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Effects of Aluminium on Growth, Chlorophyll Content, ALAD Activity and Anatomy of Root and Shoot in Azuki Bean (*Vigna angularis*) Seedlings

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The toxic effects of aluminium (Al) on growth, chlorophyll content, δ-aminolevulinic acid dehydratase (ALAD) activity and anatomy of root and shoot were investigated in 7-day-old azuki bean (*Vigna angularis*) seedlings. Significant depressions in root elongation was observed in the low concentrations of Al (50, 100 μM) and increasing Al concentrations caused a sharp decline of root and shoot growth. The degree of inhibition was dependent upon Al supply. Exposure to 50 μM Al or more inhibited root elongation within 1 day. In the 50 μM Al treatments, a recovery of root growth was seen after 7 days exposure. In contrast, lateral root initials was little affected by Al exposure. Al toxicity symptoms and growth responses were more well developed in the roots than in the shoots. Analysis of Al localization in root cells by hematoxylin staining showed that Al entered root apices and accumulated in the epidermal and cortical cells immeadiately below the epidermis. There was a good positive correlation between the level of chlorophyll and ALAD activity. Increasing Al concentrations caused a decrease in total chlorophyll contents, accompanied by proportional changes in ALAD activity, suggesting a coordinated reduction of a photosynthetic machinery. Al exerted specific influence on the morphology of root and shoot. At higher concentrations of Al the roots induced drastic anatomical changes. The epidermal cells were disorganized or destructed while the cortical cells exhibited distortion of cell shape and/or disintegration. The diameter of root and transectional area of cortical cells decreased considerably with Al treatment. In the shoot Al also enhanced reduction of diameter of shoot and cell size. Gross anatomy of leaves treated with Al did not differ significantly from the controls, except for fewer and smaller chloroplast. Our results indicate that toxic effect of Al appear to be manifested primarily in roots and secondarily on shoots, and changes in root morphology are related to changes in the root growth patterns. Results are further discussed in relation to the findings in other plant species, and it is concluded that Al causes morphological, structural and, presumably, functional damage to the roots of the species investigated.

Key words : root growth, chlorophyll content, ALAD activity, morphology, *Vigna angularis*, aluminium

1. Introduction

Heavy metal contamination of agricultural land is a widely recognized problem and studies on the harmful effects caused by heavy metals on crop plants are receiving increased attention. Aluminium (Al) toxicity is believed to be the major factors limiting plant growth and crop productivity in acid soils (Fageria *et al.*, 1988; Roy *et al.*, 1989). Due to the relationship between increased soil acidification and increased potential for Al toxicity, much attention has been focused on assessing the effect of Al on a wide range of crop species. The presence of Al is an acid rhizosphere can strongly influence many processes that determine plant growth and can cause alterations in many physiological processes in root (Foy *et al.*, 1978; Taylor, 1991). The concentration of Al at which toxicity symptoms appear depends on the plant species, its age, and the experimental conditions

employed. The primary symptom of Al toxicity in higher plants is inhibition of root growth (Wright, 1989). Structural and functional damage in the root system affect nutrient and water uptake, leading to reduced growth and mineral deficiency in shoots and leaves (Foy, 1983; Schier, 1985). Root elongation is affected within hours of Al exposure, and, as in many plant species, the primary site of Al toxicity appears to be the root apex (Bennet and Breen, 1991; Delhaize *et al.*, 1993; Ryan *et al.*, 1993). Root apex accumulate large quantities of Al and exhibit greater signs of cellular damage than other parts of the roots (Rincon and Gonzales, 1992; Ryan *et al.*, 1993). Accumulation of Al in plant tissues might decrease in mitotic activity and cell elongation inducing inhibition of root growth (Setia and Bala, 1994; Nicole and Oliveira, 1995).

