Plant and Soil **151**: 97–104, 1993.

PLSO 9858

Inhibition of nitrate uptake by aluminium in maize

R.P. DURIEUX[1], W.A. JACKSON[2], E.J. KAMPRATH[2] and R.H. MOLL[3]
[1]*Department of Agronomy, University of Vermont, Burlington, VT 05405, USA,* [2]*Department of Soil Science, North Carolina State University, Raleigh, NC 27695-7619, USA and* [3]*Department of Genetics, North Carolina State University, Raleigh, NC 27695-7614, USA*

Received 19 November 1992. Accepted in revised form 10 March 1993

Key words: aluminium, ammonium, induction, maize, nitrate, *Zea mays*

Abstract

Experiments with two maize (*Zea mays* L.) hybrids were conducted to determine (a) if the inhibition of nitrate uptake by aluminium involved a restriction in the induction (synthesis/assemblage) of nitrate transporters, and (b) if the magnitude of the inhibition was affected by the concurrent presence of ambient ammonium. At pH 4.5, the rate of nitrate uptake from 240 μM NH_4NO_3 was maximally inhibited by 100 μM aluminium, but there was little measurable effect on the rate of ammonium uptake. Presence of ambient aluminium did not eliminate the characteristic induction pattern of nitrate uptake upon first exposure of nitrogen-depleted seedlings to that ion. Removal of ambient aluminium after six hours of induction resulted in recovery within 30 minutes to rates of nitrate uptake that were similar to those of plants induced in absence of aluminium. Addition of aluminium to plants that had been induced in absence of aluminium rapidly restricted the rate of nitrate uptake to the level of plants that had been induced in the presence of aluminium. The data are interpreted as indicating that aluminium inhibited the activity of nitrate transporters to a greater extent than the induction of those transporters. When aluminium was added at initiation of induction, the effect of ambient ammonium on development of the inhibition by aluminium differed between the two hybrids. The responses indicate a complex interaction between the aluminium and ammonium components of high acidity soils in their influence on nitrate uptake.

Introduction

Aluminium has been shown to inhibit nitrate uptake in white clover (Jarvis and Hatch, 1986); sorghum (Gomes et al., 1985; Keltjens, 1987; Keltjens and Van Ulden, 1987; Galvez and Clark, 1991), rice (Van Hai et al., 1989), spruce (Peuke and Tischner, 1991), and maize (Vale et al., 1984; Cambraia et al., 1989) but it is not known how the inhibiting effect is exerted. When first exposed to nitrate, the roots of many plants progressively increase for a few hours in their capacity to absorb nitrate from an initial, low, constitutive rate (Jackson, 1978). This pattern of apparent induction implies that the rate of uptake at a given instant reflects (a) the extent to which the uptake system has been induced (viewed as the existing number of functional transporters) and (b) the effective operation of those transporters (Jackson et al. 1973). Inhibition of nitrate uptake by aluminium thus may be due to a restriction of the induction process, an inhibition of transport activity, or both. Accordingly, the primary objective of the present investigation was to determine which of these two potential effects is responsible for the inhibition by aluminium. Since nitrogen-depleted maize exhibits the induction pattern of nitrate uptake (Bergmark et al., 1992; Jackson et al., 1973; MacKown et al., 1982; MacKown and McClure,

1988), such plants were subjected to inducing conditions in the presence of aluminium. Upon removal of aluminium, a rapid increase of nitrate uptake to the rate in plants not treated with aluminium would support the hypothesis that induction had not been impaired; the corollary would be that aluminium interfered with the activity of the nitrate transporters, not with their synthesis or assembly.

Ammonium may accumulate in highly acid soils with elevated levels of soluble aluminium (Cornfield, 1952; Morrill and Dawson, 1967) and ammonium generally tends to restrict nitrate uptake and its induction in maize (MacKown et al., 1982). In some instances, ammonium has decreased the toxic effects of aluminium on root growth (Cumming, 1990; Klotz and Horst, 1988; Rorison, 1985); thus it conceivably could mitigate the effect of aluminum on nitrate uptake. Therefore, a second objective of this investigation was to determine the relative effects of aluminium on concurrent nitrate and ammonium uptake under conditions which ape those of highly acid soils, that is, when the nitrate uptake system also may be influenced by the presence of ambient ammonium.

