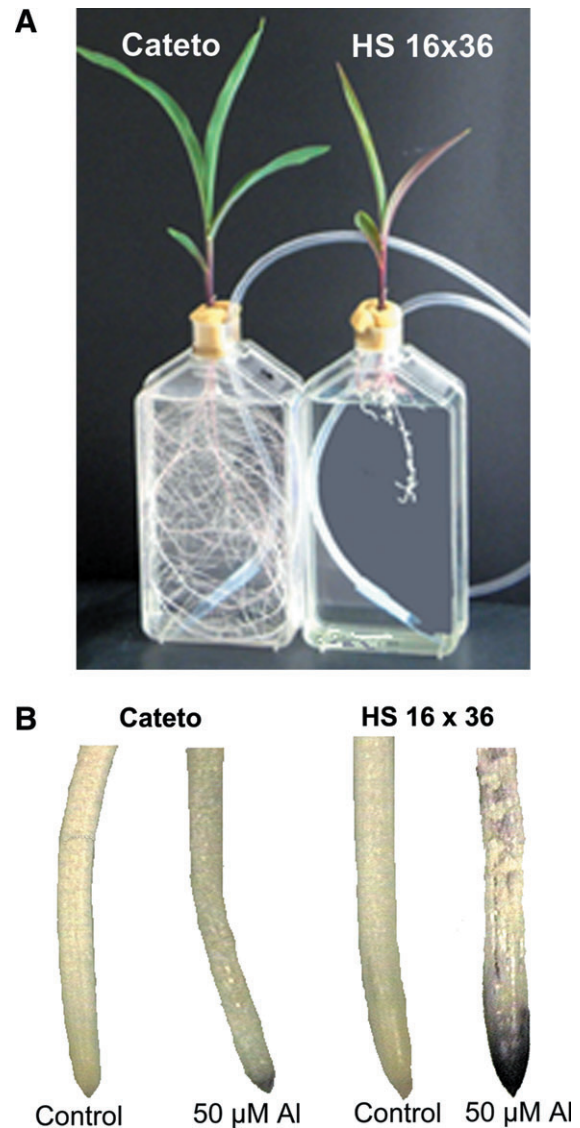


Fig. 2. (A) Seedlings of maize variety Cateto (Al-resistant) and variety HS 16×36 (Al-sensitive) exposed from day 4 after sowing to nutrient solution containing 50 μM Al (17 μM Al³⁺ activity) for 5 d. Al caused severe reduction of root elongation and abnormal root branching in Al-sensitive HS 16×36, while Al-resistant Cateto was little affected. Under control conditions HS 16×36 has an inherently higher root elongation rate than Cateto (see Fig. 1). (B) Haematoxylin staining for the accumulation of phytotoxic Al in root tips of 4-d-old maize seedlings exposed to 50 μM Al (17 μM Al³⁺ activity) for 20 h.

Influence of Al on S-phase nuclei in the root apex

S-phase nuclei in the apical root meristem of control plants of both maize varieties were clearly visible as green fluorescent spots in the laser confocal images, while nuclei that during the 120 min labelling period did not enter into S-phase remained unstained (Figs 3, 5). Under control conditions, the labelling index, i.e. the relative number of S-phase nuclei, in the meristematic zone of the root tip was higher in variety HS 16×36 than in Cateto (Fig. 4A). In control roots the labelling indexes remained constant throughout the experiment (data not shown). In the Al-sensitive variety HS 16×36, a 5 min exposure to Al was enough to block the entrance into S-phase completely of cells in the central part of the root meristem zone (250–800 µm from the apex), while the labelling index remained high in the outermost cell files of the apex (Figs 3C, 4A). After 10–180 min Al exposure, S-phase nuclei had also disappeared from this cortical zone (Fig. 3D–G). Only a few strands of single dividing cells could be detected in the procambial or cortex initials (Fig. 3D). In Cateto, the Al-tolerant variety, S-phase nuclei were abundant in the root tip meristem zone even after 180 min of Al exposure (Figs 3H, 4B).

