Asian Journal of Plant Sciences

ISSN 1682-3974

science alert

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Asian Journal of Plant Sciences 6 (8): 1246-1250, 2007
ISSN 1682-3974

Aluminium Stress Induced Alteration in Seedling Growth and Alleviation in Protein and Amino Acid Contents of *Lens culinaris*

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Abstract: The present study was conducted to investigate the growth, synthesis of amino acids and proteins of *Lens culinaris* under aluminium (Al) stress. Results showed that nutrients disorder were observed with the loss of apical dominance and both lateral and primary roots were stunted and swollen with increasing concentration of Al. All levels of Al reduced the size and weight of roots, shoots and leaves. Al altered both root and leaf architecture. At low concentration of Al, size of leaf was approximately equal to that of control plant whereas at high dose, reduced leaf expansion were observed. Investigations suggest that a number of physiological and biochemical processes in the plant cell have been affected before growth inhibition occurs. It was concluded that *Lens culinaris* is an aluminium sensitive species.

Key words: Aluminium, root, leaf architecture, protein, amino acids

INTRODUCTION

Aluminium (Al) bioavailability has raised much interest in the last two decades because Al is the most abundant metal and third most common element in the earth 's crust. Development of acid soils that limits crop production is an increasing problem worldwide. Because approximately 50% of the world's potentially arable soil are acidic (Mistrik *et al*., 2000). Acid soils are such an important constraint to agriculture, under mechanism. The target of Al toxicity is the root tips, in which Al exposure causes inhibition of cell elongation and cell division, leading to root stunting accompained by reduced water and nutrient up take (Petra and Proctor, 2000). Morphological stress was also noticed on depression in root and shoots system, chlorosis of leaves etc., stunted growth and reduced yield were other symptoms (Oleksyn *et al*., 1996). Pan *et al*. (1989) suggests that one mode of action by which Al may affect shoot growth is by inhibiting the synthesis and subsequent translocation of cytokinin to the meristematic regions of the shoot. However, the inability of applied cytokinin to counter the restriction imposed by Al on total shoot dry matter production implies the impairment by Al toxicity of other root functions, such as ion and water transport, also played an important role in altering shoot morphology (William *et al*., 1989). Neogy *et al*. (2002) reported different concentrations of $Al_2(SO_4)_3$ were found to have significant effect both on shoot and root length. Leaf area, fresh and dry weight was significantly reduced. Shamsi *et al*. (2007) found that low pH (4.0) and Al treatments caused marked reduction in root length, shoot height, dry weight, chlorophyll content, (SPAD value) and photosynthetic rate. Karina *et al*. (2006) reported no significant changes in the ammonium and protein contents in the nodules or root when the plant were treated with 50 μM but at higher concentration like 500 μM. Al-treated seedlings show identical or increased protein level compared to the control (Pietraszewska *et al*., 2001). In the nitrate added samples the effects of Al/P were less characteristic. The total amount of secreted amino acids has a specific correlation with the external Al^{3+} concentration. At first, the amino acids secrete normally, but when Al^{3+} concentration is over 10 mg L^{-1}, the amino acid constitution varies obviously (Wang *et al*., 2006). Ismail (2005) reported that Al significantly decrease in root length and dry matter yield in the shoots and roots of carrot (*Daucus carota* L.) and radish (*Raphanus sativus* L.) plants which leads to decrease in sugars and total amino acids, whereas a significant increase in the proline content of the shoots and roots was detected.

The present study focuses on an integrated assessment of Al toxicity on the weight, length, morphological and physiological changes in the *Lens culinaris*.

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MATERIALS AND METHODS

Ten to fifteen seeds of *Lens culinaris* were surface sterilized with 0.1% mercuric chloride and germinated at natural environment in petri dishes in the darkness containing Whatman No. 1 filter paper moistened with Hoagland nutrient solution. After 48 h of germination, seeds were transferred to pots containing Hoagland nutrient solution. Aluminium was given in form of aluminium chloride ($AlCl_3$) at increasing concentration (20, 30, 60, 90, 100 and 150 mg L^{-1}). Experiment was conducted during winter in January 2007 in the Department of Chemistry, Jinnah University for Women.

