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PHOTOCHEMISTRY OF LIGHT HARVESTING PIGMENTS AND SOME BIOCHEMICAL CHANGES UNDER ALUMINIUM STRESS

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Abstract

Aluminium (Al) bioavailability has raised much interest in the last two decades because of its acute toxicity, particularly at high dose, which results in the morphological and physiological disorder. *Lens culinari*s was selected to study the effects of Al on photosynthetic pigments and synthesis of carbohydrate contents in acidic soil. Growth inhibition like leaf mass ratio (LMR), stem mass ratio (SMR), root mass ratio (RMR) and ratio of dry and fresh weight of total plant DW/FW and reduced biomass production were general response of Al on seedlings. Results showed that Al altered the photochemistry of light-harvesting pigments that were decreased at all applied concentration of Al. This may be attributed, with breakdown of photosynthetic apparatus. Absorption spectrum of pigments showed that concentrations of chlorophyll a and b were decreased without shift in wavelength of pigments, which may be due to the decrease in absorption of photon of light that ultimately alter the synthesis of starch contents. Aluminum enters the cells, it reacts with phosphorus compounds, and upsets the plant phosphorus metabolism, and consequently the reduction in plant phosphorus was observed.

Introduction

Aluminium (Al) is a widespread and most abundant metal. Aluminium (Al^{3+}) toxicity is regarded as one of the major causes of nutritional disorders of upland crops on acid soils. Al concentration in the soil solution is high, when the soil has low pH (Akaya & Takenaka 2001). As the pH increases in the submerged soil the Al concentration in the soil solution decreases below the critical level for Al toxicity.

General effects of Al toxicity in plants include root alterations, leaf roll, chlorosis, and reduced growth. More specifically, Al interacts with water balance, damages the photosynthetic apparatus, lowers chlorophyll and carotenoid content, inhibits stomatal opening, affects the activity of several enzymes through replacement of other metal ions, and produces oxidative stress (Tripathi & Gaur 2006; Rai *et al*., 2004).

Al accumulation in plants may be dangerous for animal life and human health because Al enters the food chains in this way. Chen *et al*., (2005) observed that under high photon flux, excitation energy may be in excess in aluminum (Al)-treated leaves, which use a smaller fraction of the absorbed light in electron transport due to decreased CO_2 assimilation compared with normal leaves and antioxidant enzymes. Potentially, Al might alter the synthesis and breakdown of particular components and so affect the mechanisms of energy dissipation in leaves. Low pH (4.0) and Al treatments caused marked reduction in root length, shoot height, dry weight, chlorophyll content, (SPAD value) and photosynthetic rate. Todd *et al*., (1997) reported that environmental affects may results in deactivation of photosynthesis light availability and metabolic rate. Akaya & Takenaka (2001) observed that all Al-treated seedlings of *Quercus glauca* displayed greater leaf Al contents, decreased leaf phosphorus (P) contents and decreased water and nutrient absorption ability in the roots. Pereira *et al*., (2006) reported that Al inhibits enzyme δ-aminolevulinic acid

dehydratase (ALA-D), activity which results in the reduction of chlorophyll and also greatly impairs plant growth. Lidon & Barreiro (2002) observed that Al toxicity decreased significantly the concentrations of N, Mg, P and Fe, and hypothesized that P deficiency specifically triggers the reduction of biomass production.

Påhlesson (1990) reported that toxicity of Al in beech (*Fagus sylvatiea*) lowers the most nutrients (P, Ca, Mg, Zn and Cu) however concentration of starch in both the shoots and roots were significantly increased and elevated concentration and contents of starch in the seedlings may partly be related to the reduced shoot growth. Carvalho *et al*., *(*1980) observed growth reduction due P deficiency in acidic soil, whereas Thornton (1980) found that Al toxicity in honey locust resulted in increase in P and Ca level which causes reduction in growth of species.

The objective of the present study was to analyze the effects of aluminum on the growth, some efficiency parameters of photosynthetic pigments and their effects on synthesis of carbohydrate. Phosphorus metabolism of *Lens culinari*s have been discussed in the appropriate section of this article.