Results are reported for two maize hybrids, Pioneer hybrid 3320, a nonprolific commercial hybrid adapted to the southeastern United States, and an experimental prolific hybrid (I202 × Mo17) developed for improved nitrogen use efficiency in that region (Moll et al., 1987). The data in general indicate that activity of the nitrate transporters was impaired by aluminium but that the capacity for induction was largely unaffected. In addition, the pattern of inhibition by aluminium appeared to be affected differentially by ammonium in the two hybrids.

Methods and materials

Results of two experiments following the same general procedure are reported. Caryopses of the nonprolific Pioneer hybrid 3320 and the prolific experimental hybrid I202 × Mo17 were incubated at 30° C and 95% relative humidity in germinating paper moistened with 0.1 m*M* $CaSO_4$. After two days, the secondary roots were excised, and the primary roots of four seedlings (one culture) were guided through holes in the bottom of hollow polyethylene cups. Moist cotton was placed around the seed, and the stoppers were filled with black polypropylene pellets. The cultures were placed in 15-L tanks of aerated, nitrogen-free nutrient solution. Each tank contained 12 cultures. The tanks were placed under sodium-vapor and metal-halide lamps with a 16/8 hour light/dark cycle and a photosynthetic photon flux density of 1050 μmol $m^{-2}s^{-1}$ at the surface of the containers. The temperature was 27 ± 2° C during the light and 20 ± 1° C in darkness. The nitrogen-free nutrient solution contained 1 m*M* $MgSO_4$, 2.5 m*M* K_2SO_4, 1 m*M* $CaSO_4$, 1 mg Fe L^{-1} (as FeEDTA) and 0.4 × Hoagland solution for the micronutrients (Hoagland and Arnon, 1950). The acidity was adjusted twice daily to pH 6 with Ca $(OH)_2$, and the nutrient solution was replaced each day.

On the eight day, four cultures of each hybrid were harvested after rinsing the roots in 0.1 m*M* $CaSO_4$, and the root and shoot fresh weights were determined. The remaining cultures were divided into four replications each consisting of one culture of four plants for each treatment.

Each culture was placed in a styrofoam beaker with 250 ml of uptake solution that contained 1 m*M* $CaSO_4$, 240 μ*M* KCl, and micronutrients at 0.4 × strength Hoagland solution (Hoagland and Arnon, 1950). The acidity was adjusted using HCl or $Ca(OH)_2$. Aluminium was supplied as $AlCl_3$. Nitrogen also was present at a concentration of 240 μmol L^{-1} either as NH_4NO_3 (Experiment 1) or KNO_3 (Experiment 2). After each hour the cultures were placed in a new beaker with fresh uptake solution. The acidity of each spent solution was recorded, and a sample was frozen for subsequent analysis.

After the experiment, the plants were harvested, and root and shoot fresh weight were measured. The treatment solutions were analyzed for nitrate by a non-automated modification of the method of Lowe and Hamilton (1967). Ammonium was analyzed by the procedure of Cataldo et al., 1974. Net uptake of the ions was determined from the extent of depletion of the solution during each uptake period and was normalized to hourly rates per unit root fresh weight. The data presented are the average

of four cultures ± the standard error of the mean.

Experiment 1

The effects of 100 μM $AlCl_3$ at pH 4.5 on the rate of nitrate and ammonium uptake from 240 μM NH_4NO_3 were examined with hybrids 3320 and I202 × Mo17. Hourly uptake rates were measured starting 8 hours after the onset of the photoperiod.

Experiment 2

The rapidity of response to aluminium withdrawal or addition in the absence of ammonium was examined by initially exposing cultures to 240 μM KNO_3 ± 100 μM $AlCl_3$ at pH. 4.5 (KCl was not present). After six hours, four of the cultures were transferred from aluminium to aluminium-free solution while another four cultures remained in aluminium. Similarly, four of the cultures that had been in aluminium-free solution were transferred to aluminium and another four remained in aluminium-free solution. Hourly rates were measured during the first six hours. Following the transfers to and from aluminium, uptake rates were measured during 6–6.5 and 6.5–7.0 hours, and then for 5 hourly periods thereafter. Hybrids 3320 and I202 × Mo17 were both employed.

