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Physiological aspects of aluminium tolerance associated with the long arm of chromosome 2D of the wheat (*Triticum aestivum* L.) genome

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Abstract Aluminum (Al) uptake in roots of wheat near-isogenic lines having differing tolerances to aluminium toxicity was studied using roots and root segments immersed in a nutrient solution at a controlled pH and temperature. At low Al concentrations a mechanism preventing root tips from accumulating too much Al was observed in an Al-tolerant isoline and a 'BH1146' euploid. This mechanism was more efficient when divalent cations of calcium or magnesium were present in the nutrient medium. Al accumulation steadily increased in root tips of the Al-sensitive wheat isoline during all 24 h of incubation, and the presence of divalent cations in the medium even increased Al concentration in root tissue. However, at higher Al concentrations in the medium the mechanism preventing the root tips of Al-tolerant genotypes from accumulating too much Al was not observed, and in effect Al concentration in root tips of both Al-tolerant and Al-sensitive isolines increased. It is concluded that genetical factors are located on the long arm of chromosome 2D from the BH1146 euploid that control the mechanism preventing root apical meristems from accumulating too much Al at low Al concentrations in the medium. However, there must be other genetical factors also located on this chromosome segment that control Al detoxication in root tips of Al-tolerant lines at higher external Al concentrations.

Key words Aluminium · Isogenic lines · Tolerance · Wheat

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Introduction

Aluminium (Al) ions are regarded as a main toxic factor affecting plant growth in mineral soils at a pH below 5.5 (Foy et al. 1978). While the effect of Al on plant physiological processes is well-known the mechanism of the toxic effect of Al on root growth is not yet fully understood (Horst et al. 1983). Still less is known about the mechanism of aluminum tolerance (for a recent review see Taylor 1991).

Aluminium toxicity is primarily manifested by the inhibition of root growth, and this effect is used to determine Al tolerance (Moore et al. 1977; Konzak et al. 1977; Poole et al. 1978). Plants differ in their reaction to Al, and genetic variation has been found between plant species as well as between crop cultivars (Foy and Fleming 1978; Anioł and Gustafson 1984). A differential response of wheat (*Triticum aestivum* L.) to aluminum has been reported, and several attempts have been made to determine the inheritance of this character. Major genes, controlling wheat tolerance to Al were located on chromosomes of the A and D genomes of hexaploid wheat (Anioł 1990), but the physiological processes controlled by these genes are still unknown. The majority of the observed variability with respect to Al tolerance in wheat could be explained by the hypothesis of two or three gene pairs, each gene affecting the same character, with complete dominance of each gene pair. Studies on Al tolerance in aneuploid series of wheat cultivar 'Chinese Spring' (CS) revealed that genes controlling this character are located on the short arm of chromosome 5A and the long arm of chromosome 2D and 4D (Anioł and Gustafson 1984). There are numerous data on Al accumulation in roots of Al-tolerant and Al-sensitive wheat genotypes (Anioł 1983; Petterson and Strid 1989; Zhang and Taylor 1988, 1989), however, it is not clear whether the differences in Al tolerance are due to differential Al accumulation in root tissue.

In this paper, near-isogenic wheat lines were used to study Al uptake in roots. These lines differ markedly in

Al tolerance. Possible differences in Al uptake and Al accumulation between near-isogenic wheat lines could be reasonably ascribed to the activity of the genes by which these lines differ.

Materials and methods

Plant material

Previous studies indicated that one of major factors controlling Al tolerance in wheat cv 'Chinese Spring' (CS) is located on the long arm of chromosome 2D (Aniol 1991). The effect of this or these factors were studied using:

- CS ditelosomic line 2Ds; e.g., with both of the long arms of chromosome 2D missing. Seeds of this line were obtained from Dr. E. Sears, USDA, Columbia, M. USA;
- the CS allo 2Dl line developed in our laboratory. In this line the long arm of chromosome 2D from Al-tolerant wheat cv 'BH1146' was transferred into the CS genome using 'Chinese Spring' ditelosomic line (ditelo 2Ds) in the reciprocal monosomic method as described by Snape et al. (1983);
- the euploid CS cultivar, obtained from Dr. E. Sears;
- the euploid BH1146 cultivar obtained from the USDA Beltsville collection.