Materials and Methods

Ten to fifteen seeds of *Lens culinari*s were surface sterilized with 0.1% Mercuric chloride and germinated in natural environment in Petri dishes in darkness containing Whatman No.1 filter paper moistened with Hoagland nutrient solution for 15 days. 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 (20,30,90,100,150 mgL^{-1}). Experiment was conducted during January 2007 in the Department of Chemistry, Jinnah University for Women, Karachi.

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 at interval of 48 h. The plant material was dried at 60^{o}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).

Chlorophyll contents were extracted in 80% acetone and spectra was scanned in the range of 200-800nm on Schimadzo 160 A UV- Visible spectrophotometer. Phosphorus was determined by dry ashing method; extract was shaken with ammonium molybedate and stannous chloride. Absorbance of blue colored complex was measured at 660 nm by spectrophotometer.

Carbohydrates were analyzed in protein free filtrate extract of plants in water using anthrone reagent and complex absorbance was observed by spectrophotometer at 650 nm. Statistical analyses were performed on Microsoft Excel 2000 and standard error were calculated.

Results and Discussion

Values of measured parameters showed decline and the visual symptoms of Al toxicity were accompanied by a reduction in plant growth, expressed as a relative growth rate (RGR) (Table 1). These showed that Al toxicity is the major growth – limiting factor for crop cultivation on acid soil. Low pH level decreased leaf area and dry mass of shoot and root (Table 1). At 20 ppm of Al concentration, growth was approximately same to that of control plant but at 30 ppm and above, *L. culinari*s become more sensitive

especially in the last experimental days. These results suggest that *L. culinari*s could protect them self against toxic metal concentration in the beginning of treatment and at low concentration or by adopting defense mechanism. In response to Al stress, plants can resort to a number of defense systems, such as exudation of complexing agents, immobilization in the cell wall, exclusion through the action of the plasma membrane, compartmentalization in the vacuole, formation of metal resistant enzymes, and synthesis of phytochelatins and stress proteins (Azmat *et al*., 2007). Growth inhibition, leaf mass ratio (LMR), stem mass ratio (SMR), root mass ratio (RMR), ratio of dry and fresh weight of total plant DW/FW and reduced biomass production were visual symptoms of Al of toxicity (Table 1). Growth inhibition and reduction of biomass production are general response of plants to Al toxicity, which results due to inhibition in elongation and division of cells by metal addition. It has been observed that growth of root is more sensitive because root could play an important role in retention of metal by preventing an excess of toxic accumulation in the shoot. In the same way root of higher plants were considered as barrier against heavy metal translocation to the top parts, reflecting a potential tolerance mechanism operating in the root. Al affected the growth, in dose and time dependent manner. Seedling exposed to higher concentration and for long time shows drastic reduction in morphological and biochemical parameters (Thornton *et al*., 1986; Mossor-Pietraszewska, 2001).

Photochemistry of pigments under Al stress: Results showed that Al induced adverse affects on photosynthetic apparatus of *L. culinari*s prevented photosynthetic light harvesting in the affected antenna pigments (Table 2). The inhibition of chlorophyll synthesis may be due to physical presence of Al in the chloroplast and this might be the reason or the observed damaged in chloroplast structure (Fig. 1). A total decrease in pigment contents of photosynthetic apparatus was also reported as toxicity of Cd and Pb as observed in this investigation. It seems that metals have no specific affects on chlorophyll content. Reduced leaf size (LMR) also support this mechanism which leads to decrease the percentage of absorbing photon of light in reduced leaf area (Table 1). Aluminum (Al)-treated leaves, which use a smaller fraction of the absorbed light in electron transport due to decreased CO_2 assimilation compared with normal leaves (Table 2). Toxicity of metals in leaves suggests that this component at high level, can act as an efficient generator of toxic oxygen specie, such as O_2^{*-} and H_2O_2 by inhibiting photosynthetic electron transport. This highly cytogentic specie of oxygen (O_2^{*-}) can seriously disrupt normal metabolism through oxidative damage to photosynthetic apparatus. The damages of chloroplast structure during the treatment with high level imply functional changes of integral photosynthesis process and observed symptoms were same as could detect under influence of stress factor as water and temperature.