Results

Experiment 1

The induction pattern of each hybrid for the rate of nitrate uptake from 240 μM NH_4NO_3 at pH 4.5 is shown in Figure 1. With 3320 (Fig. 1A), the rate increased from 3.0 ± 0.4 μmol g^{-1} h^{-1} during the first hour to 8.8 ± 0.5 μmol g^{-1} h^{-1} during the eight hour. Corresponding values for I202 × Mo17 (Fig. 1B) were 2.5 ± 0.3 and 10.0 ± 1.0 μmol g^{-1} h^{-1}. In both hybrids, the presence of 100 μM aluminium inhibited the rate of nitrate uptake although an increase in rate from the first hour still was evident (Figs. 1A, B). Inhibition by presence of aluminium was more severe for 3320 than for I202 × Mo17.

The rate of ammonium uptake was greatest during the first hour and declined moderately during the next few hours (Fig. 2). There was little difference between the hybrids and no discernable effect of aluminium on the rate of ammonium uptake. Because of the inverse pattern with time for uptake of the two ions (Figs. 1 and 2), the proportion of the nitrogen absorbed as nitrate increased steadily during the induction period from about 20% to about 55% (Fig. 3). Presence of aluminium restricted this increase to less than 40% in 3320. With I202 × Mo17, how-

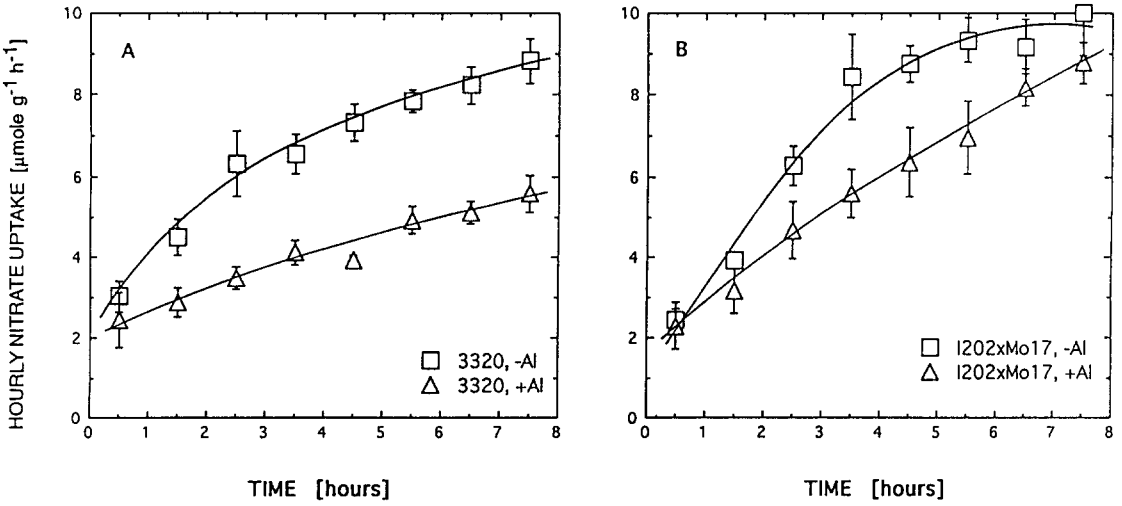

Fig. 1. The effect of aluminium (100 μM) during induction with 240 μM NH_4NO_3 on the rate of nitrate uptake by nitrogen-depleted 3320 (**A**) and I202 × Mo27 (**B**). Experiment 1.