Al is known to affect directly membrane structure and permeability (Chen *et al.*, 1991) and alterations of the root-cell plasma membrane and membrane transport proteins (Taylor, 1988). Distruption of membrane transport processes can severely limit nutrient accumulation in long term. Al can also influence plant hormone synthesis (Massot *et al.*, 1994), cell membrane fluidity (Zel *et al.*, 1993), membrane composition (Lindberg and Griffiths, 1993), photosynthesis (Moustakas *et al.*, 1995) and respiration (Collier *et al.*, 1993; de Lima and Copeland, 1994). Photosynthetic processes are very sensitive to heavy metals such as cadmium, lead and copper and many data have been reported on chlorophyll content and activities of a number of enzymes essential for various photosyntheitc functions (Lidon and Hendriques, 1993; Stoyanova and Tschakalova, 1993). In contrast, scant information is available about the influence of Al on plastid development and photosynthesis.

Plants differ in their reaction to Al toxicity. Plant species and varieties vary greatly in Al tolerance and varieties of the same species often show different degrees of sensitivity to Al (Scott *et al.*, 1991, Rincon and Gonzales, 1992; Huang *et al.*, 1993). Azuki bean (*Vigna angularis*) is one of the most important crops and is relatively sensitive to heavy metals. However, little is known of the plants response to Al, so we have evaluated the response of azuki bean to the supply of the metal to the root and shoot system. In the work presented here we treated the intact roots and shoots of azuki bean seedlings with Al for long periods and then examined the growth responses, chlorophyll content, ALAD activity, and anatomical changes in root and shoot to clarify the mode of action of the direct effect of Al.

2. Materials and Methods

2.1 Plant material and growth conditions

Seeds of azuki bean (*Vigna angularis*) were surface sterilized for 20 min in 1% sodium hypochlorite solution, rinsed five times in sterile distilled water, and soaked in running tap water for 12 h. The seeds were germinated in the dark for 7 days at 25±1℃ on two layers of moistened filter papers. 7-day-old seedlings were placed in 10 cm glass Petri dishes lined with filter paper moistened with 10 ml of one of the different aluminium concentrations (0, 50, 100, 200 and 500 μM) from times ranging from 1 to 7 day. $AlK(SO_4)_2 \cdot 12H_2O$ was used as the source of aluminium (Al^{3+}). Control seeds received no Al. Seedlings were grown at 25±1℃ and 70% relative humidity in the growth chamber with a 16-h light/ 8-h dark regime. The solutions (pH 4.6) were renewed twice a day to prevent depletion.

2.2 Measurements of root and

shoot growth

Root and shoot growth measurements were determined every 24 h. Each seedling was placed under a dissecting microscope equipped with a calibrated eyepiece micrometer for the initial measurements. Otherwise, when the roots grew longer, measurements were conducted using a calibrated picture of the micrometer ruler. Lateral root initials at a distance of approximately 10 cm from root tip were also counted under a dissecting microscope. Twenty seedlings were used and three replicates carried out per test. Data represent the means and SE from three replicate roots.

2.3 Localization of Al in root cells

Roots exposed to Al solutions for various times were thoroughly washed in distilled water with several changes of water before being stained with hematoxylin. Roots were stained using modifications of the method described by Polle *et al.* (1978). The staining solution consisted of 2 g hematoxylin and 0.2 g $NaIO_3$ dissolved in a liter of distilled water (Gill *et al.*, 1974). The hematoxylin binds with Al ion found in the cells, giving a purple stain. Cross sections of the stained roots were made by free hand. The sections were mounted with 70 % glycerine and examined with light microscope.

2.4 Chlorophyll determination

To determine chlorophyll content the first leaves of the seedlings were collected. Each preweighed leaves were homogenized with a mortar and pestle in 80% (v/v) aceton and centrifuged at 10,000×g for 15 min at 0℃ to 4℃. The supernatant was brought up to 10 ml volume and the absorbance of the acetone extract was measured at 663 and 645 nm with UV-visible spectrophotometer (UV-260, Shimadzu).

2.5 δ-Aminolevulinic acid dehydratase(ALAD) assay

The first leaves were homogenized with a prechilled mortar and pestle in 5 ml of 50 mM Tris-HCl buffer (pH 8.2) containing 0.1 mM dithiothreitol(Naito *et al.*, 1980). The homogenate was centrifuged at 27,000×g for 20 min at 0℃. The supernatant was assayed for enzyme activity.