Seeds were sterilized with 0.1% $HgCl_2$ aqueous solution for 10 min, rinsed excessively with water, and germinated overnight on filter paper in petri dishes. Sprouted seeds were sown the next day on a polyethylene net fixed in lucite frames. Styrofoam blocks were attached to the frames with rubber bands and floated on the surface of a vigorously aerated nutrient solution. Containers of nutrient solution were placed in a waterbath at 25 °C with 16 h per day of illumination with incandescent low intensity light from Polam incandescent tubes.

For the screening tests, nutrient solution was used as described earlier (Aniol 1984). Al-uptake experiments were performed in simplified nutrient solutions, e.g., Al^{3+} added at different concentrations in an aqueous solution supplemented with Ca^{2+} or Mg^{2+} at 100 μM. Four-day-old seedlings were used. After each exposure to Al, (as indicated in the Figures) the seedlings were removed, thoroughly washed for 2–3 min in running tap water, and then placed for 30 min in a 0.5 mM aqueous EDTA solution at 4 °C (approximately 130 seedlings per replication). After the desorption with EDTA, each sample was divided into two groups. One, of approximately 30 seedlings, was washed again for 2–3 min in running tap water and then transferred to Al-free medium for 48 h. The root regrowth or additional root growth after Al shock was easily assessed by staining the roots with a 0.1% aqueous solution of Eriochrome cyanine R for 10 min. After staining, the excess dye was removed by washing under tap water. When the aluminum treatment did not destroy the root apical meristem, the part of the root which grew after Al treatment was white (unstained) and contrasted with the heavily stained root part that had been exposed to aluminum. When the apical meristem had been irreversibly damaged, the root tips remained intensively stained after 48 h of growth in Al-free medium. During all stages of growth, and particularly during the Al treatment the nutrient solution was maintained at pH 4.5 ± 0.02. At approximately 20 ml of nutrient solution per seedling changes in the pH of the medium did not exceed 0.02 during the 24 h of aluminium treatment. In the second group of the sample, roots (from approximately 100 seedlings) were separated into root tips (apical 5 mm) and the remainder of the root and the fresh weight of tips and roots was estimated.

Each experiment was replicated at least three times, and in each replication three root tips and root samples were mineralized and the Al content determined.

Aluminum determination

The roots and root tips were mineralized in the acid mixture as described by Jones and Thurman (1957). The acid mixture consisted of sulfuric, perchloric, and nitric acids in a proportion by volume of 1:2:7. Fresh root samples were transferred to digestion tubes, and the acid mixture was added (5 ml/g fresh weight). Samples were mineralized in a Tecator heating block. The Al content was estimated using the catechol violet method (Wilson 1984).

Analysis of data

Statistical analysis of data were performed using multifactor analysis of variance available on statistical package Statgraphics Version 2.6. Comparisions among means were performed using Turkey's procedure. Significance of the statistics is on a confidence level of at least 95%.

Results

Aluminium tolerance of tested wheat lines

The transfer of the long arm of chromosome 2D from Al-tolerant cv 'BH1146' into Al-sensitive 'Chinese Spring' via the CS 2Ds ditelosomic line increased the tolerance of the obtained CS allo 2Dl isogenic line markedly (Table 1). The CS ditelosomic line as well as C S euploid seedlings showed irreversible damage of root apical meristems after incubation for 24 h in a medium containing 8 ppm Al (0.296 mM) this damage was manifested as a lack of root growth after Al treatment. Root apical meristems of the CS isogenic line (CS allo 2Dl line) were affected by aluminium only when exposed to the highest Al concentration used, e.g., 0.592 mM. However, the Al tolerance of the 'BH1146' cultivar, which is the donor of the chromosome 2D segment to the CS isogenic line, was much higher: no damage to root apical meristems was observed even after incubation in 0.593 mM Al. Therefore, the 'Chinese Spring' wheat euploid and its ditelo 2Ds line should be classified as being Al sensitive, while the near-isoline of 'Chinese Spring' (CS) allo 2Dl, with long arms of chromosome

Table 1 Aluminium tolerance of investigated wheat lines. Data represent percent of seedlings with undamaged apical meristems after 24 h of Al treatment of the indicated concentration in the standard nutrient solution. Number of tested seedlings in parentheses

Al concentration	0.148 mM	0.296 mM	0.444 mM	0.593 mM
Chinese Spring Ditelo 2Ds	60% (143)	0% (142)	0% (140)	0% (136)
Chinese Spring Allo 2Ds	100% (147)	92% (138)	74% (135)	41% (134)
Chinese Spring	70% (143)	0% (132)	0% (130)	0% (133)
BH 1146	100% (139)	100% (131)	100% (135)	100% (128)

[a] Standard nutrient solution: 0.4 mM $CaCl_2$; 0.65 mM KNO_3; 0.25 mM $MgCl_2 \times 6H_2O$; 0.01 mM $(NH_4)_2SO_4$; 0.04 mM NH_4NO_3

2D from 'BH1146' and the 'BH1146' euploid are Al-tolerant.