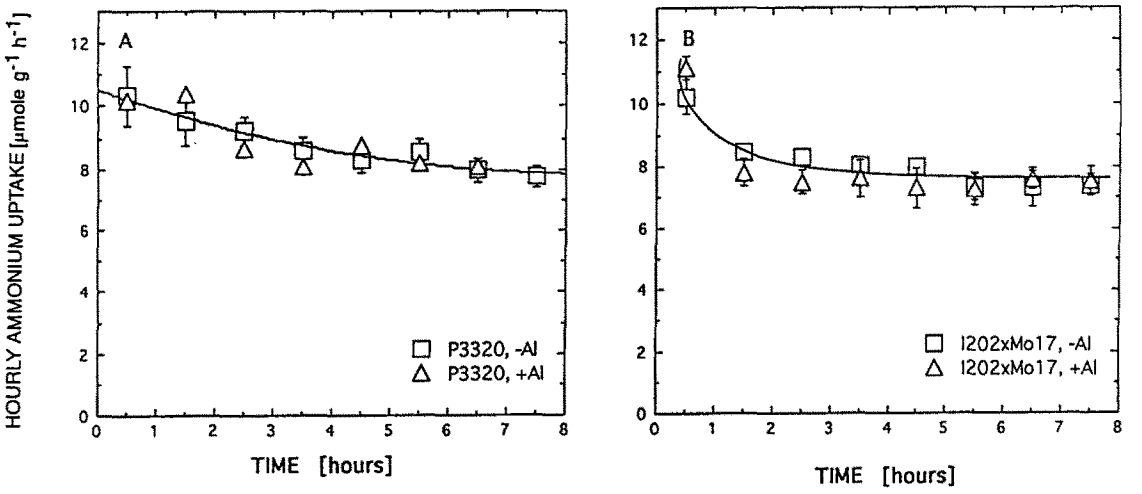

Fig. 2. The effect of aluminium (100 μM)during induction with 240 μM NH_4NO_3 on the rate of ammonium uptake by nitrogen-depleted 3320 (**A**) and I202 × Mo27 (**B**). Experiment 1.

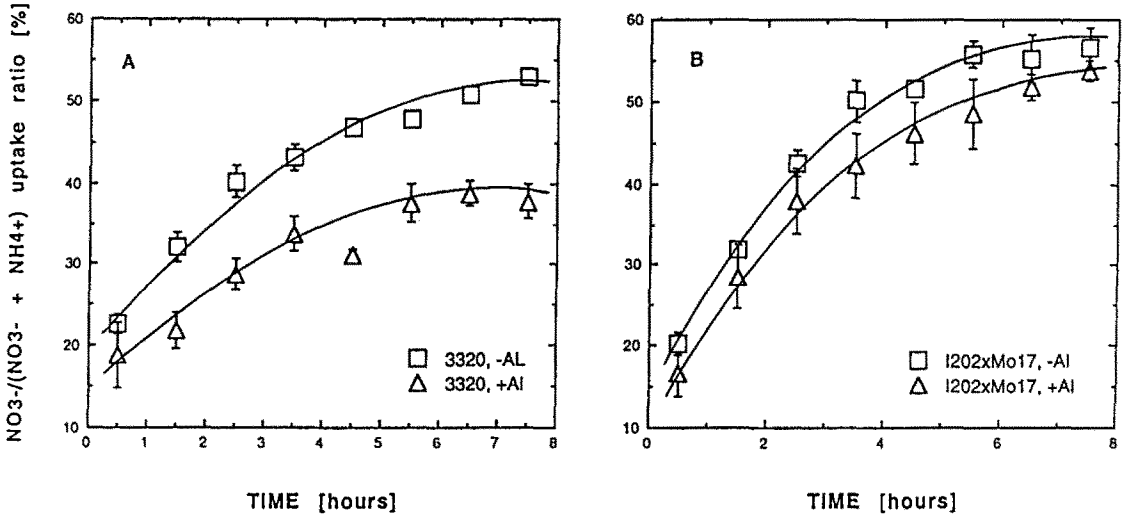

Fig. 3. The effect of aluminium (100 μM) on the percentage of total nitrogen uptake (nitrate + ammonium) that was absorbed as nitrate in each hourly period by nitrogen-depleted 3320 (**A**) and I202 × Mo17 (**B**) during an eight-hour induction period with 240 μM NH_4NO_3. Experiment 1.

ever, there was less of an aluminium-induced inhibition in the proportion absorbed as nitrate.

The rate of nitrate uptake was maximally inhibited by 100 μM aluminium under these conditions and neither nitrate nor ammonium uptake were appreciably affected at pH 4.5 compared to pH 6.0 (data not presented). The inhibitory effects of the $AlCl_3$ additions appear to be due to aluminium because previous experiments with maize root systems indicated that ambient chloride at these concentrations did not interfere significantly with nitrate uptake (Longmire, 1982). Moreover, 300 μM NaCl at pH 4.5 did not provide the inhibition that resulted from 100 μM $AlCl_3$ at pH 4.5 (data not presented).