While the Al treatment quickly stopped BrdU incorporation into nuclei in the proximal meristem zone of root apex of HS 16×36 (250–800 µm from the apex), 10 min exposure to Al was enough to induce the incorporation of BrdU into nuclei in a small area located in the distal elongation zone (DEZ) at 2.5–3.1 mm from the tip (Figs 4C, 5A). The position of the DEZ in control and Al-treated plants was determined by the observation of near isodiametric cells containing perinuclear microtubules (Baluska *et al.*, 2001) visualized by immunofluorescence techniques (data not shown). After 30 min of Al treatment, a lateral swelling containing many S-phase nuclei was observed in the DEZ (Fig. 5B). After 180 min, the protrusion of a new lateral root initial was clearly distinguishable (Fig. 5C). The labelling index in this subapical root meristem located at 2.5–3.1 mm from the tip (Fig. 4C) was about the same as that found in the apical meristem of controls at 250–800 µm (Fig. 4A).

Influence of NPA on S-phase nuclei in the root apex

In plants with 10 µM NPA locally supplied to the distal transition zone (DTZ) at 1–2 mm from the apex for 30 min, the meristematic activity was found in cells in the central part of the root tip at a more distal position (1.6–2.0 mm from apex) (Fig. 5D, arrow) than in controls (250–800 µm from apex) (Fig. 3A). Compared with controls (Fig. 3A), a lower number of dividing cells was observed (Fig. 5E) and the labelling index was only $20 \pm 3.5\%$ ($n=5$), compared with the 45% observed in controls (Fig. 4A). In the NPA-treated tips, however, abundant S-phase nuclei were observed in the DEZ at 2.5–3 mm from the tip (Fig. 5D).

