Indian Journal of Fundamental and Applied Life Sciences ISSN: 2231-6345 (Online)
An Online International Journal Available at http://www.cibtech.org/jls.htm
2013 Vol. 3 (2) April-June, pp.252-258/ Babu et al.
Research Article

'ALUMINIUM' INDUCED PHYTOTOXIC EFFECT ON ANTIOXIDANT ENZYME ACTIVITIES IN PEARL MILLET VARIETIES NANDI 32 AND NIRMAL 9

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ABSTRACT
The phytotoxic effect of various concentrations (50-500µM) of Al on Lipid peroxidation, pectin methyl esterase enzyme activity and some anti-oxidant enzyme activities like SOD, POD, Catalase were investigated in increasing concentrations of Aluminium (Al) in two Pearl millet (*Pennisetum glaucum* (L.) R. Br. of family Poaceae, varieties Nandi 32 (Al resistance) and Nirmal 9 (Al sensitive). The results were clearly showed that pectin methyl esterase and lipid peroxidation increased significantly with the increased concentration of Al. Whereas the activities of anti-oxidant enzymes were severely altered during the Al treatments. SOD and POD levels were increased at 250 µM concentration and then reduced. Catalase enzyme activity was decreased with the increasing concentration of Al.

Key Words: *Aluminium, Pearl Millet, Lipid Peroxidation, POD, Proline, SOD*

INTRODUCTION
All living organisms under normal physiological conditions, the cells produce ROS by reducing molecular oxygen (Boscolo *et al.,* 2003). However, under environmental stress conditions this production is increased. Various physical and chemical environmental stresses including temperature, mechanical shock, UV light, water deficiency, an excess of metallic ions and wastage of chemicals from industries showed their effect on physiological and biochemical and molecular changes in plants. Aluminum (Al) is the most abundant metal in the earth crust, comprising about 7% of its mass. Most of the A1 is bound by ligands or occurs in other non phytotoxic forms such as aluminosilicates and precipitates. However, solubilization of this A1 is enhanced by low pH and become phytotoxic (Matustumoto, 2000). Al toxicity is the most important factor, being a major constraint for crop production on 67% of the total acid soil area (Eswaran *et al.,* 1997). Since a large part of the world's total land area consists of acid soil, much attention has been given to Al toxicity (Matustumoto, 2000; Delhaize *et al.,* 2001; Yamamoto *et al.,* 2001; Foy, 1992; Barcelo and Poschenrieder, 2002). Al is a non-essential element for plant growth and can be dangerous for plants at low pH. Al is most severe in soils with low base saturation, poor in Ca and Mg (Vitorello *et al.,* 2005). It has been estimated that over 50% of the world's potentially arable lands are acidic (Uexküll and Mutert, 1995). Furthermore, up to 60% of the acid soils in the world occur in developing countries in South-America, Central Africa and Southeast Asia, where food production is critical. Although the poor fertility of acid soils is due to a combination of mineral toxicities (Al and Mn) and deficiencies (P, Ca, Mg and Mo).

Aluminium affected seed germination of different varieties of wheat (*Triticum aestivum* L.). And the inhibitory effect increased with the increase of Al^{3+} concentration (Alamgir And Akhter, 2009; Shen *et al.,* 1993). Al reduced the shoot and root length of *Vigna radiata* Land, in Soybean (Haider *et al.,* 2007), in Sorghum genotypes (Baligar, 1995) and in Wheat (Nasr *et al.,* 2011). Al showed its effect on protein content, free proline content, electrolyte leakage and membrane integrity (Yang and Chen 2001), Al ion interact with the lipid components of the plasma membrane (Akeson *et al.,* 1989). The binding of the Al to the membrane lipids cause the rigidification of the plasma membrane (Deleers *et al.,* 1986). A different environmental condition induces

the formation of reactive oxygen species (ROS) in plant cells (Vierstra and Ubiquitin, 1987). Al effected lipid peroxidation and activities of enzymes related to production of activated oxygen species (Cakmak and Horst 2006; Qin *et al.,* 2010). Al showed its effect on pectin methyl esterase enzyme (Yang *et al.,* 2008). Al also showed its effect on relative water content (Silva *et al.,* 2012).

The objective of this work was to evaluate the toxic effect of Al on pectin methyl esterase, lipid peroxidation and certain anti-oxidant enzyme activities like SOD, POD and Catalase in two pearl millet cultivars with differential tolerance to Al.