Experiment 2

This experiment was designed to determine if the inhibition of nitrate uptake by aluminium resulted from a restriction in the rate of induction

of the nitrate uptake transporters, or whether the effect could be accounted for entirely by inhibited activity of the existing transporters. Ammonium was omitted from the ambient media to minimize its influence (MacKown et al., 1982) on the induction and activity of the nitrate uptake system. When aluminium was present at the onset of induction, inhibition of nitrate uptake in 3320 was evident after the third hour (Fig. 4B vs. 4A). In 1202 × Mo17, the inhibition was evident during the first hour (Fig. 5B vs. 5A). These responses (Figs. 4, 5) thus differed from those in the presence of ammonium (Fig. 1) in which inhibition by aluminium occurred by the second hour with 3320 but not until after that with I202 × Mo17.

Transfer of aluminium-treated 3320 to aluminium-free solution resulted in an increased rate of nitrate uptake within the next hour (Fig. 4B) to the rate (~11 μmol g^{-1} h^{-1}) of plants

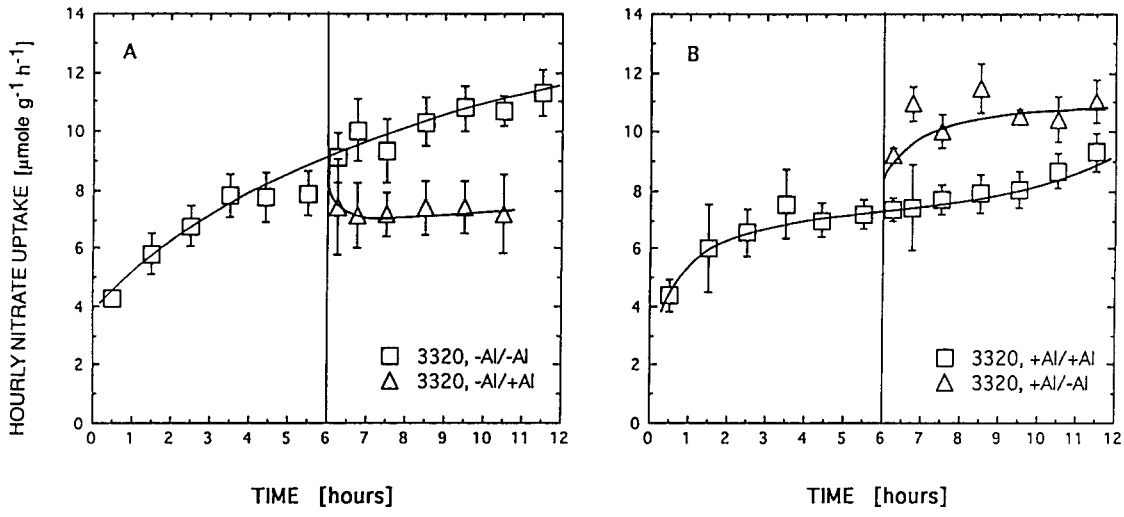

Fig. 4. The effect of aluminium (100 μM) addition on the rate of nitrate uptake by nitrogen depleted maize hybrid 3320 that had been induced in 240 μM KNO_3 for 6 h (**A**), and the effect of aluminium withdrawal when 3320 had been induced in the presence of 100 μM aluminium (**B**). Experiment 2.