Five uniform plants were selected and dissected in roots and shoots. Root length was recorded individually for each plant by measuring total length of all the roots from root base to maximum of a root and shoot length were measured using standard centimeter scale an interval of 48 h. The plant material was dried at 60°C to achieve a constant weight. Ratio of root length to weight was calculated for each plant and averaged for pot and replications of a treatment (Akmal *et al*., 2005).

Protein contents in root and shoot extract in water, were treated with Folin cicalteau phenol reagent (half diluted). The extract was left for 30 minutes at room temperature. A blue colored complex was developed. Absorbance of the complex was observed at 650 nm on Schimadzo 160 A UV- Visible spectrophotometer. Amino acids were determined by treating the extract of root and shoot with ninhydrin solution in 10% ethanol and heated to 50 to 70°C for few minutes till purple color appeared. Optical density was recorded at 566 nm.

RESULTS AND DISCUSSION

The toxicity of Al depends upon a number of factor, including, pH and organic matter content. Results showed that (Al) treatment caused a significant decrease in root and shoot length and weight (Fig. 1) (Loboda and Wolejko, 2006). Compared to shoot growth, an adverse effect of the increasing metal concentration to the plant root has already been observed (Ayala-Silva and Al-Hamdani, 1997).

Statistically significant changes in the root biomass, shoot biomass and total plant mass were observed. Root biomass after 2 weeks was reduced by 11% compared with plants grown without Al (Fig. 2-3).

Root W = 7×10^{-5} Al +0.0252 (R^2 = 0.0166)
Shoot W = -7×10^{-5} Al+ 0.0294 (R^2 = 0.6132)
Total Plant Dry Mass (TPDM) = -7×10^{-6} Al+0.556 (R^2 = 0.0002)

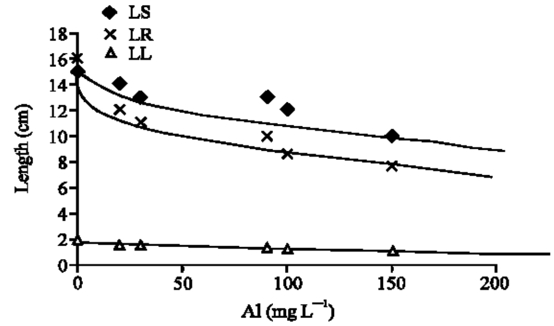

Fig. 1: Length of shoot, root and length of *Lens culinaris* under Al stress, LS = Length of Shoot, LR = Length of Root, LL = Length of Leaf

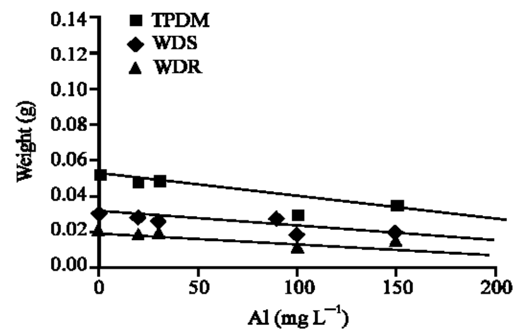

Fig. 2: Total plant dry mass, weight of dry shoot and weight of dry root of *Lens culinaris*, TPDM = Total Plant Dry Mass, WDS = Weight of Dry Shoot, WDR = Weight of Dry Root