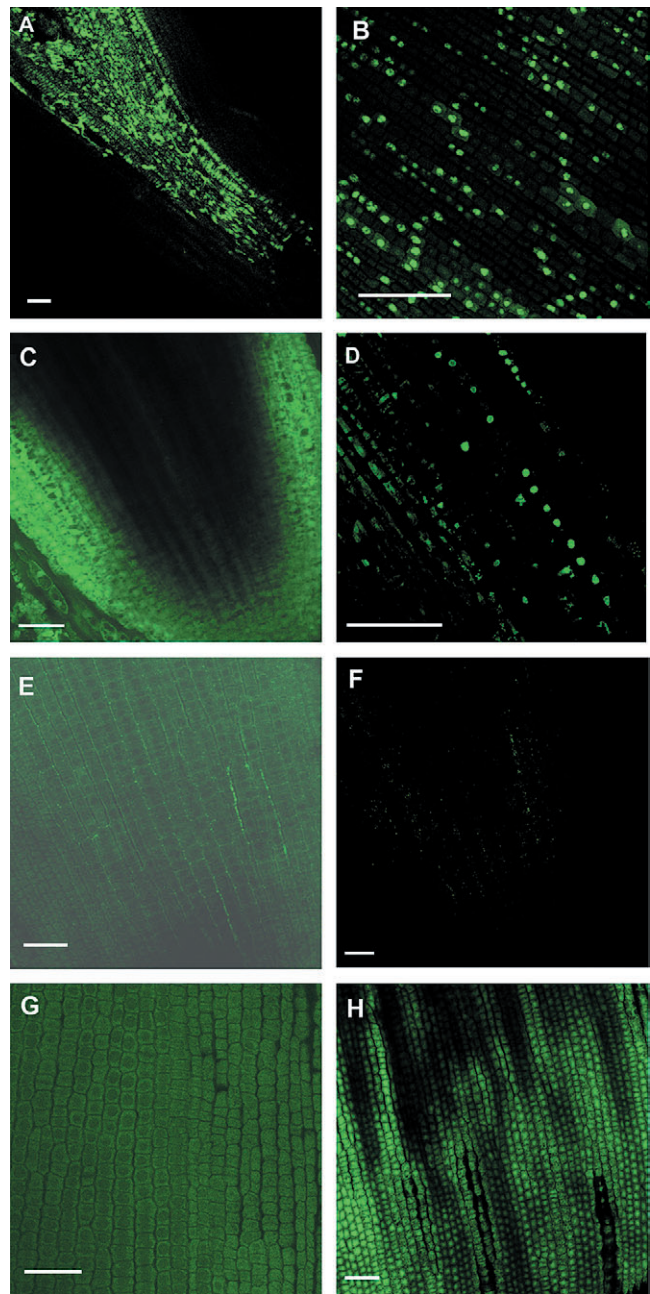


Fig. 3. Laser confocal micrographs of longitudinal sections of root tips (approximately 250–800 µm) of 4-d-old maize seedlings exposed to nutrient solution with 50 µM Al (17 µM Al³⁺ activity) or without Al (control) for 5–180 min. Roots of intact seedlings were labelled for 120 min with BrdU and S-phase nuclei were visualized by immunofluorescence staining. (A–G) Tips from the Al-sensitive variety HS 16×36. (A) Proximal meristem of a plant exposed to control solution for 30 min. (B) Proximal meristem of a plant exposed to control solution for 30 min followed by 0.1 mM citrate wash for 10 min. (C) Root tip after 5 min Al treatment. Note lack of S-phase nuclei in the central part of the meristem, while abundant S-phase nuclei can be seen in the periphery of the tip. (D–G) Root tips after Al supply for 30 min (D), 10 min (E), 30 min+10 min 0.1 citrate (F), or 180 min (G). (H) Proximal meristem of Al-resistant Cateto after 180 min Al treatment. (Scale bars=50 µm). (A) A picture summarizing a series of nine sections taken at 4 µm intervals. (B) A picture summarizing four sections taken at 2 µm intervals. All the others are individual snapshots.

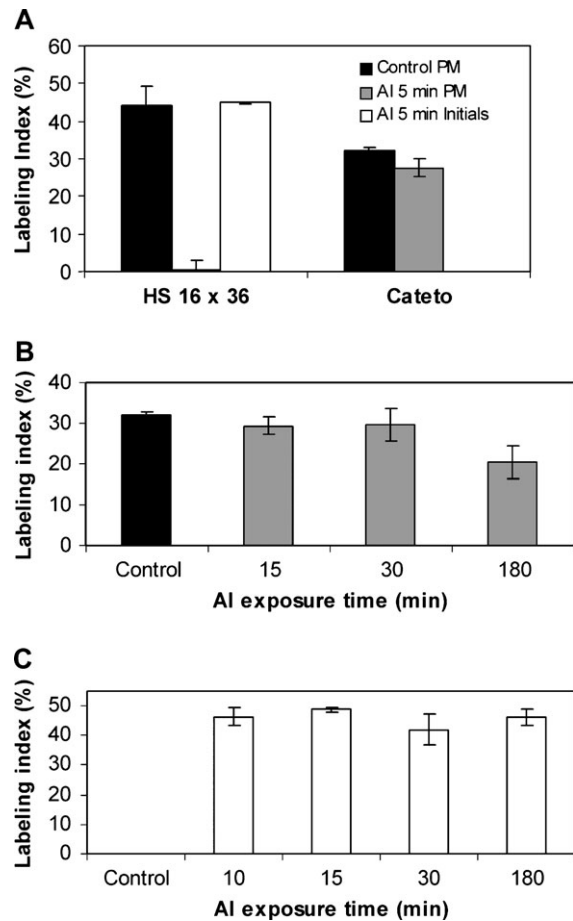


Fig. 4. (A) Labelling index (% of S-phase nuclei) in maize varieties Cateto (Al-resistant) and HS 16×36 (Al-sensitive) exposed for 5 min to control or 50 μM Al-containing nutrient solution (17 μM Al^{3+} activity) (PM, proximal meristem; Initials, cortical or procambial initials). (B) Labelling index in the proximal meristem of Cateto after 10–180 min exposure to Al. (C) Labelling index in the Al-induced meristem in the distal elongation zone located at 2.5–3.1 mm from apex in the Al-sensitive variety HS 16×36 (mean \pm SE; $n \geq 3$).

Discussion

Maize varieties HS 16×36 and Cateto displayed substantial varietal differences in the inherent root elongation rate under control conditions (Fig. 1). Labelling of S-phase nuclei with BrdU revealed a higher proportion of dividing cells in the fast elongating control roots of HS 16×36 (Fig. 4A) than in the slow growing Cateto (Fig. 4B). Root elongation is a complex process, which involves cell division and cell expansion. Taking into account the constancy of cell division rate in root meristems in the absence of inhibitors (Baskin, 2000) the higher root expansion rate, under control conditions, in HS 16×36 than in Cateto can be expected to be a consequence of the higher cell production rate.