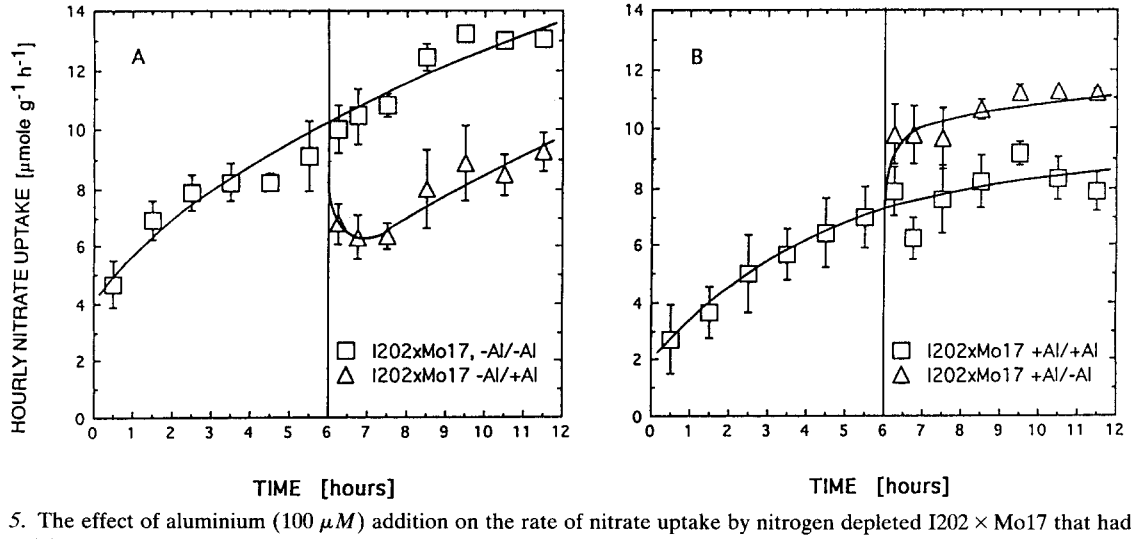

Fig. 5. The effect of aluminium (100 μM) addition on the rate of nitrate uptake by nitrogen depleted I202 × Mo17 that had been induced in 240 μM KNO_3 for 6 h (**A**), and the effect of aluminium withdrawal when I202 × Mo17 had been induced in the presence of 100 μM aluminum (**B**). Experiment 2.

which had been exposed to aluminium-free media throughout (Fig. 4A). Rapid recovery also occurred with I202 × Mo17 (Fig. 5B) but the maximal rate obtained after withdrawal of aluminium (~11 μmol g^{-1} h^{-1}) was slightly less than the maximal rate (~13 μmol g^{-1} h^{-1}) of fully induced plants (Fig. 5A) that had never been exposed to aluminium.

Inhibition of the rate of nitrate uptake occurred quickly when aluminium was added to plants that had been induced for 6 hours in the absence of aluminium (Figs. 4A, 5A). For 3320, inhibition occurred within the first 30 minutes and the rate of nitrate uptake was sustained at the inhibited level to the end of the experiment (Fig. 4A). The initial inhibition appeared to be more severe in I202 × Mo17 (Fig. 5A) but, by the second hour after aluminium addition, the inhibition had lessened such that the rate of nitrate uptake was similar to that of plants that had received aluminium throughout. With both hybrids, the rate of nitrate uptake following the addition of aluminium to induced plants remained greater than the rate during the first hour of induction in the aluminium-free treatment (Figs. 4A, 5A).

Discussion

Effects of aluminium on activity and induction of the nitrate uptake system

The nitrogen-depleted maize seedlings used in this investigation absorbed nitrate as effectively at pH 4.5 as at pH 6.0 (data not presented) and, at pH 4.5, they exhibited the characteristic pattern of induction in nitrate uptake (Figs. 1A, B, 4A, 5A,) observed under less acidic conditions (Bergmark et al., 1992; Jackson et al., 1973; MacKown et al., 1982; MacKown and McClure, 1988).

The rate of nitrate uptake at a given time can be visualized to be a function of the activity of existing nitrate transporters and the number of transporters present (Jackson et al., 1973; Jackson, 1978). The latter presumably is determined by the effectiveness of induction. The data indicate that, while the presence of ambient aluminium inhibited activity of the nitrate transporters, it either did not restrict (3320) or only slightly restricted (I202 × Mo17) the induction process. After induction, addition of aluminium restricted nitrate uptake within 30 minutes but not to the initial (0–1 h) rate (Figs. 4A, 5A). Following removal of ambient aluminium, the induced transporters rapidly became fully functional (Figs. 4B, 5B). In this interpretation, therefore, the nitrate that was absorbed in the presence of ambient aluminium was effective in the induction process and the transporters assembled in the presence of aluminium were capable of rapid restoration to a functional state in the absence of ambient aluminium. The inhibition exerted by the aluminium thus appears to have been through a direct but loose interaction with the nitrate transporters. No significant restriction occurred in ammonium uptake (Fig. 3) indicating a specific effect on the nitrate transport system.