The effect of Al on root growth in the Al-uptake experiments

Aluminium uptake experiments were performed using very low Al concentrations in order to avoid Al from complexing with other ions (Kinraide 1993). The effect of the addition of calcium and magnesium ions on Al accumulation in root tissue was also investigated.

Root growth in all of the tested genotypes was affected by aluminium ions at a concentration of 30 μM. However, this Al concentration was not toxic to root apical meristems, except for CS ditelosomic line seedlings incubated in an aqueous solution of Al. Aluminium toxicity measured as the reduction of root regrowth after Al treatment was alleviated when divalent cations of Ca or Mg were present in medium containing Al and was expressed as longer root regrowth after Al shock. Higher Al concentrations in the medium, e.g., 80 μM and 320 μM, evidently inhibited root growth even in the Al-tolerant wheat genotypes tested, while root apical meristems of the Al-sensitive 'Chinese Spring' isoline were irreversibly damaged (Fig. 1) at these external Al concentrations.

It is interesting to note that a short (1–6 h) exposure to aluminium, particularly at higher concentrations, caused a clear stimulation of root growth after Al shock. However, in the Al-sensitive isoline this stimulation of root growth was followed by the irreversible destruction of the apical meristem.

Aluminium uptake into the root tips

The pattern of aluminium accumulation in the root tips of Al-tolerant cv 'BH1146' and the Al-tolerant CS isogenic line was different than in the root tips of the Al-sensitive CS euploid and its 2Ds ditelosomic line.

Two phases of Al accumulation could be observed in root tips from Al-tolerant wheat genotypes; first, during the first 6 h of incubation, when the increase in Al concentration in the root tips took place; and the second phase from 6 to 24 h of Al treatment, when Al concentration in root tips did not increase and even showed some tendency to decrease. In root tips of Al-sensitive lines, however, Al accumulation increased continuously with length of incubation.

The addition of divalent cations Ca or Mg influenced Al accumulation in the root tips of the wheat lines tested differently. Divalent cations reduced Al concentration in