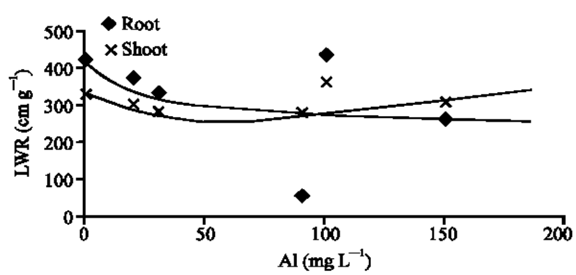

Fig. 3: Length weight ratio of root and shoot of *Lens culinaris* under Al stress

Leaf Weight Ratio (LWR) for all dose showed a reduction with plant age (Akmal *et al*., 2005). Moreover, a drastic reduction in the root density was recorded with stunted, compact and swollen structure (Ayala-Silva and Al-Hamdani; 1997; Goransson and Eldhuset, 1991; Clark, 1977). Root length to weight ratio of *Lens culinaris* treated with different concentration of Al showed reduction. LWR is the ratio of dry mass and average maximum length of all roots and shoots of a plant (Fig. 3).

LWR = −1.0359 Al+385.21 (R^2 = 0.179, root)
LWR = 0.0512 Al+305.36 (R^2 = 0.0076, shoot)

The values correspond to R^2 indicates that aluminum toxicity is the primary factor limiting crop productivity in acidic soils, which comprise large areas of the worlds' land. The reduction in root and shoot elongation with an increasing concentration of Al has also been observed for many other crops, as the first sign of Al toxicity appears in the root system which becomes stubby as a results of inhibition of elongation of root main axis (Pan *et al*., 1989).

Aluminum (Al) is widely known to inhibit root growth, however it also affects many physiological processes like synthesis of amino acids and protein (Clark, 1977). The concentration of these metabolites increased in roots and shoots of the seedlings in the nutrient solution with added Al. The absorption spectrum of total amino acids and protein contents were shown in Fig. 4-7 and results were reported in Table 1 where a uniform increase in total protein contents was observed with the increase concentration of Al as compared to control plant. These results suggest that soluble protein contents in plant are an important indicator of physiological state as reported earlier that time course experiments for *Arabidopsis* plants, respond to aluminum toxicity by altering their protein expression and also Al-treated seedlings show identical or increased protein level compared to the control (Pietraszewska, 2001).

The total amino acids contents found increased in roots and shoots with plant exposure to Al. The concentration of these metabolites indicates that *Lens culinaris* accumulated more metabolites under Al stress than in control.

A board range absorption spectrum of amino acid were observed as compared to non treated plant (Fig. 6 and 7) which may be due to affinity of amino acids towards Al. The rhizotoxic Al^{+3} ions, prevailing at lower pH often enhanced growth at low concentration under acidic conditions that reduced root elongation whereas with regarding protein and amino acids in Al treated plants, it may be related to the different changes in cytoplasmic and membrane proteins which is an agreement with the reported data of different plants (Walter *et al*., 1999; William *et al*., 1989; Hu *et al*., 1995).

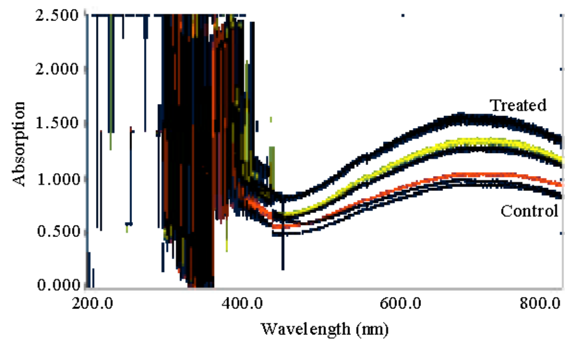

Fig. 4: Absorption spectrum of proteins of shoot of *Lens culinaris* under Al stress