Both varieties largely differed in their response to Al. Relative root elongation rates that allow varietal differences in Al resistance to be distinguished, regardless of the inher-

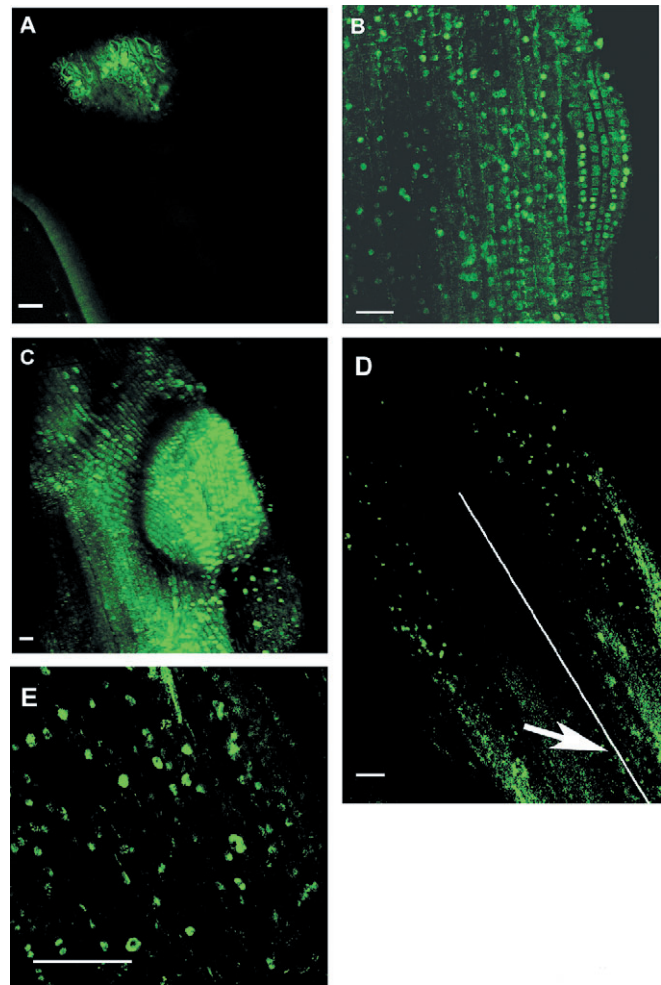


Fig. 5. Laser confocal micrographs from root tips of 4-d-old maize seedlings. Roots of intact seedlings were labelled for 120 min with BrdU and S-phase nuclei were visualized by immunofluorescence staining. (A–C) Root tips from plants exposed to nutrient solution with 50 μM Al (17 μM Al^{3+} activity) for 10 min (A), 30 min (B) or 180 min (C). Al induced cell division activity in the distal elongation zone at approximately 2.5–3.1 mm from the apex leading to the protrusion of a new lateral (B, C). No S-phase nuclei were found in controls in this root tip zone (not shown). (D, E) S-phase nuclei in tips after a local supply for 30 min of 10 μM NPA to the distal transition zone (1–2 mm from the apex). (D) Snapshot with reference line. NPA supply induced abundant S-phase nuclei in the cortex of the distal elongation zone (2.5–3.1 mm from apex). In the central part of the apex, NPA induced both a shift of the meristematic activity to a more distal position (arrowhead points to the upper border of the central meristem located at 1.6–2.0 mm from apex) and a 50% reduction in the labelling index in this central meristem (labelling index $20 \pm 3.5\%$; $n=5$). (E) This shows a higher magnification of the central meristematic zone adjacent to the arrowhead in (D). Scale bar in (D) = 100 μm ; all others = 50 μm .

ent varietal differences in growth (Llugany *et al.*, 1994; Poschenrieder *et al.*, 1995; Kim *et al.*, 2001), were much more affected by Al in HS 16×36 than in Cateto (Fig. 1). Differences in haematoxylin staining (Fig. 2B) showed that better Al exclusion plays a role in the high Al resistance of Cateto. This is in line with findings by Piñeros *et al.* (2002)

who reported enhanced citrate exudation over the first 5 cm of the root tip in this maize variety. The exceptionally high difference in Al resistance between both varieties was even more evident after longer Al exposure (Fig. 2A).