For the determination of ALAD activity, 1 ml of extract was incubated with 0.3 ml of 1 $mg \cdot ml^{-1}$ ALA, 1.3 ml of 50 mM Tris-HCl buffer (pH 8.2) containing 0.1 mM dithiothreitol and 0.8 ml of 0.2 mM $MgCl_2$ for 2.5 hr at 37℃ (Naito *et al.*, 1980). The enzymatic reaction was stopped with 0.3 ml of 3 M trichloroacetic acid containing 0.1 M $MgCl_2$ (Schneider, 1970). Samples were then centrifuged at 400×g for 10 min. PBG formed in the supernatant was measured according to Mauzerall and Granick (1956) : 1 ml of supernatant was mixed with an equal volume of a modified Ehrlich's reagent. The absorbance at 555 nm was determined after 15 min and the concentration of PBG was calculated using an extinction coefficient of 61 $mM^{-1} \cdot cm^{-1}$. ALAD activity was expressed in mmole PBG formed per h and per leaves.

2.6 Light microscopy

The root, shoot and leaf segments of control and Al-treated plants were harvested after exposure and processed for light microscopy. The samples were fixed in FAA, dehydrated in graded ethanol series and embedded in paraffin according to techniques described by Berlyn and Miksche (1926). The embedded specimens were sectioned at 10 μm on a rotary microtome. Sections were stained with a combination con-

sisting of hematoxylin, safranin and light green. From the serial sections anatomical structure of the root, shoot and leaf were examined.

3. Results

3.1 Effects of Al on growth responses

The toxic effect of Al on azuki bean seedlings were evident both morphologically and physiologically. Measurements of main root growth made in a solution containing a range of Al concentrations indicated that root growth rate was correlated with external Al concentration. Expose to 50 μM Al or more inhibited root elongation within 24 h (Fig. 1). The degree of inhibition was dependent upon the Al supply. Significant depressions in root elongation was observed in the low concentration of Al, however, after 7 days treatment a recovery of growth was seen in the 50 μM treatment. Exposure to 200 or 500 μM Al almost completely inhibited root elongation, and no recovery of growth was seen over the duration of treatment period. In Al concentrations of 200 and 500 μM, the root growth dropped by approximately 58% and 66%, respectively, after 7 days of treatment. There was a decrease in the percentage of surviving seedlings in the 500 μM treatment. In contrast, no necrotic phenomena were observed at the lower concentrations.

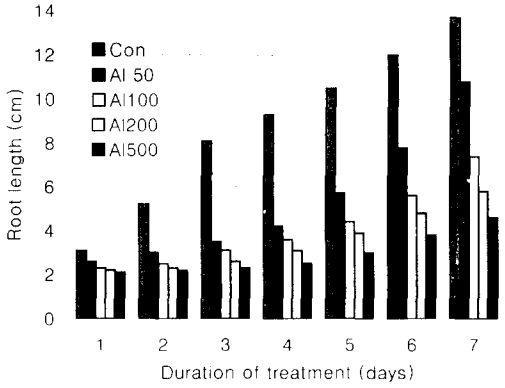

Fig. 1. Time course of root elongation of azuki bean seedlings exposed to 0, 50, 100, 200 or 500 μM Al concentrations. Values represent the average of three replicate tests.

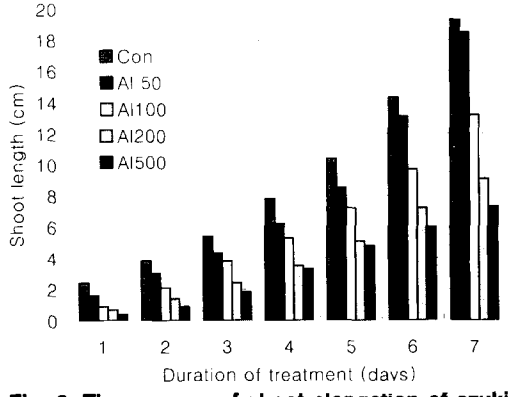

Fig. 2. Time course of shoot elongation of azuki bean seedlings exposed to 0, 50, 100, 200 or 500 μM Al concentrations. Values represent the average of three replicate tests.

The effect of Al on shoot growth is shown in Fig. 2. In the exposure to relatively low Al concentration (50 μM) for 7 days, little drastic decline of shoot growth was observed over the duration of the treatment period. However, significant depressions in shoot length was observed from 200 to 500 μM Al treatment. The highest Al level (500 μM) caused significant inhibition of shoot elongation by 60% of the control.