Huang et al. (1992) have shown a marked inhibition of calcium uptake in wheat root apices upon aluminium addition and a swift recovery upon aluminium removal. They suggest that Al interacted at the outer face of a calcium channel in the plasmalemma of the root cells. The relatively rapid inhibition of nitrate uptake upon aluminium addition after plants that had been induced for 6 hours (Figs. 4A and 5A) and the rapid recovery upon aluminium removal (Figs. 4B and 5B), also imply that the inhibitory effect was exerted at the external cell surface. This interpretation is supported by the findings of Bennet et al. (1985), in which hematoxylin staining indicated no aluminium inside epidermal maize root cells after several hours of exposure by which time peripheral root cap cells were penetrated by aluminium and morphological alterations had occurred (see also Bennet and Breen, 1991). Recovery from inhibition of nitrate uptake after removal of ambient aluminium (Figs. 4B, 5B) has been reported previously for two other maize genotypes (Vale et al., 1984). Both that investigation and the present one imply a loose association between aluminium and its site of interaction with the nitrate transporters.

The influence of ambient ammonium

Nitrate uptake was inhibited by aluminium in the presence (Fig. 1A, B) as well as absence (Figs. 4 and 5) of ammonium. In the presence of ammonium, increasing aluminium concentration from 100 to 500 μM did not result in further inhibition (data not presented) which implies that a significant component of the nitrate uptake system was resistant to inhibition by aluminium. That there is a component resistant to ambient aluminium is further supported by the lack of a progressive decline in the rate of nitrate uptake after the immediate, but partial, inhibition that occurred upon addition of aluminium to plants that had been induced for six hours (Figs. 4A, 5A).

Addition of aluminium at initiation of induction did not have a marked effect on the initial (0–1 h) nitrate uptake rate whether or not ambient ammonium was present (Figs. 1, 4, 5). The data thus indicate that aluminium had relatively little effect on activity of the constitutive nitrate uptake system and, instead, exerted its dominant effects on activity of a component of the induced system.

Development of inhibition when aluminium was added at the start of induction appeared to differ between the two hybrids (Fig. 4 vs. Fig. 5), and the nature of that difference was altered by the presence of ammonium (Fig. 1). The difference between the hybrids in the response to aluminium in the presence of ammonium was not associated with measurable differences in ammonium uptake (Fig. 2) which implies a genotypic difference at the level of the nitrate transporters. An enhanced tolerance of soybean root growth to aluminium when ammonium was present has been reported (Klotz and Horst, 1988) and similar results have been observed in several sorghum genotypes (Tan et al., 1992). The soybean results were interpreted to reflect an interaction in which ammonium interfered with binding of aluminium to membrane entities, thus offsetting the inhibitory influence of aluminium (Klotz and Horst, 1988). This reasoning appears not to be entirely satisfactory for the present observations on inhibition of nitrate uptake because ammonium increased the development of the inhibitory effect in 3320 (Fig. 1A vs. Fig. 4) but decreased the early inhibition in I202 × Mo17 (Fig. 1B vs. Fig. 5).

Acknowledgements

We thank P Longmire for help in the conduct of the experiments and J Wahab for help in preparation of the manuscript.

References

Bennet R J and Breen C M 1991 The recovery of the roots of *Zea mays* L. from various aluminum treatments: Toward elucidating the regulatory processes that underlie root growth control. Environ. Exp. Bot. 31, 153–163.

Bennet R J, Breen C M and Fey M V 1985 Aluminium uptake sites in the primary root of *Zea mays* L. S. Afr. J. Plant Soil 2, 1–7.

Bergmark C L, Jackson W A, Volk R J and Blum U 1992 Differential inhibition by ferulic acid of nitrate and ammonium uptake in *Zea mays* L. Plant Physiol. 98, 639–645.

Cambraia J, Pimenta J A, Estevao M M and Sontánna R 1989 Aluminum effects on nitrate uptake and reduction in sorghum. J. Plant Nutr. 12, 1435–1445.

Cataldo D A, Schrader L E and Youngs V L 1974 Analysis by digestion and colorimetric assay of total nitrogen in plant tissues high in nitrate. Crop Sci. 14, 854–856.

Cornfield A H 1952 The mineralization of the nitrogen of soils during incubation: Influence of pH, total nitrogen, and organic carbon contents. J. Sci. Food Agr. 3, 343–349.