The time frame for a measurable inhibition of root elongation in variety HS 16×36, observed after 45 min Al exposure, is similar to that observed in former studies which reported Al-induced inhibition of root elongation 30–120 min after the start of the Al treatment (Llugany *et al.*, 1995; Barceló and Poschenrieder, 2002). The shortness of the time required for Al to inhibit root elongation provided a major argument for focusing investigations into the primary mechanisms of Al toxicity on root cell expansion rather than on cell division (Gunsé *et al.*, 1997; Kollmeier *et al.*, 2000). The root elongation rate depends on both cell division and cell expansion. Although cell division *per se* does not increase root length, both the rate of cell division and the time period that mitotic cells remain active determine the supply of cells to the elongation zone and hence the elongation rate (Beemster and Baskin, 1998). As the residence time of cells in the meristem zone is several hours (Beemster and Baskin, 2000), the Al-induced inhibition of the root elongation rate, measurable after 45 min in the Al-sensitive variety HS 16×36, must be attributed to an Al-induced decrease of root cell expansion rather than to the fast inhibition of root cell division. The toxic effect of Al on root cell expansion is well established (Ryan *et al.*, 1993; Kochian, 1995; Gunsé *et al.*, 1997) and, no doubt, root elongation is a primary target of Al toxicity. This, however, does not preclude fast Al effects on root cell division.

In this work it is shown for the first time that in Al-sensitive maize an Al supply of only 5 min is able to inhibit the incorporation of BrdU into S-phase nuclei in the central meristem of root tips within the following 120 min. The requirement of a labelling period hampers an exact timing of the Al-induced inhibition of cell division. The possibility cannot be excluded that the Al absorbed into the cells during the treatment period (5–180 min) is toxic to cell division not only within the exposure time, but also acts during the following 120 min of the labelling period. The fact that no differences in toxic effects on cell division between samples washed with distilled water or desorbed with citrate for 10 min were observed makes it unlikely that apoplastic Al had caused such an after-effect during the labelling period. However, the citrate wash may not have eliminated all the Al associated with the apoplast. A further argument against toxic after-effects of Al during the labelling period provides the observation that Al-treated HS 16×36 restarted root elongation when transferred to labelling solutions without Al, although at a lower rate than Al-resistant Cateto.

Salt stress has recently been found to disrupt mitotic activity after 120 min of exposure. Blockage of cell cycle progression under stress was proposed as a general mechanism to prevent entrance of the cells to vulnerable stages (e.g. M-phase) and to allow the cellular defence system to

be activated (West *et al.*, 2004). Under Al stress, not only entrance into the S-phase was inhibited in the proximal meristem, but cell division was stimulated in the DEZ where a new lateral initial was formed. In plants exposed to excess Cu, damage in the root tip meristem was swiftly accompanied by the proliferation of laterals. Such a response may prevent the extension of the main root into soil patches with high ion toxicity and favour extension of lateral roots into the less toxic top soil (Llugany *et al.*, 2003).

As far as is known, this is the first report on such a rapid inhibition of cell division by a relatively low Al activity (17 μM). It is now recognized that Al can enter plant cells quite quickly (Lazof *et al.*, 1996; Taylor *et al.*, 2000). Aluminium was found in nuclei of root meristematic cells of Al-sensitive soybean after only 30 min exposure to a low Al^{3+} activity (Silva *et al.*, 2000). In view of this, the possibility cannot be excluded that the rapid Al-induced inhibition of root cell division in this study was caused by a direct interaction between Al and nuclei (Matsumoto *et al.*, 1976). However, these results provide strong arguments against a general cariotoxic action after short Al exposure. After 5 min exposure to Al, cell division was inhibited in the central part of the root tip but not in the root periphery where cells are expected to be in direct contact with Al. Despite the fast and intense inhibition by Al of mitotic activity in the apical 250–800 μm , some cells in the procambial and cortical initials maintained cell division capacity (Fig. 3D). Moreover, at 2.5–3.1 mm from the tip, S-phase cells appeared after 10 min exposure to Al and the mitotic activity was maintained even after 180 min exposure to Al when an incipient lateral was distinguishable in this zone (Fig. 5C). This root zone which has been described as DEZ (Ishikawa and Evans, 1995) or the transition zone (Baluska *et al.*, 1996) is located between the most Al-sensitive DTZ (1–2 mm from apex) (Sivaguru and Horst, 1998) and the main elongation zone (>3 mm from the tip). The DEZ is a specialized root zone where both dividing and elongating cells can be found. Growth in the DEZ seems highly responsive to environmental factors (Ishikawa and Evans, 1995). Dividing cells were not observed in the DEZ in controls, but abundant S-phase cells were found there both after Al and after NPA treatment (Fig. 5).