3.2 Effects of Al on lateral root initiation

The initiation of lateral roots by the approximately 10 cm of root tissue, which grew after the initiation of Al treatment, was not significantly affected by Al exposure. Al treatments that affected main root elongation also influenced lateral root growth and development (Table 1). The lateral root initials developed into very short laterals increased, but not significantly, by 35%, at lower concentrations of Al after 7-day treatment period. The initiation of lateral roots, however, was not increased at concentrations as high as 200 μM Al. A progressive decrease of lateral root initials with increasing Al supply was observed at

Table 1. The effect of Al on the initiation of lateral roots at a distance of approximately 10 cm from root tip in azuki bean seedlings. Values are means ±SD (n=3).

Treatment	Number of lateral root initials
Control	14 ± 2.17
50 μM Al	19 ± 1.41
100 μM Al	15 ± 3.56
200 μM Al	14 ± 2.35
500 μM Al	10 ± 1.63

Table 2. Chlorophyll content in leaves of azuki bean seedlings exposed to different concentrations of Al for 7 days. Values are means ±SD (n=3).

Treatment	Total Chlorophyll (mg/g fr. wt.)
Control	12.03 ± 0.75
50 μM Al	11.24 ± 0.35
100 μM Al	6.29 ± 0.25
200 μM Al	4.74 ± 0.45
500 μM Al	3.67 ± 0.27

higher Al concentrations.

3.3 Effects of Al on chlorophyll determination

Segments prepared from the primary leaves of 7-day-old seedlings subjected to various concentrations of Al. After 7-day exposure to Al the chlorophyll in azuki bean leaves was determined. A progressive reduction of chlorophyll content in leaves was observed with increasing Al supply (Table 2). A sharp decline of chlorophyll content was observed from 100 to 500 μM Al treatment, a reduction equivalent from 48 to 71% of the control.

3.4 Effects of Al on ALAD activity in leaves

Since Al has been shown to lower the amount of chlorophyll it was considered worthwhile to measure the effect of Al on the ALAD activity in leaves. The activity of ALAD which is necessary enzyme for chlorophyll synthesis and photosynthesis and locates in proplastids or chloroplasts declined with increasing Al concentraion (Table 3). ALAD activity was most affected by Al, and the activity consistently declined with increasing Al concentration up to 500 μM. Al concentrations, even the lowest one, resulted in a large decline in ALAD activity in leaf tissues. The highest ALAD activity reduction, 95%, occurred in seedlings exposed to 500 μM Al for 7 days. There was similar pattern of changes in chlorophyll content and ALAD activity. At higher Al, however, the inhibition of ALAD activity was much more severe than that of chlorophyll content.

Table 3. ALAD activity in leaves of azuki bean seedlings exposed to different concentrations of Al for 7 days. Values are means ±SD (n=3).

Treatment	ALAD activity (mmole PBG · h^{-1} · $leaves^{-1}$)
Control	2.83 ± 0.07
50 μM Al	1.67 ± 0.13
100 μM Al	0.32 ± 0.14
200 μM Al	0.22 ± 0.18
500 μM Al	0.15 ± 0.04

3.5 Localization of Al in the root tissues

The distribution of Al in the root cells is indicated by the appearance of purple stain (Fig. 3). Seedlings exposed to 100 μM Al for 72 h and stained with hematoxylin at the root apices. The staining intensify increased as time of Al exposure progressed, and this was accompanied by a gradual accumulation of Al in root apices.

Al was mainly localized on the epidermal and cortical cells and hardly in the vascular system. Al deposition was observed in the cell walls of epidermal and cortical cells. Abnormal cortical cells were observed in the high Al concentrations. The

