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Tolerance to Aluminum Toxicity: Certain Basic Biochemical Aspects

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Excessive acidity in cultivable soils is a serious challenge to crop production. One of the important toxic factors which become severe in acid soils is aluminum (Al) stress (Foy et al. 1978). Aluminum is a major constituent of mineral soils where it is present as aluminosilicates and other precipitated forms such as gibbsite (Lindsay 1979). When the pH of soil solutions falls below 4.5 the concentration of Al increases exponentially (Bergkvist 1987) since its solubility is increased. Thereby, acid soils normally have more amounts of Al available for uptake by plants. While the nutritional role of Al in plants is still under question, its toxic effects have been investigated and reviewed extensively (Foy 1988). The characteristic symptoms of Al toxicity include restriction of root proliferation, RNA synthesis and altered nutrient metabolism, which ultimately limit crop productivity. Though the existence of intraspecific and interspecific differences in tolerance and/or susceptibility to Al toxicity have been reported in many crops, the biochemical processes which contribute to such differences still continue to be potential areas of research. In the present investigation we have attempted to analyze some basic biochemical aspects related to Al tolerance. The influence of two metabolic inhibitors, 2,4-dinitrophenol (DNP) and cycloheximide, and induction of tolerance to Al by Al pretreatment are envisaged.

MATERIALS AND METHODS

Inasmuch as Al is a rhizotoxic ion (Kinraide and Parker 1989), we chose the modified Allium test proposed by Fiskesjo (1985) in which root growth can be easily monitored. Equal-sized bulbs were chosen from a population of common onion (**Allium cepa** L.) procured from a local market and a series of onions were grown in each experiment. The bulbs were grown in boiling tubes containing tap water as the basal medium in all experiments. Our preliminary experiments revealed that the optimal pH for onion root growth is 4.75 and the effect concentration of Al causing 50% (EC 50) damage effect (growth restriction was 0.93 mM. These two factors were used throughout our experiments.

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Al was supplied in the form of $AlK(SO_4)_2.12\ H_2O$. The experiments were performed at a constant room temperature of about 30°C and protected against direct sunlight.

In the experimental treatments the basal medium contained EC 50 Al plus 10 μg ml^{-1} each of 2,4-dinitrophenol (DNP) and cycloheximide. For nitrate treatement 10 mM KNO_3 was incorporated in the basal medium in addition to EC 50 Al. In Al pretreatment experiments, one set of onions was grown for the first three days in tap water containing 5 μM Al and another set in normal tap water (control), both at pH 4.75. On the fourth day, after measuring the root length, both of these (pretreated with 5 μM Al and control) were exposed to EC 50 level of Al and stressed for three days and the final length of roots was measured. From these two values the regrowth of roots was derived by substracting the root length before exposure to EC 50 Al from the root length measured after exposure. Each treatment was given for five days and on the sixth day root length was measured in whole root bundles and the mean values of five replicates were calculated. The nitrate content in the root cells was estimated according to Cataldo et al. (1975). The in vivo nitrate reductase (NR) activity in the root tissue was assayed using the method of Jaworski (1971). Total soluble protein content in the root tissue was estimated according to Lowry et al. (1951) and elemental analysis of Al by using an atomic absorption spectrophotometer (AAS).

RESULTS AND DISCUSSION

Data presented in Fig. 1 show that 2,4-dinitrophenol (DNP) and cycloheximide had drastic effects on root growth, registering an additive injurious effect along with Al. DNP caused a 77% reduction in root growth with a concomitant increase in Al uptake by 60% (Fig. 1). The differences in Al uptake and root growth brought about by DNP were significant ($P < 0.05$). DNP has been attributed to increased membrane permeability, thereby allowing Al to move from an exchangeable position of cell wall to the inner parts of the cortical cells and xylem parenchyma (Foy 1988). Though the actual mechanism by which Al traverses the plasmalemma is unknown, this could be the plausible means of the accumulation of excess concentrations of Al in the root tissues of onions under the supply of DNP. However, cycloheximide was totally inhibitory on root growth when supplied along with EC 50 Al. Total inhibition of root growth in the presence of cycloheximide can be ascribed to some mechanism related to the blocking of protein synthesis, which may allow direct binding of Al on its targets inside the root cells such as the phosphate in nucleic acids (Matsumoto et al. 1976). This mechanism is hypothesized since a shift of Al from cytosol to nuclei is possible, thereby restricting cell division and eventually resulting in total arrest of root growth.

Table 1 shows that the supply of nitrate significantly protects the roots from Al injury. Root growth was increased by 22% and