MATERIALS AND METHODS

Plants and treatments

Seeds of Pearl millet verities Nandi 32 (Al resistance) and Nirmal 9 (Al sensitive) were fully rinsed with distilled water and then germinated at 25°C with 14-hr photoperiod. The germinated equal length of seedlings were transferred to a net tray floated on a container filled with of 0.5 mM $CaCl_2$ solution at pH 4.5 containing 0, 50, 100, 250 and 500 µM $AlCl_3$ for 72hr. The solution was renewed daily. All the experiments were conducted in the growth chamber with a 14-h photoperiod, day/night temperature of 26/23°C, irradiance of 150 µmol (photon) m^{-2} s^{-1} and a relative humidity of 70%.

PME Activity Assay

For extraction of PME, cell wall materials (50 apices for each sample) were suspended in 1M NaCl solution (pH 6.0) at 4^0C for 1 h with repeated vortexing (20s for 10 min each). Extracts were centrifuged (14,000g, 10 min) and the supernatant was collected. A highly sensitive colorimetric assay method based on the condensation of an aldehyde with a molecule of MBTH under neutral conditions was used to analyze cell wall PME activity according to Anthon and Barrett (2004). Incubation contained 100 µL of 100 Mm Tris-HCl (pH 7.5) containing 0.4 mg mL^{-1} of pectin, 10 µL of alcohol oxidase (AO) at 0.01 units μL^{-1}, 40 µL of MBTH (3 mg/mL, dissolved in water) and 100 µL of sample or 1 M NaCl (as blank). After addition of AO, samples were incubated for 20 min at 30^0C and then 200 µL of a solution containing 5 mg mL^{-1} each of ferric ammonium sulfate and sulfamic acid was added. After 20 min at room temperature, 550 µL of water was added to give a final volume of 1.0 mL and A_{620} determined.

Examination of antioxidant enzyme activities

The fresh roots were homogenized in a pestle and mortar with 0.05 M sodium phosphate buffer (pH 7.8) at the end of the Al treatment. The homogenate was centrifuged at 10,000 × g for 20 min and the supernatant was used for analyzing SOD, POD and CAT. The above steps were carried out at 4°C (Meng *et al.,* 2007).

SOD assay

The SOD activity was estimated according to the modified method of Zhang *et al.* 2005. The reaction mixture was made of 54 mL methionine, 2 mL nitroblue tetrazolium chloride (NBT), 2 mL EDTA-Na_2, 2 mL riboflavin. Appropriate quantity of enzyme extract was added to the reaction mixture. The reaction started by placing tubes below two 15 W fluorescent lamps for 15 min. Reaction stopped by keeping the tubes in dark for 10 min. Absorbance was recorded at 560 nm. One unit of SOD enzyme activity was defined as the quantity of SOD enzyme required to produce a 50% inhibition of reduction of NBT under the experimental conditions and the specific enzyme activity was expressed as units per gram fresh weight.

POD assay

The activity of POD was determined as described by Zhang *et al.,* 2005. The reaction mixture in a total volume of 50 mL 0.1 M sodium phosphate buffer (pH 6.0) containing 19 µL H_2O_2 (30%), 28 µL Guaiacol was prepared immediately before use. Then 1 mL enzyme extract was added to 3 mL reaction mixture. Increase in absorbance was measured at 470 nm at 0.5 min intervals up to 2 min using a UV-Vis spectrophotometer (UV-2550, Shimadzu, Kyoto, Japan). Enzyme specific activity is defined as units (one peroxidase activity unit defined as absorbance at 470 nm changes per minute) per g of fresh weight.

CAT assay

CAT activity was assayed according to the method of Zhang *et al.* (2005). CAT activity was determined by a UV-Vis spectrophotometer (UV-2550, Shimadzu, Kyoto, Japan) in 2.8 mL reaction mixture containing 1.5 mL

0.05 M sodium phosphate buffer (pH 7.8), 1 mL deionized water and 0.3 mL 0.1 M H_2O_2 prepared immediately before use, then 0.2 mL enzyme extract was added. The CAT activity was measured by monitoring the decrease in absorbance at 240 nm at 0.5 min intervals up to 2 min as a consequence of H_2O_2 consumption. Activity was expressed as units (one catalase activity unit defined as absorbance at 240 nm changes per minute) per gram of fresh weight.

Examination of MDA content

Level of lipid peroxidation was expressed as the content of Malondialdehyde (MDA) according to Zhang *et al.* (2005). The fresh samples from each treatment were homogenized in 5 mL of 10% Trichloroacetic acid (TCA) with a pestle and mortar at the end of each time interval (3 d). Homogenates were centrifuged at 4000 × g for 20 min. To each 2 mL aliquot of the supernatant, 2 mL of 0.6% 2-thiobarbituric acid (TBA) in 10% TCA was added. The mixtures were heated in boiled water for 15 min and then quickly cooled in an ice bath. After centrifugation at 4000 × g for 10 min, the absorbance of the supernatant was recorded at 532 nm and 450 nm. Lipid peroxidation was expressed as the MDA content in n mol per gram of fresh weight.