The time sequence of Al-induced changes in the position of S-phase nuclei in HS 16×36 (Figs 3, 4, 5) shows a shift of cell division activity from the central part of the meristem to the periphery of the root tip and to the DEZ. This reflects a change in root cell patterning rather than a direct toxic effect of Al blocking cell division in general. Similar changes in root cell patterning have been described by others in plants exposed to phytotropins like NPA or TIBA (Sabatini *et al.*, 1999; Doerner, 2000; Jiang *et al.*, 2003). Here it was also observed that NPA-stimulated cell division in the DEZ, while it induced both a shift to a more distal position of the dividing cells in the central part of the apex and a lower number of S-phase nuclei therein. Jiang *et al.*

(2003) found that auxin transport inhibitors induce a shift of maximum auxin concentrations from the columella and quiescent centre to the procambial and cortical initials and the proximal meristem. In the proximal meristem, cell division is inhibited and after prolonged exposure to NPA a mirror image duplicated meristem can be found away from the apex. So the relative position in root tips of the quiescent zone and meristematic activity is largely determined by auxin levels and the polarity of auxin transport; in turn, polar auxin flux seems to be determined by the relative position of meristem activity (Doerner, 2000).

In the Al-treated plants the induction of an apparent lateral root initial was found in the DEZ. Also the local supply of the auxin transport inhibitor NPA induced cell division in this region. Early lateral root primordia initiation can arise close to the root tip and extensive dedifferentiation of pericycle cells seems not always to be required for the formation of these primordia (Dubrovsky *et al.*, 2000). Auxins and other hormones are considered to be essential factors for the initiation of lateral root primordia (Charlton, 1996; Zhang and Hasenstein, 1999; Francis and Sorrell, 2001).

The similarity of effects on root cell patterning between Al toxicity and phytohormones supports the hypothesis that inhibition of auxin flow plays an important role in the Al toxicity syndrome. Inhibition by Al of the basipetal auxin flux has been found in maize roots (Kollmeier *et al.*, 2000). The observed depletion of IAA in the elongation zone was interpreted as a mechanism of Al-induced inhibition of root elongation. Here it is shown that both Al and NPA can inhibit cell division in root tip meristems after a few min of exposure, probably not by a direct cytotoxic effect but by changing cell patterning. Both Al and NPA induce cell division in the DEZ. Auxin seems to play a major role in these Al effects on cell division. However, interaction with other hormonal factors cannot be excluded in view of the rapid Al effects on root cytokinin and ethylene concentrations (Massot *et al.*, 2002).

Taking together the finding that Al induces, within minutes, a change in the position of cell division activity in root tips and the inhibitory effect of Al on basipetal auxin transport in the tips (Kollmeier *et al.*, 2000), there is quite a strong support for the view that the Al-induced alteration of root architecture is a very early step in the Al-toxicity syndrome. Abnormal branching of the root system as already described by Clarkson (1969) and observed in this investigation (Fig. 2A) are only visible after several days of exposure to Al. This investigation is the first to show that the cellular events that underlie these morphogenetic alterations are induced by Al within a few min. So root cell patterning has to be considered as a primary target of Al in maize.

Acknowledgements

The authors wish to thank EMBRAPA, Sete Lagoas (Brazil) for the maize seeds and Ms Mercè Martí for helpful technical advice

on immunocytochemistry. This work was supported in part by the European Research Council (Contract number: ICA4-CT-2000-30017) and by the Spanish and Catalan Governments (DGICYT project number BFU2004-02237-CO2-01/BFI and 2001 SGR 00200). SD was supported by a fellowship of the NATO Scientific Committee and MA by a predoctoral grant from the Spanish Ministry of Education.

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