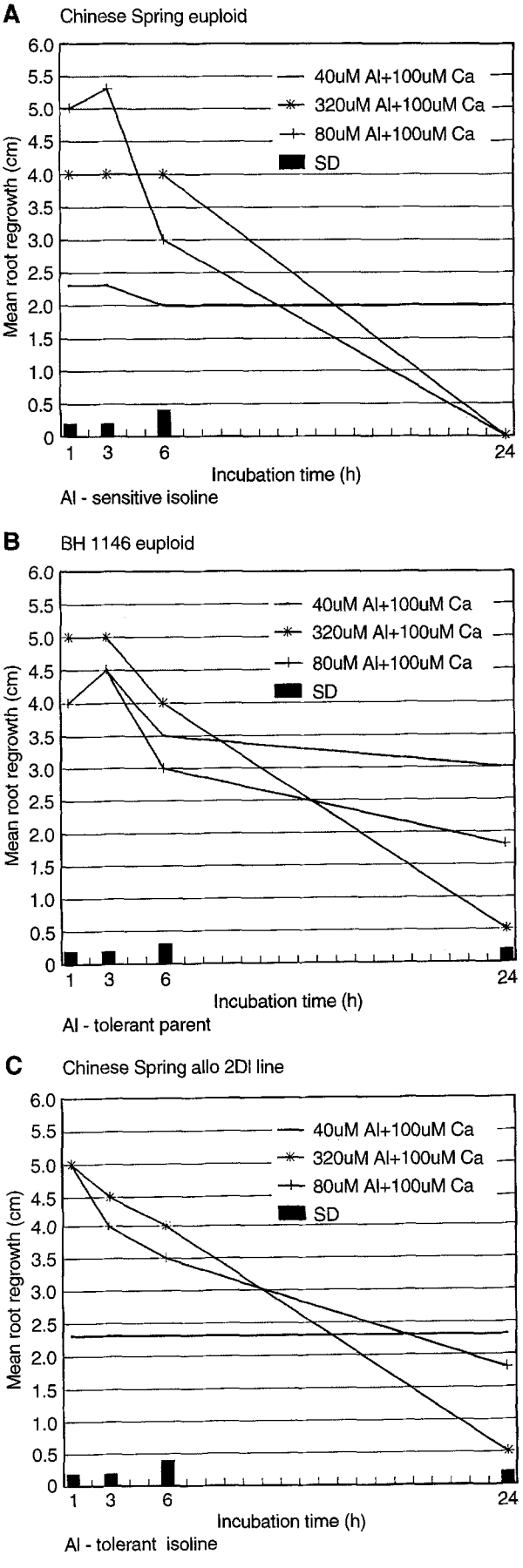
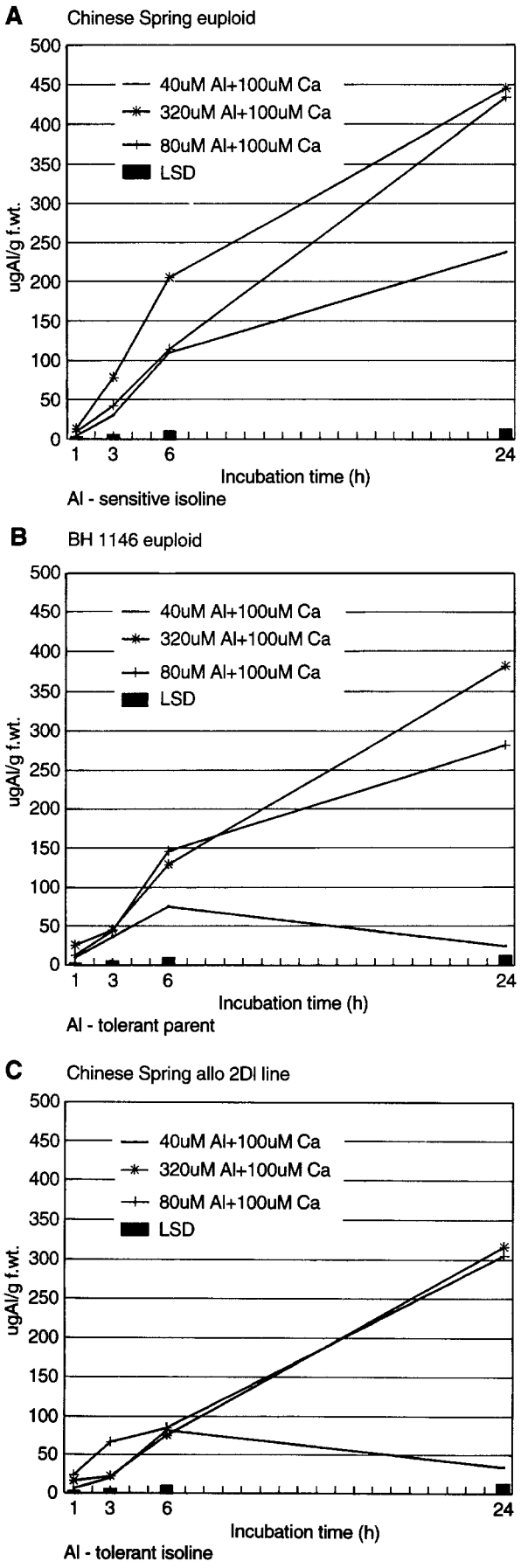


Fig. 1A–C The effect of increased Al concentratioins on mean root regrowth of tested wheat lines. Data are means from measurements of approximately 30 seedlings



root tips of Al-tolerant isolines: magnesium ions were a little more effective than those of calcium in reducing Al accumulation in root tips of the Al-tolerant CS isoline, while the differences in effectiveness between these cations were insignificant in the Al-tolerant 'BH1146' cultivar. On the contrary, Al accumulation in root tips of Al-sensitive wheat lines increased significantly when divalent cations of calcium or magnesium were present in the medium together with aluminium. However, the effect of both cations was different in different wheat genotypes; there was no effect of calcium on Al accumulation in root tips of the Al-sensitive CS euploid, while magnesium ions caused a significant increase in Al accumulation. The reverse effect was observed in Al-sensitive the CS ditelo 2Ds line – the calcium ions caused a significant increase in Al concentration, while magnesium ions had little effect. This suggests, that besides genes controlling Al tolerance/toxicity mechanisms, the long arm of chromosome 2D also carries factors regulating root response to divalent cations.