RESULTS AND DISCUSSION

Two Pearl millet(*Pennisetum glaucum*) varieties Nandi 32 (Al resistance) and Nirmal 9 (Al sensitive) were screened to find out Al resistance based on biochemical assays such as lipid peroxidiation, pectin methyl esterase assay and antioxidant enzyme system. From these two verities of Pearl millet, Nandi 32 showed resistance where as Nirmal 9 remain sensitive. Nandi 32 (Al resistance) and Nirmal 9 (Al sensitive) were used for further work.

Effect Al on lipid peroxidation in Pearl millet

The effects of Al on MDA concentration are presented in Fig-1. The MDA contents increased with increasing concentration of Al. The MDA contents in roots exposed to 50 to 500 µM Al were higher significantly ($p< 0.05$) than the control. Nirmal 9 (Al sensitive) had higher MDA content than Nandi 32 (Al resistance).

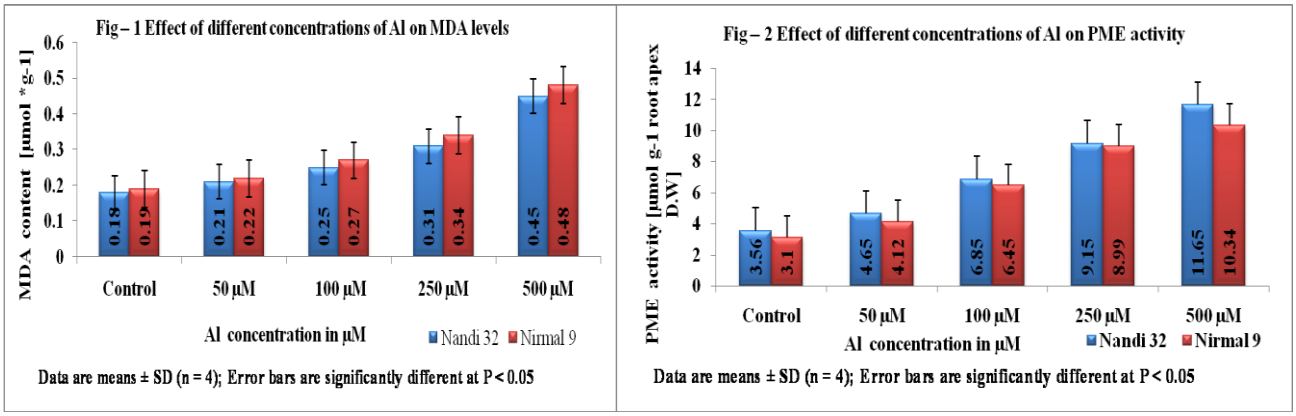
Our results suggest that the Al induces membrane damage in root of finger millet varieties that might be attributable to the accumulation of Al and it may leads to generation of reactive oxygen species (ROS). These ROS involve in a cyclic cascade of reactions causing a distortion of the lipid bilayer, membrane proteins and disruption of the membrane by the Al. The polyvalent cations of the Al ions may interact with S and N groups present in cell proteins and cause an alteration of the ionic channels of the membrane, which promotes a higher flow of ions of the cell into the leaf discs. Similar results were reported by Xiong and Wang (2005) and Tamas *et al.* (2006). In our study, peroxidation of lipids was increased along with increasing concentration. Some studies was reported that the aluminum-enhanced peroxidation of lipids in root tips of soybean it resulted that the inhibition of root elongation (Horst *et al.,* 1992). It has been shown that Al exposure is associated with peroxidative damage of membrane lipids due to the stress-related increase in the production of highly toxic oxygen free radicals. Phosphatidylserine is the most susceptible substrate for Al to facilitate lipid peroxidation (Xie and Yokel, 1996). A close relationship existed between lipid peroxidation and inhibition of root elongation rate. Enhanced lipid peroxidation by oxygen free radicals is a consequence of the primary effects of Al on membrane.