Photosynthetic organisms from both terrestrial and aquatic environments may be exposed to Al stress, which would cause severe damage in basic metabolic processes (Heim *et al*., 1999). These studies have documented reductions in chlorophyll content and growth, inhibition of PSII activity, increase in lipid peroxidation, and changes in primary metabolism, mineral contents and thus inhibits electron transfer in the reaction center which finally results in the break down of photosynthesis. This mechanism has a rapid and severe impact on the photosynthesis light reaction, whereas other parts of metabolism are affected much later. Absorption spectrum (Fig. 1) showed decreased in concentration of chlorophyll pigments with increasing Al dose without change in wave length. Results reported in the table 2 showed a decrease in carotenes, xanthophyll concentration due to accumulation of Al.

Table 1. Visual symptoms of Al toxicity in *Lens culinaris*.

Conc. of Al (ppm)	LMR	SMR	RMR	Dw/Fw	RGR
0	-	-	-	0.203 ± 0.01	0.025 ± 0.01
20	0.963 ± 0.3	0.77 ± 0.1	0.69 ± 0.1	0.177 ± 0.02	0.039 ± 0.02
30	0.927 ± 0.5	0.74 ± 0.1	0.662 ± 0.1	0.180 ± 0.02	0.0399 ±0.01
90	0.854 ± 0.2	0.693 ± 0.3	0.445 ± 0.2	0.187 ± 0.03	0.038 ± 0.01
100	0.818 ± 0.2	0.682 ± 0.3	0.453 ± 0.2	0.186 ± 0.02	0.037 ± 0.01
150	0.505 ± 0.2	0.656 ± 0.3	0.657 ± 0.2	0.198 ± 0.02	0.0284 ±0.0s1

Table 2. Photosynthetic pigments of *Lens culinaris* under Al stress.

Al (ppm)	% Decrease in absorption	Chl a (mg/gm)	Chl b (mg/gm)	Xanthophyll (mg/gm)	Carotenes (mg/gm)
0	-	4.927 ± 0.19	4.347 ± 0.2	0.034 ± 0.01	2.539 ± 0.01
20	5.882 ± 02	4.637 ± 0.20	4.057 ± 0.2	0.024 ± 0.01	2.524 ± 0.03
30	11.764 ± 03	4.347 ± 0.15	3.768 ± 0.2	0.015 ± 0.01	2.491 ± 0.05
90	17.647 ± 04	4.057 ± 0.0.09	3.478 ± 0.15	0.014 ± 0.02	0.503 ± 0.03
100	21.764 ± 03	4.147 ± 0.08	3.188 ± 0.1	0.107 ± 0.01	0.949 ± 0.9
150	23.529 ± 04	3.768 ± 0.12	2.898 ± 0..1	0.036 ± 0.02	2.486 ± 0.5

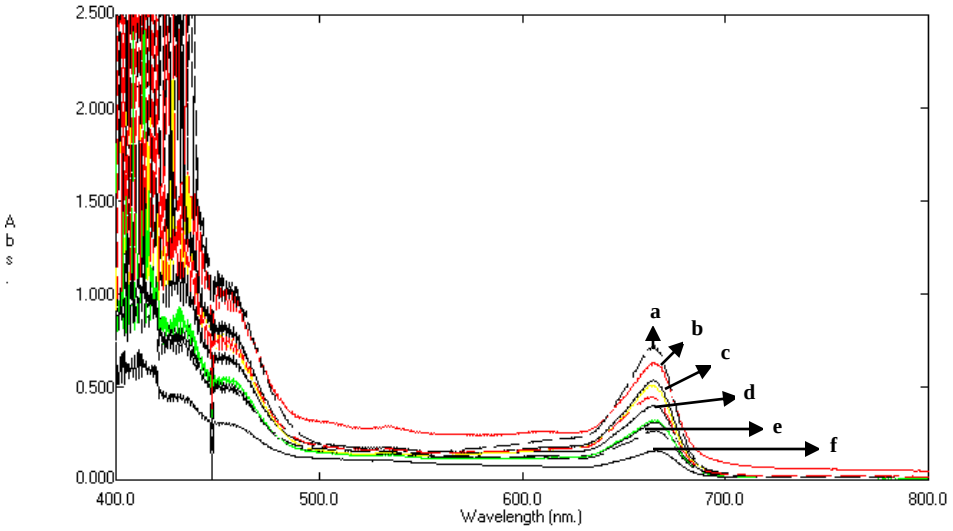

Fig. 1. Absorption spectrum of photosynthetic pigments under Al stress.