It is worth noting that the apical meristems of the CS ditelosomic line were still viable after Al treatment in the presence of Ca or Mg cations, as measured by root regrowth, despite the fact that the Al concentration in the root tips was much higher than that in the root tips of seedlings incubated in an Al aqueous solution, in which the apical meristems were irreversibly damaged. Also, while the Al concentration in root tips of the Al-tolerant CS iso-line incubated in the Al aqueous solution was at the same level as that found in root tips of the CS ditelo 2Ds line, the roots of the Al-tolerant iso-line were able to continue growth after Al shock while those of the Al-sensitive aneuploid were not.

Aluminium accumulation in the basal parts of the roots

The Al accumulation in the basal parts of the roots was not as clearly different in Al-tolerant and Al-sensitive wheat lines as it was in the root tips. The time course of Al accumulation in the roots of the lines tested was very similar; only in the roots of the CS cultivar incubated in the aqueous Al solution supplemented with Mg^{2+} was Al concentration in the roots significantly higher. Divalent cations caused a reduction in Al accumulation in basal parts of the roots. In contrast to Al accumulation in the root tips, the effects of Ca and Mg ions on Al concentration in basal root parts did not differ in Al-tolerant and Al-sensitive wheat lines; divalent cations caused a reduction in the Al concentration irrespective of the Al tolerance level of the wheat line tested. However, calcium ions were significantly more effective than

Fig. 2A–C Aluminum accumulation in root tips of CS near-isogenic lines and the BH1146 euploid at increased Al concentrations in nutrient solution. Data are means form at least three replications

magnesium ions in reducing Al-accumulation in the roots of Al-sensitive genotypes while there was no difference between the effect of these ions on Al-accumulation in the roots of Al-tolerant genotypes.

The differences in Al accumulation as affected a by Al concentration and divalent cation content in the nutrient solution become more marked with time of incubation in both root tips and roots.

The effect of increased Al concentration in the nutrient solution

The existence of the mechanism preventing the root tips of Al-tolerant seedlings from accumulating too much Al when the medium contained 30 μM Al was also evident when the Al concentration was increased to 40 μM. This effect was not present, however, when seedlings were incubated in nutrient solution with Al concentrations of 80 μM and 320 μM Al (Fig. 2): the aluminium content in root tips increased in both Al-tolerant and Al-sensitive genotypes at these Al concentrations. In effect, the Al concentration in the root tips was only a little higher in the Al-sensitive CS euploid than in the Al-tolerant isoline after 24 h of incubation; but the apical meristems of the Al-sensitive line were irreversibly damaged, while those from the Al-tolerant isoline were still viable although a marked reduction in root regrowth was observed (Fig. 1).

Aluminium accumulation increased along with increased Al concentration in the nutrient solution only in the root tips of the Al-tolerant 'BH1146' cultivar, while in the root tips of both Al-tolerant and Al-sensitive CS -isogenic lines Al accumulation was the highest at 80 μM Al in the medium and did not increase at higher Al concentrations.

It is interesting to note that despite the fact that no significant differences were found in the Al concentration in root tips from the Al-tolerant CS isoline when incubated with 80 μM or 320 μM Al in the medium, the Al tolerance level as measured by root regrowth ability was markedly different at those Al concentrations (Fig. 1).

The Al uptake pattern in the root tips described above contrasts with that observed in the basal root parts; Al concentratioin in root tissue from the Al-tolerant 'BH1146' euploid and the Al-tolerant CS isoline was not affected by Al concentration in the medium. Only in the case of the Al-sensitive CS isoline was the Al accumulation pattern in the roots different. Al accumulation in the roots was highest when there was 80 μM Al in the medium, while it was significantly lower at 320 μM. However, at both of these Al concentrations, the root apical meristems were irreversibly damaged in this line.

Discussion

The near-isogenic lines of the 'Chinese Spring' wheat cultivar differ markedly in tolerance to aluminium (Table 1). Since the CS near-isogenic lines tested differ only in the long arm of chromosome 2D, it is logical to assume that the observed differences in Al tolerance could be ascribed to the activity of genetical factors located on this chromosome.

In an interesting experiment in which the spatial sensitivity of maize roots to aluminium was investigated, it was shown that the root apical meristem is a primary site of Al toxicity (Ryan et al. 1993). The hypothesis of the root cap providing protection from Al stress as suggested by Bennet and Breen (1991) was not confirmed since the removal of the root cap had no effect on the Al-induced inhibition of root growth. Therefore, any mechanism protecting the root apical meristem from Al injury would be a very important component of the Al tolerance mechanism.