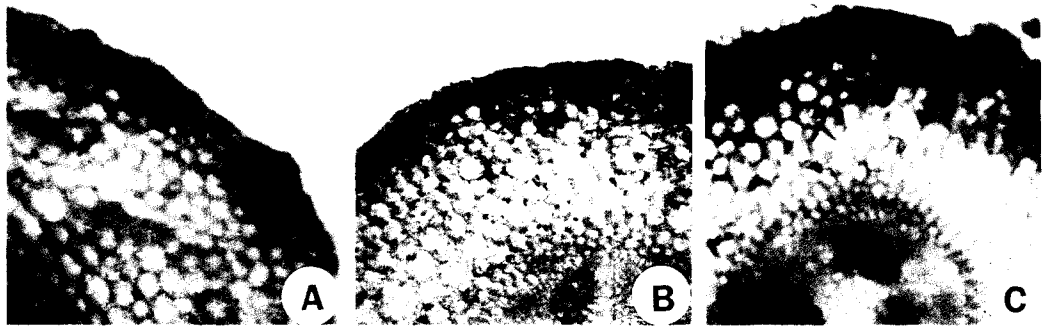

Fig. 3. Distribution of Al in the root cells of azuki bean seedlings exposed to 100 μM Al for 72 h with hematoxylin staining. ×40.

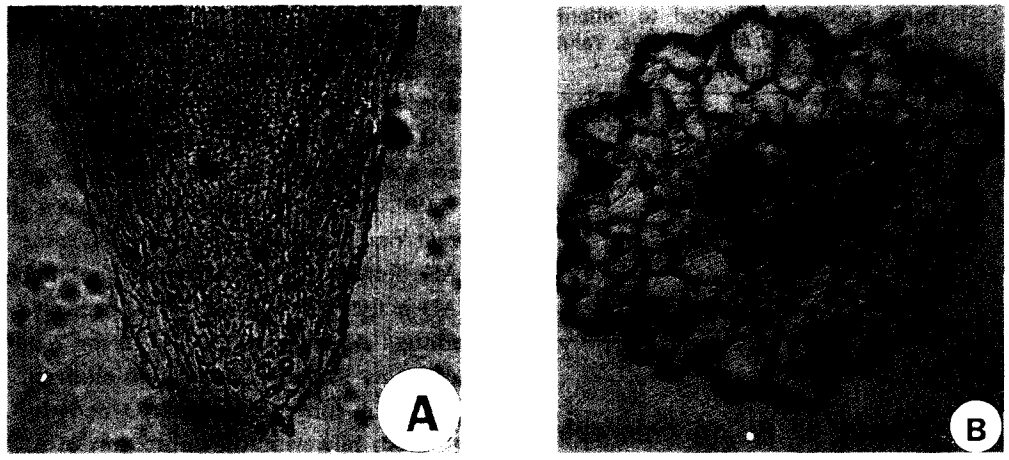

Fig. 4. Light micrographs of longitudinal sections of root tips (A) and transverse sections of root (B) of azuki bean seedlings exposed to 500 μM Al for 72 h. ×40.

appearance of the abnormal cells coincided with marked root length reduction.

3.6 Anatomical changes in root and shoot in response to Al

Because macroscopic differences were more pronounced between control plants and Al-stressed plants, much attention was placed on comparing the morphology of the two extremes. Roots growing in the presence of Al, displayed a number of morphological and structural malformations. Al-induced root tips were shoot, thick and brownish.

The toxicity on root morphology became progressively intensified with increased exposure to Al. Thus more attention was placed on the 7 days exposure. Transverse sections cut at different distances from the root apex and median longitudinal sections of the roots were investigated by light microscopy. Although no statistical analysis was carried out, the root cap appeared to be decreased in size and the meristematic region occupied a shorter area than the control (Fig. 4). The diameter of root and transectional area of cortical cells decreased considerably with Al treatment. The epidermal and the outer cells of the cortex in the meristematic and elongating regions of the roots appeared detached, distorted or even collapsed. The increased con-

centrations of Al induced serious damage to the cells of root epidermis.

In the roots of 500 μM Al grown plants, the epidermal cells disorganized (Fig. 4). In cortex most of the cells appear irregularly shaped and show disintegration. Some darkly stained particles are seen on the root surface and also in the disorganized cortical region. In the shoot grown in 500 μM Al, the epidermal cells are more thick walled as compared to the control (Fig. 5). The cells in the ground tissue show signs of disintegration and are separated from one another. Other gross anatomical malformations were not detected except for reduction of diameter and cell size.