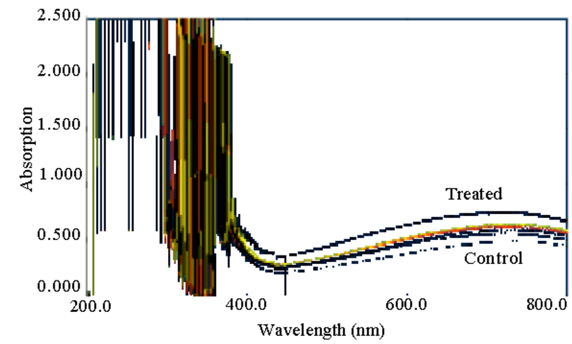

Fig. 5: Absorption spectrum of proteins of root of *Lens culinaris* under Al stress

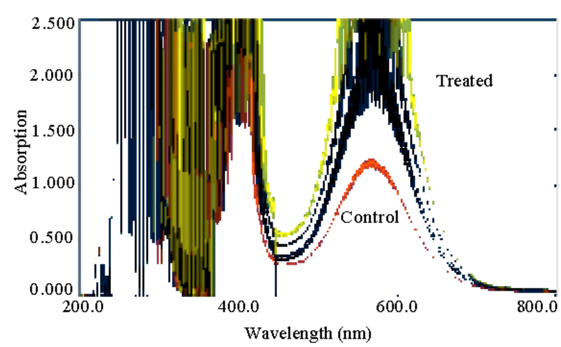

Fig. 6: Absorption spectrum of amino acids of root of *Lens culinaris* under Al stress

Mechanism: The exact mechanism of root growth inhibition is uncertain but two main mechanisms to tolerate high soil Al-including the soil solution Al and inactivating absorbed Al. Recent observation of a plant disorder in *Lens culinaris* on acid soils, was associated with low leaf that plant communities on very acid soils tend to be slow growing and relatively unproductive, even if they do tolerate the conditions. Current evidence indicates the tolerance mechanisms have a cost to the plant. This cost can eventually be expected to show up as reduced yield potentials. In the second tolerance mechanism the plant inactivates the

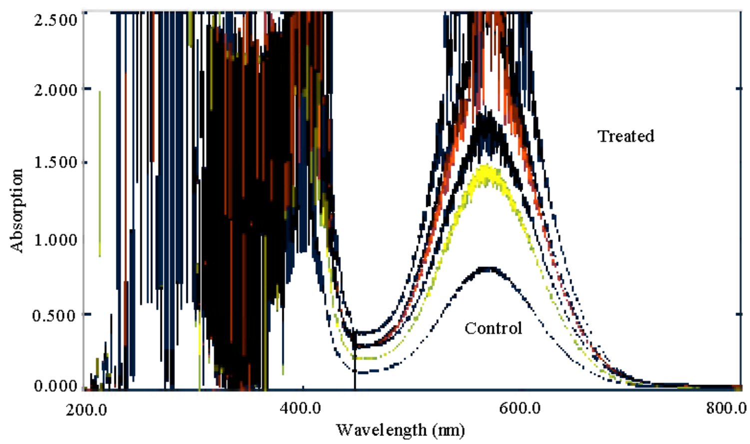

Fig. 7: Absorption spectrum of amino acids of shoots of *Lens culinaris* under Al stress

Table 1: Some morphological and physiological parameters of *Lens culinaris* under Al stress

Al (mgL^{-1})	Germination (%)	Average root length (cm)	Average shoot length (cm)	Amino acid (%) Shoots 10^5	Amino acid (%) Roots 10^6	Protein (%) Shoots	Protein (%) Root
0	90.0±12.0	8.00±2.2	10.0±1.00	6.90±1.2	1.68±0.3	1.45±0.1	1.68±0.2
20	75.0±10.3	7.00±1.9	8.50±0.60	5.08±1.0	2.76±0.2	1.60±0.2	2.76±0.1
30	67.5±5.20	6.50±1.0	8.00±0.80	4.31±0.9	2.36±0.1	1.80±0.1	2.36±0.1
90	62.5±5.00	5.00±0.9	7.75±0.81	4.23±0.6	2.11±0.3	2.02±0.1	2.11±0.2
100	55.0±3.00	4.50±0.6	7.00±0.40	6.73±0.6	2.32±0.2	2.15±0.3	2.32±0.2
150	30.0±2.00	3.75±0.1	5.95±0.50	6.70±0.5	2.16±0.2	2.52±0.2	2.16±0.3