Cumming J R 1990 Nitrogen source effects on Al toxicity in nonmycorrhizal and mycorrhizal pitch pine (*Pinus rigida*) seedlings. II. Nitrate reduction and NO_3^- uptake. Can. J. Bot. 68, 2653–2659.

Galvez L and Clark R B 1991 Nitrate and ammonium uptake and solution pH changes for Al-tolerant and Al-sensitive sorghum (*Sorghum bicolor*) genotypes grown with and without aluminum. Plant and Soil 134, 179–188.

Gomes M M S, Cambraia J, Santánna R and Estevão M 1985 Aluminium effects on uptake and translocation of nitrogen in sorghum (*Sorghum bicolor*), L. Moench. J. Plant Nutr. 8, 457–465.

Hoagland D R and Arnon D I 1950 The water culture method for growing plants without soil. Calif. Agric. Exp. Sta. Circ. 347.

Huang J W, Shaff J E, Grunes D L and Kochian L 1992 Aluminum effects on calcium fluxes at the root apex of aluminium-tolerant and aluminum-sensitive wheat cultivars. Plant Physiol. 98, 230–237.

Jackson W A 1978 Nitrate acquisition and assimilation by higher plants: Processes in the root system. *In* Nitrogen in

the Environment. Vol. 2. Eds. D R Nielsen and J G MacDonald. pp 45–88. Academic Press, New York.

Jackson W A, Flesher D and Hageman R H 1973 Nitrate uptake by dark-grown corn seedlings: Some characteristics of apparent induction. Plant Physiol. 52, 120–127.

Jarvis S C and Hatch D J 1986 The effects of low concentrations of aluminium on the growth and uptake of nitrate-N by white clover. Plant and Soil 95, 43–55.

Keltjens W G 1987 Nitrogen source and Al toxicity of two sorghum genotypes differing in Al susceptibility. J. Plant Nutr. 10, 841–856.

Keltjens W G and Van Ulden P S R 1987 Effect of Al on nitrogen (NO_3^- and NH_4^+) uptake, nitrogen reductase activity and proton release in two sorghum cultures differing in Al tolerance. Plant and Soil 104, 227–234.

Klotz F and Horst W J 1988 Effects of aluminium and nitrate nutrition on aluminium tolerance of soybean (*Glycine max* L.). Plant and Soil 111, 59–65.

Longmire P B 1982 Nitrate-chloride interactions during transport in corn root tissue. MS Thesis, North Carolina State University, Raleigh, NC.

Lowe R H and J L Hamilton 1967 Rapid method of determination of nitrate in plant and soil extracts. J. Agric. Food Chem. 15, 359–361.

MacKown C T and McClure P R 1988 Development of accelerated net nitrate uptake. Plant Physiol. 87, 162–166.

MacKown C T, Volk R J and Jackson W A 1982 Nitrate assimilation by decapitated corn root systems: Effects of ammonium during induction. Plant Sci. Lettr. 24, 295–302.

Moll R H, Kamprath E J and Jackson W A 1987 Development of nitrogen-efficient prolific hybrids of maize. Crop Sci. 27, 181–186.

Morrill L G and Dawson J E 1967 Patterns observed for the oxidation of ammonium to nitrate by soil organisms. Soil Sci. Soc. Am. Proc. 31, 757–760.

Peuke A D and Tischner R 1991 Nitrate uptake and reduction of aseptically cultivated spruce seedlings, *Picea abies* (L.) Karst. J. Exp. Bot. 42, 723–728.

Rorison I H 1985 Nitrogen source and the tolerance of *Deschampsia flexuosa*, *Holcus lanatus*, and *Bromus erectus* to aluminium during seedling growth. J. Ecol. 73, 83–90.

Tan K, Keltjens W G and Findenegg G 1992 Effect of nitrogen on aluminum toxicity in sorghum genotypes. J. Plant Nutr. 15, 1383–1394.

Vale F R, de Novais R F, de Barros N F and Milagres B G 1984 Absorção de nitrato e amônio por raízes intactas de milhao pré-tratadas com alumínio. R. Bras. Cienc. Solo 8, 215–218.

Van Hai T, Thi Nga T and Laudelout H 1989 Effect of aluminium on the mineral nutrition of rice. Plant and Soil 114, 173–185.

Section editor: A C Borstlap