Light micrograph sections through leaves treated with 500 μM Al for 7 days are shown in Fig. 5. Leaf tissues revealed little anatomical differences among the samples examined. Leaf thickness and cell arrangement appeared unaffected by Al. The only apparent differences in light micrographs between the control and 500 μM Al treatment were the number and size of mesophyll chloroplasts.

4. Discussion

Root length and elongation rate are essential for plants exploring for water and nutrients and for the establishment of seedlings. A decrease in root elongation due to heavy metals have been reported for many plant species. Although the root elongation test for assessing sensitivity to metals was originally developed for grasses (Wilkins, 1978), it has been applied to plants of diverse root morphology to reveal that one of the most rapid responses to toxic metal level is the inhibition of root growth (Eleftheriou and Karataglis, 1989; McQuattie and Schier, 1990). Our results revealed that roots were the main site of Al injury in azuki bean seedling and are in good agreement with this conclusion.

The results presented show that a supply of 50 μM Al or more drastically depressed the root growth of azuki bean seedlings estimated as the daily elongation rate (Fig.1). The results are consistent with the findings (Nichol and Oliveira, 1995) that root growth inhibition was only apparent after 24 h. In contrast, inhibition of root growth was detected by Kochan and Staff (1991) within 2-3 h after exposure to Al in an Al-sensitive cultivar of wheat. Periods as short as 1 h exposure to Al were reported to inhibit root growth in several other Al-treated plants (Ownby and Popham, 1989; Horst *et al.*, 1991; Ryan *et al*, 1992; Van *et al.*, 1994).

The lower sensitivity of seedilings grown in Al-solutions may be due, in part, to the ability of seedlings to rely on reserve materials stored in the seed to support early growth. This would minimize the requirement of nutrients from external sources, making Al-interference with transport mechanisms and other plasma membrane associated processes less critical at this stage of the germination process (Nichol *et al.*, 1993)

In azuki bean seedlings the recovery of root elongation after the initial Al shock is similar to that in *Vigna unguiculata* (Horst *et al.*, 1983). After the initial shock of the Al exposure almost all the seedlings were able to tolerate an Al supply of up to 50 μM.

High Al supply induced drastic decline in taproot elongation affected lateral root initials. Exposure to higher concentrations of Al showed a smaller reduction in the initiation of lateral roots (Table 1). A more compact and branched root system has often been observed in heavy metal treated plants considering a typical consequence of damage to the root tip (Arduini *et al.*, 1994).

In the present study, root growth of azuki bean was more inhibited than shoots. Our results are in agreement with recent re-