a = Absorbtion Spectrum of Chlorophyll at 0 ppm Al, b = Absorbtion Spectrum of Chlorophyll at 20 ppm Al, c = Absorbtion Spectrum of Chlorophyll at 30 ppm Al, d = Absorbtion Spectrum of Chlorophyll at 90 ppm Al, e = Absorbtion Spectrum of Chlorophyll at 100 ppm Al, f = Absorbtion Spectrum of Chlorophyll at 150 ppm Al

Table 3. Carbohydrate and P contents of *Lens culinari*s under Al stress.

Al (ppm)	% Carbohydrates (shoots) 10^4	% Carbohydrates (roots) 10^4	% Phosphorous (shoots)	% Phosphorous (roots)
0	4.510 ± 0.1	1.75 ± 0.1	0.701 ± 0.01	0.512 ± 0.01
20	4.120 ± 0.1	1.23 ± 0.2	0.262 ± 0.01	0.228 ± 0.01
30	2.590 ± 0.4	7.82 ± 0.2	0.331 ± 0.01	0.178 ± 0.01
90	4.980 ± 0.3	9.77 ± 0.2	0.516 ± 0.01	0.045 ± 0.01
100	4.330 ± 0.2	1.04 ± 0.2	0.258 ± 0.01	0.017 ± 0.01
150	4.510s ± 0.02	1.56 ± 0.2	0.204 ± 0.01	0.548 ± 0.01

Starch contents and P metabolism: Results reported in the Table 3 shows acute toxicity of Al on starch and phosphorus contents of *L. culinari*s. At 20 and 30 ppm of Al concentration soluble starch contents decrease but at higher dose of Al, carbohydrate contents increases as compared to control plant which shows that Al disturb the metabolic function of seedling at high concentration and this increased in concentration of carbohydrate in the species may partly be related to the reduced shoot growth of *Lens culinari*s (Table 3) as observed by earlier investigators (Påhlesson, 1990; Carvalho *et al.*, 1980) on the other hand in the roots starch contents were found to be increased as compare to control root but as the concentration increase the sugar contents decrease which may be due reduction in water uptake and less absorption of CO_2 during photosynthesis. Mechanism of this reduction may be well thought-out that some aluminum enters the cells, probably after damaging the root cell membranes. Once within the cell it reacts with phosphorus compounds and upsets the plant phosphorus metabolism. Aluminium also interferes in the process of cell division and inhibits the nucleic acid metabolism (i.e. inhibits reproduction of the plants genetic material) of the plant. The visual symptoms of phosphorus deficiency are that the plant tops of aluminum toxic plants appear typically phosphorus deficient. This reflects aluminum disruption of the plant phosphorus metabolism. The occasional observation of yellow spots or pale flecking of the leaves, may reflect effects of aluminum on other metabolic processes as phosphorus present in the root may serve as possible ligand for Al to adsorbed or precipitated at root in a new exchangeable form (Heim *et al*., 1999). Phosphorus in the plant influence activity of different enzymes e.g., NAD Kinaz (ATP-NAD2-phosphotransferase), which phosphorylates in the presence of ATP. It is probable that the formation of different soluble compound of phosphorus with aluminum on the surface of roots and inside the roots leads to phosphorus deficient in the plant. Pietraszewska (2001) reported that 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.

Conclusion

Al^{+3} toxicity may be due to physical presences that can damages the root structure**.** Al^{3+} forms complexes with nucleotides, with the cell wall and with other biomolecules, reducing the growth and development of the plant.

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