absorbed Al, by forming organic complexes with the damaging aluminum ions. It is worth to note that both the tolerance mechanisms seem to involve compromises. A different explaination for the stimulation of plant growth by low Al dose may be based on speciation effects related to the complex solution chemistry of Al that may influence the bio availability of other ions in a way that stimulate plant growth. Decrease in plant growth may be the results of alleviating of proton (H^+) toxicity by Al^{+3}. Absorption spectrum of protein and amino acids offered an in increase concentration of protein and amino acid contents in the presence of Al, which may be due to the affinity of protein and amino acids towards Al. Al tends to bind to the phosphate or carboxyl groups rather than to SH groups characteristic for chelatins (Gunse *et al*., 1997; Khan *et al*., 2000). However, Snowden *et al*. (1995) and Wu *et al*. (2000) suggested that plant metallo-thionein-like protein and phytochelatins may play a role in Al tolerance this may be attributed with the increase in concentration of proteins in leguminous plant under investigation. An Al-induced polypeptide (TA1-18) was identified in wheat that shows homology to a pathogenesis associated (PR) protein (Cruz-Ortega and Ownby, 1993) due to strong interaction of Al^{+3}, the main Al toxic form, with oxygen donor ligands (proteins, nucleic acids, polysaccharides) results in the inhibition of cell division, cell extension and transport of water. The response of the studied material at low pH (4.5) and different Al concentration may be in accordance with Kinraide (1993, 1997) hypothesis visualized Al^{+3} and H^+ competing for common apoplast binding sites. Slaski (1989) reported an increase in protein NAD-kinase activity in root apical meristems of various crops under Al stress while decreased in some most sensitive plants.

CONCLUSIONS

The above investigation revealed that Al inhibits plant growth by interfering with many physiological processes prevailing at low pH, would be capable of alleviating proton toxicity results in the increase in protein and amino acids content of *Lens culinaris.*

REFERENCES

Akmal, M., M. Asif and G.D. Khan, 2005. Root length to weight ratio and water use efficiency of perennial rye grass in different water and nitrogen supplies. The Nucleus, 43: 207-211.

Ayala-Silva, T. and S. Al-Hamdani, 1997. Interactive effects of Polylactic acid with different Al concentrations on growth, pigment concentrations and carbohydrate accumulation of Azolla. Am. Fern. J., 87: 120-126.

Clark, R.B., 1977. Effect of Al on growth and mineral elements of Al-tolerant and Al-intolerant corn. J. Plant Sci., 47: 653-662.

Cruz-Ortega, R. and J.D. Ownby, 1993. A protein similar to (PR pathogenesis related) a protein is elicited by metal toxicity in wheat roots. Physiol. Plant., 89: 211-219.

Gunse, B., C. Poschenrieder and J. Barcelo, 1997. Water transport properties of roots and root cortical cells in proton and Al-stressed maize varieties. Plant Physiol., 113: 595-602.

Goransson, A. and T.D. Eldhuset, 1991. Effects of aluminium on growth and nutrients uptake of small *Picea abies* and *Pinus sylvestris*. Plants J. Trees Str. Fun., 5: 136-142.

Hu, H., H.Q. Li and X.H. Wuhan, 1995. The effect of different concentration aluminum on the secretion of amino acids and carbohydrates by the wheat root system. Turang Tongbao, 26: 15-17.

Ismail, M., 2005. Aluminium-phosphorus interactions on growth and some physiological traits of carrot and radish plants. J. Acta Agronomica Hungarica, 53: 293-301.