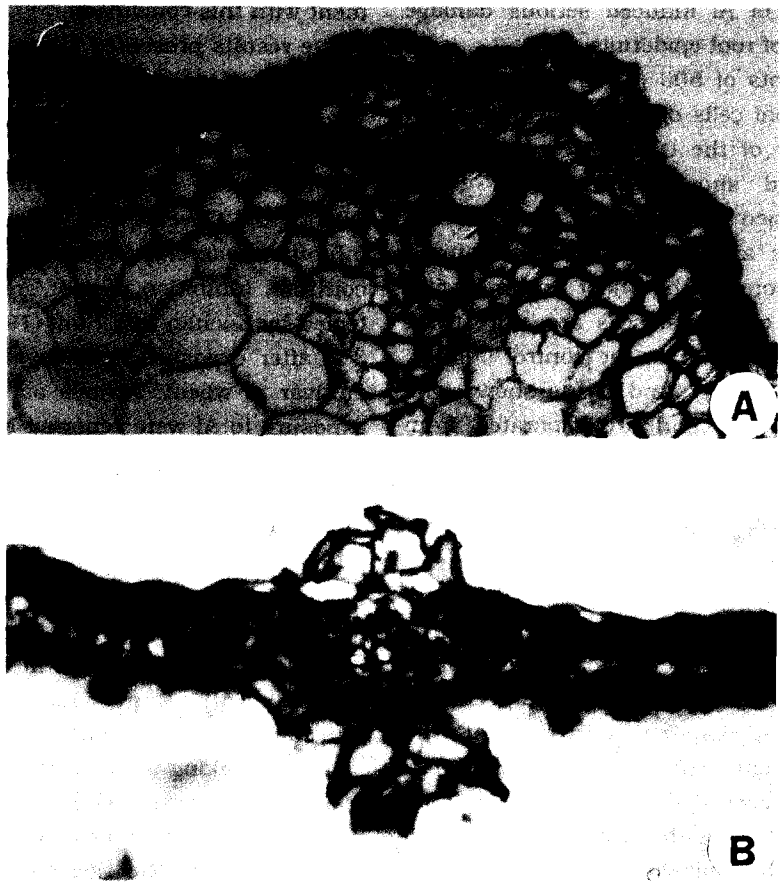

Fig. 5. Light micrographs of transverse sections of shoot (A) and leaf (B) of azuki bean seedlings exposed to 500 μM Al for 72 h. $\times$100.

ports which indicates that metal accumulate more in the roots than in the shoot-leaves and Al toxicity symptoms were more developed in the roots than in the shoots (McQuattie and Schier, 1990). This is accord with studies on the uptake of metals from culture solutions and their translocation from roots to shoots, which typically indicate that metals accumulate more in the roots than in the shoots. Uptake of Al ions can be carried out via several mechanism. Since the entry of Al into cytoplasm has toxic effects on membrane structure and function, supply of Al could have caused membrane failure, resulting in a loss of ion uptake activity and membrane leakness (Taylor, 1988; Chen *et al.*, 1991; Zel *et al.*, 1993; Lindberg and Griffiths, 1993). It is suggested that the reduced shoot growth of Al-treated wheat is due to a retarded Ca transport from roots to shoots (Strid, 1996). The impairment of shoot growth in Al-treated plants is apparently not a direct effect of Al within shoots since no significant amounts of Al could be detected in the shoots. Al supply not only influenced root growth patterns but also affected root morphology and architecture. As indicated by Foy *et al.* (1978) and Marschner (1991), one of the primary sites of Al toxicity is the meristem. Evidence obtained in the present study in-

dicates that the most serious effects of Al toxicity occurs in the peripheral cells of the root and the root cap. These findings are in accordance with other investigations regarding the primary site of Al injury (Wagatsuma *et al.*, 1987; McQuattie and Schier, 1990; Hodson and Wilkins, 1991; Eleftberios *et al.*, 1993). The root apex is known to accumulate large quantities of Al and to exhibit greater signs of cellular damage than other parts of the root (Rincon and Gonzales, 1992). Ryan *et al.* (1993) demonstrated that Al must be applied to the root cap and meristem for root growth to be inhibited. When the roots growing in the presence of Al were stained with hematoxylin, differential staining preceded differences observed in root elongation (Fig. 3). The difference became more marked with time. Analysis of Al localization showed that Al in the root cells were mainly localized on the epidermal and cortical cells. The appearance of the abnormal cells in cortical cells coincided with marked root length reduction. The distribution of Al agreed with the previous work showing that Al accumulates rapidly in the outer cells of the root cap and epidermis (Matsumoto *et al.*, 1977; Bennet *et al.*, 1985). Al deposition in cell walls of epidermal and cortical cells in roots was observed in rice (Coronel *et al.*, 1990). The primary effect of Al is established - it inhibits root elongation by restricting cell division and elongation (Roy *et al.*, 1989; Setia and Bala, 1994). The amount of Al absorbed by the cells is a determing factor in the inhibition of growth by Al (Yamamoto *et al.*, 1994). The distruption of both cell division in the meristematic region and cell expansion in the elongation zone of the root may be one factor attributing to the retardation to normal root growth (Nichol and Oliveira, 1995). Al rapidly reduced squash root growth by inhibiting cell elongation and altering metabolism of cell wall polysaccharides in the nonelongating zone as well as in the elongation zone (Van *et al.*, 1994)

Symptoms characteristic of Al toxicity such as swelling and damage of the root cells and disintegration of the epidermal and outer cortical cells have been observed in azuki bean (Fig. 4). These results were observed in other plant species including wheat (Henning, 1975), barley (Hecht-Bichholz and Foy, 1981), Norway spruce (Hecht-Buchholz *et al.*, 1987) and soybean (Hecht-Buchholz *et al.*, 1990).