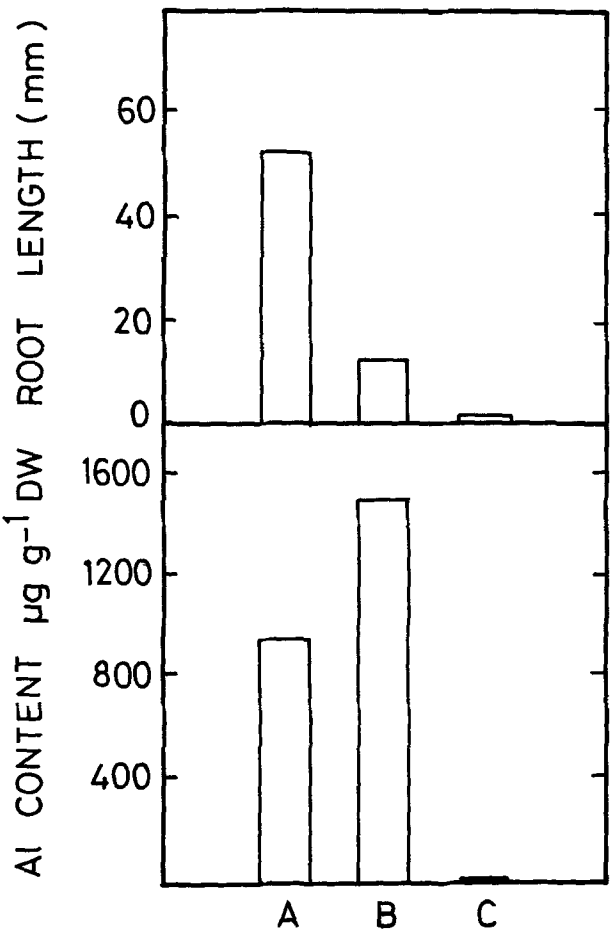

Figure 1. Effects of metabolic inhibitors (2,4-dinitrophenol and cycloheximide supplied at 10 µg ml^{-1} each) on Al tolerance by onion roots. Data on root length are mean ± SE (n = 5) and Al determinations are mean of three independent replicates. A - EC 50 Al; B - EC 50 Al + DNP; C - EC 50 Al + cycloheximide.

there was a 3.6-fold increase in cellular nitrate content and a 4-fold increase in the in vivo nitrate reductase (NR) activity. Likewise, the soluble protein content in the root was also enhanced by 46%. The large increases in nitrate uptake, and subsequent increase in NR activity and protein content showed positive and significant correlation ($r = 0.92$; $P < 0.01$). In the presence of nitrate the uptake of Al was inhibited by 31% and the ratio of protein/cellular Al content was doubled, as compared to those which were not supplied with nitrate (Table 1). Mugwira and Patel (1977) concluded that uptake and assimilation of nitrate in wheat contributed to differences in Al tolerance. Though uptake of nitrate is an adaptive strategy to tolerate Al (Keltjens and van Ulden 1987), the results of the present study, however, do not support the suggestions of a

Table 1. Influence of NO_3^- on Al tolerance in onion root growth and the accumulation of Al in the root tissue. Data on root length are means ± SE (n = 5) and Al determinations are the means of three independent replicates. Values in parantheses indicate percent change over the treatment EC 50 Al alone.

Parameters	Treatment	
	EC 50 Al + 0 mM KNO_3	EC 50 Al + 10 mM KNO_3
Root length (mm)	52.2 ± 2.4	63.6 ± 3.6 (+22)
Cellular NO_3^- content (μmol g^{-1} FW)	0.5	1.8 (+260)
NR activity (μmol NO_2^- g^{-1} FW)	0.3	1.2 (+300)
NR/NO_3^- ratio (%)	60.2	66.3
Protein content (mg g^{-1} FW)	1.3	1.9 (+46)
Cellular Al content (μg g^{-1} DW)	937.5	650.5 (-31)
Protein/cellular Al content ratio	1.4	2.9

direct Al-induced reduction of NR activity in a plant (Gomes et al. 1985). Increase in nitrate absorption can also be a mechanism to enhance the rhizosphere pH (Taylor and Foy 1985), consequently minimizing Al toxicity.

Pretreatment with low concentrations of Al significantly increased Al tolerance on subsequent exposure to toxic concentrations of Al (Table 2). The Al preexposed onions registered 60% more root regrowth after exposure to EC 50 Al. Further, though Al pretreated onions accumulated 13% higher amounts of Al in their roots as compared to the non-pretreated ones, the injurious effects of Al on root regrowth in the former were remarkably lesser. Pretreatment with sublethal microquantities of Al before exposing to toxic amounts indicates an inducible mechanism of Al tolerance to exist in plants. This might involve the induction of synthesis of Al-binding proteins, which on later exposure to toxic levels bind with Al and exclude it from active metabolic processes. The role played by inducible proteins in the mechanism of Al tolerance has been elucidated earlier by Aniol (1984) who showed that Al immunization in plants is possible with sublethal quantities of Al. The total inhibition of root growth by

Table 2. Effects of Al pretreatment on Al tolerance in onion root growth. Onions were exposed to 0 and 5 μM of Al for three days and later subjected to toxic levels of Al (EC 50) for three days. Regrowth means growth after exposure to toxic levels of Al. Values in parantheses are indicate percent change over tap water treatment.

Treatment	Initial root growth (mm)	Root regrowth (mm)	Al content ($\mu g\ g^{-1}$ DW)
Tap water	37.6 ± 2.1	18.2 ± 1.6	802.5
Al (5 μM)	35.4 ± 1.8 (-6)	29.2 ± 3.1 (-60)	904.6 (+13)

cycloheximide recorded in the present study provides additional evidence for this mechanism. Though the molecular mechanisms operating behind the synthesis of similar proteins are not clear, it has been found that they are determined by multiple genes (Lafever and Campbell 1978) and control the existence of different degrees of Al tolerance in plants. More studies on genetically determined tolerant and sensitive plants are warranted to unravel further the biochemical mechanisms of Al tolerance in plants.

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