Karina, B., G.M. Susana and M.L. Tomaro, 2006. Aluminium stress affects nitrogen fixation and assimilation in soya bean. Plant Growth Regul., 48: 277-281.

Khan, A.A., T. McNeilly and J.C. Collins, 2000. Accumulation of amino acids, proline and carbohydrates in response to aluminum and manganese stress in maize. J. Plant Nutr., 23: 1303-1314.

Kinraide, T.B., 1993. Aluminum enhancement of plant growth in acid rooting media. A case of reciprocal allevation of toxicity by two toxic cations. Physiol. Plant, 88: 619-625.

Kinraide, T.B., 1997. Reconsiderating the rhizotoxicty of hydroxyl, sulphate anf flouride complexes of aluminium. J. Exp. Bot., 48: 1115-1124.

Loboda, T. and E. Wolejko, 2006. Effect of pH and Al^{3+} concentration on growth of spring brewer's barley. Agron. Res., 4: 517-529.

Mistrik, I., L. Tamas and J. Huttova, 2000. Quantitative changes in maiz membrane induced by aluminium. Biol. Plant, 43: 85-91.

Neogy, M., J. Datta, A.K. Roy and S. Mukheri, 2002. Studies on phytotoxic effect of aluminium on growth and some morphological parameters of *Vigna radiata* L. Wilczek. J. Environ. Biol., 23: 411-116.

Oleksyn, P., M.J. Karolewskil, A. Giertych, M.G. Werner, Tjoelker and P.B. Reich, 1996. Altered root growth and plant chemistry of *Pinus sylvestris* seedlings subjected to aluminium in nutrient solution. J. Trees Str. Fun., 10: 135-144.

Pan, W.L., A.G. Hopkins and W.A. Jackson, 1989. Aluminum inhibitions of shoot lateral branches of *Glycine max* and reversal by exogenous cytokinin. Plant Soil, 120: 1-9.

Petra, S.K. and J. Proctor, 2000. Effects of aluminium on the growth and mineral composition of *Betula pendula* Roth. J. Exp. Bot., 51: 1057-1066.

Pietraszewska, T.M., 2001. Effect of Aluminium on plant growth and metabolism. Acta Biochem. Polonica, 48: 667-686.

Shamsi, I.H., K. Wei, G. Jilani and G. Zhang, 2007. Interactions of cadmium and aluminum toxicity in their effect on growth and physiological parameters in soybean. Ziran Kexueban, 8: 181-188.

Slaski, J.J., 1989. Effect of aluminum on calmodulin dependent and calmodulinim dependent NAD knase activity in wheat (*Triticumn aestivm* L.) root tips. J. Plant Physiol., 133: 696-701.

Snowden, K.C., K.D. Richards and R.C. Gardner, 1995. Aluminum-induced genes. Introduction of toxic metals, low calcium and wounding and pattern of expression in root tips. Plant Physiol., 107: 341-348.

Walter, J.H., N. Schmohl, M. Kollmeier, Franti, ek Balu ka and M. Sivagurul, 1999. Does aluminium affect root growth of maize through interaction with the cell wall-plasma membrane-cytoskeleton continuum. J. Plant Soil, 215: 163-174.

Wang, P., B. Shuping, W. Shi and D. Qiuyan, 2006. Variation of wheat root exudates under aluminum. J. Agric. Food Chem., 54: 10040-10046.

William, G., Keltjens and E.V. Leone, 1989. Effects of Aluminium and mineral nutrition on growth and chemical composition of hydroponically grown seedlings of five different forest tree species. J. Plant Soil, 119: 39-50.

Wu, P., C.Y. Liao, B. Hu, K.K. Yi, W.Z. Jin, J.J. Ni and C. He, 2000. QTLs and *Epistasis foraluminum* tolerance in rice (*Oryza sativa* L.) at different seedling stages. Theor. Applied Genet., 100: 1295-1303.