The results described in this paper indicate that Al induce differential anatomical changes in the root and shoot of azuki bean. The alterations in the shoot structure could be related with the structural changes via-a-vis disfunctioning of the root system following Al treatments. Insufficient supply of essential nutrients and hormones from the roots adversely influences the differentiation of tissues in shoot (Davies, 1991).

The observed growth reductions are probably due to a number of different effects of Al on plant processes. While perturbations in root structure and function appear to be involved, our results show that leaf function is also affected. A severe reduction in chlorophyll content with increasing Al concentrations was observed (Table 2). The result show that Al is a potent inhibitor in the biosynthesis of chlorophyll (Ohki, 1986). Toxic metal concentrations may reduce chlorophyll content or inhibit its biosynthesis (Onzounidou *et al.*, 1992), thus seriously affecting photosynthesis (Clijsters and Van Assche, 1985). This would be particularly pronounced during seedling development and in the growth of new leaves where active chlorophyll production occurs. The inhibition in chlorophyll biosynthesis, therefore, represent a primary event in the

toxicity of Al to plant growth.

A progessive decrease in ALAD activity with increasing Al supply was observed at all levels tested (Table 3), suggesting that Al interfere with the synthesis of structural proteins of the chloroplast thylakoids. ALAD activity on a protein level is closely correlated with the chlorophyll level in etiolated cotyledons exposed to light (Steer and Gibbs, 1969).

There was parallel progressive reduction of ALAD activity and chlorophyll content in Al-stressed leaves. These observations suggest that there is a regulatory system for ALAD activity which may participate in regulating chlorophyll synthesis and supprot the hypothesis of a coordinated reduction of a photosynthetic machinery (Moustakas *et al.*, 1994). The constant relationship between ALAD activity and chlorophyll content also suggest a common intracellular site and common control over development for 2 systems.

Leaf tissues of azuki bean showed no significant anatomical differences between the control and Al-treated plants (Fig. 5). Similarities in leaf anatomy among the samples indicate that excess Al does not affect such gross structural parameters, even with 7 days exposure. Similar results were also obtained with Cu-treated plants (Ouzounidou *et al.*, 1992).

Further physiological and ultrastructural study of Al-tolerant and Al-sensitive plants may lead a better understanding of how plants respond to the presence of potentially toxic concentrations of Al in the medium or environment.

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Aluminium이 팥(*Vigna angularis*) 유식물의 생장, 엽록소함량, ALAD활성 및 뿌리와 경엽부의 형태에 미치는 영향

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발아후 7일된 팥유식물에서 aluminium(Al)이 생장, 엽록소 함량, ALAD 활성 및 뿌리와 경엽부의 형태에 미치는 영향을 조사하였다. 저농도(50, 100 μM)의 Al처리에 의해 뿌리와 경엽부의 신장이 매우 감소되었으며 농도가 증가함에 따라 생장이 더욱 억제되었다. 따라서 생장억제는 농도의존적이었다. 뿌리신장은 Al 처리 24시간에서 감소되었으며 7일간의 저농도처리에 의해 억제효과가 회복되는 경향을 보여주었다. Al의 독성증상과 생장반응은 경엽부에 비해 뿌리에서 더 크게 나타났다. Hematoxylin 염색법에 의해 Al 분포를 조사한 결과 Al은 근단을 통해 표피와 피층세포에 축적되어 있음을 알 수 있었다. 한편 Al처리는 엽록소함량을 감소시켰으며, ALAD 활성 또한 억제시켰다. 엽록소 함량과 ALAD 활성간에는 양의 상관관계가 나타났다. Al 처리에 의한 뿌리의 형태변화를 보면 표피세포 및 피층세포의 변형 또는 파괴가 관찰되었으며, 뿌리직경과 피층의 체적도 매우 감소되었다. 경엽부에서도 Al 처리는 직경과 세포크기의 감소를 보여주었다. 그러나 잎에서의 형태적 변화는 엽록체수와 크기변화 이외에는 거의 관찰되지 않았다. 이와 같은 결과에서 Al의 독성효과는 1차적으로 뿌리에서 나타나며, 뿌리형태의 변화는 뿌리의 생장패턴과 관련이 있음을 알 수 있었다. 따라서 Al은 팥유식물에서 특히 뿌리의 형태와 기능적 손상을 일으키는데 큰 영향을 미치는 것으로 생각된다.