When the Al concentration in nutrient solution is low, 30 μM and 40 μM, a mechanism blocking Al accumulation in the root tips operates in genotypes carrying the 2Dl chromosome fragment from 'BH1146' (Fig. 2, B and C). This mechanism does not operate in genotypes with this 2Dl chromosome fragment from the CS euploid or in the CS line without this chromosome fragment (CS ditelo 2Ds line).

A similar mechanism of Al tolerance based on the inhibition of Al accumulation in root tips has been described Al-tolerant wheat cv 'Atlas 66' by Rincon and Gonzales (1992), and these authors suggest that this mechanism operates as a metabolism-dependent exclusion of Al from the root apical meristem.

Studies on Al uptake and distribution in root apices of near-isogenic wheat lines differing in Al-tolerance at a single locus, *Alt-1*, revealed that this gene from the Al-tolerant 'Carazinho' cultivar encodes a mechanism responsible for malic acid excretion in root tips under Al stress. Malic acid in turn, chelates and excludes Al from root apices (Delhaize et al. 1993a,b). It is for further experiments to investigate whether the mechanism of Al-accumulation inhibition controlled by genetical factors located on the long arm of chromosome 2D from the 'BH1146' wheat cultivar is also manifested in a tested isogenic line as a malic acid excretion and Al chelation. However, data reported in this paper already indicate that the described Al-accumulation inhibition mechanism is efficient only at very low external Al concentrations and that some other Al-tolerance mechanism must operate at higher Al concentrations in external medium as well as inside the root tissue. This second Al-tolerance mechanism seems to be also encoded by gene or genes located on 2Dl chromosome fragment.

It is interesting to note that root growth stimulation was observed at short incubation times (Fig. 1), particularly at higher Al concentrations in the incubation medium, and that this stimulation was later followed by severe root growth inhibition and even by the irreversible destruction of the apical meristems. This phenomenon has been described many times in the literature (see Foy 1984). According to Kinraide (1993) it is a general phenomenon that polyvalent cations (charge > 2) are

generally rhizotoxic at low concentrations, while they may enhance growth at subtoxic levels under H^+ stress.

The disturbances in Al accumulation in root tissue of Al-tolerant and Al-sensitive CS near-isogenic lines at higher external Al-concentrations, which is manifested as the lack of increase or even decrease in Al content in root tissue, might be explained by the fact that Al^{3+} causes structural damage in root apices when present in sub-lethal or lethal doses (Ikeda and Tadano 1993). The above supposition is confirmed by the fact that in roots of the very Al-tolerant 'BH1146' euploid a steady increase of Al-content in the tissue was observed along with increased external Al concentratioins (Fig. 2B). It can be assumed that no Al-induced damage occurred in this Al-tolerant genotype.

The ameliorative effect of divalent cations on Al toxicity is evident from the data reported in this paper and has also been found by many other authors (Kinraide and Parker 1987; Rengel 1992, just to mention the recent ones). But the role of these cations, particularly Ca^{2+}, in Al-toxicity/tolerance mechanisms is still disputed. Some authors suggest that the Al disruption of Ca^{2+} transport resulting in calcium deprivation at the root apex may play an important role in the mechanism of Al toxicity (Huang et al. 1992, 1993; Rengel and Elliott 1992). Calcium deprivation might induce disturbances in the Ca^{2+} homeostasis in the cytosol, which in turn would alter the pattern of Ca^{2+} – calmoduline binding affecting the activity of this regulatory protein (Ślaski 1989, 1990; Rengel 1992). Other authors, however, report that severe Al-induced inhibition of root growth can occur without affecting Ca^{2+} uptake (Ryan et al. 1994) and that the observed ameliorative effect of cations occurs because of decreased membrane-surface negativity and the consequent decrease of the membrane-surface activity of Al^{3+} (Miyasaka et al. 1989; Kinraide et al. 1994).

The results presented in this paper confirm the ameliorative effect of calcium and magnesium ions on Al rhizotoxicity, but their effect on Al accumulation in root apices is puzzling. While Ca^{2+} caused increased Al accumulation in the root tips of the Al-sensitive isogenic line and at the same time alleviated Al-toxicity, in Al-tolerant genotypes these ions caused a reduction in Al-accumulation in root apices. The results discussed above suggest that the divalent cations, particularly Ca^{2+}, might play a important role not only in the processes occurring on the membrane surfaces but also in the mechanisms preventing cell damage, even at high Al accumulation inside the root tissue. This will be the subject of further investigations that might lead to the identification of other Al-tolerance mechanisms encoded by gene or genes located on the long arm of the 2D wheat chromosome.

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