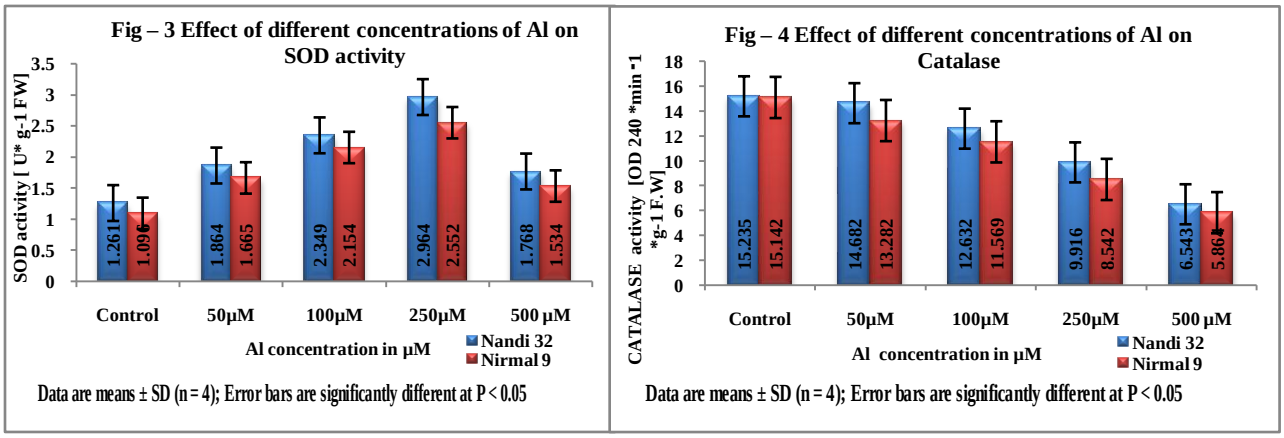
Effect of Al on Pectin methyl esterase enzyme

The degree of pectin methylation is an important factor affecting the properties of the cell wall. We analyzed pectin methyl esterase (PME) activity using a sensitive colorimetric assay method based on the amount of methanol released from cell wall pectin extracted from the finger millet root apex (0–10 mm). As shown in Fig-13, PME activity was significantly higher in Al-sensitive Nirmal 9 (Fig-2). In the absence of Al, and Al treatment resulted in an increase in PME activity in both cultivars. According to Mimmo *et al. (*2003, 2005 and 2009) the polyvalent cations bind with root cell apoplasm under acidic conditions and the pectin matrix is the main target of Al accumulation and thus Al toxicity. The pectin matrix with its different degree of pectin esterification (DE) seems to play a fundamental role in the expression of Al toxicity and resistance. High degree of esterification of pectins would thus be an indicator of Al-resistance as the high DE decreases the binding strength of Al to the pectin matrix and favours it release/desorption by organic acids. The aluminum sorption

affects the conformation of the Caepectates complex, both aluminum and Calcium seem to interact with the carboxylate groups as well as to the anomeric oxygen of pectins (Yang *et al.*, 2008).

Figure 1 & 2: Effect of different concentrations of Al on MDA, PME and Catalase enzymatic activity in the root apex of pearl millet varieties after three days Al treated plant Three-day-old seedlings. Data are means ± SD (n = 4). Error bars are significantly different at P < 0.05.





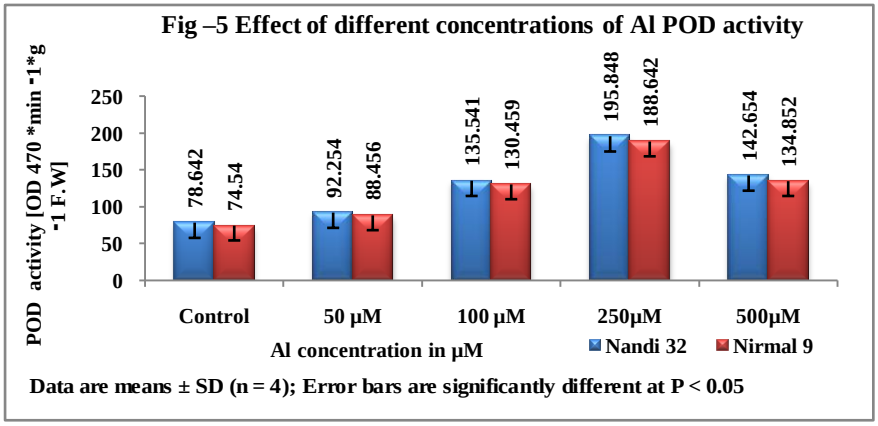


Figure 3, 4 & 5: Effect of different concentrations of Al on antioxidant enzyme activities such as SOD, Catalase, POD levels in pearl millet verities, exposed to Al (50,100, 250 and 500µM) stress after 3 days. Data are means ± SD (n = 4). Error bars are significantly different at P < 0.05.

Effect Al on SOD, Catalase and Peroxidase Activity

Effects of Al on SOD activities of Pearl millet roots varied with the different concentrations (50 to 500 µM) of Al. The maximum SOD activity was observed at 250µM in roots of both varieties of Nandi 32 (resistance) and Nirmal 9 (sensitive) Pearl millet (Figure 3). The CAT activity was found to be inhibited significantly ($P< 0.05$) and to be the lowest in the roots at 500 µM Al in both pearl millet varieties (Figure 4). The POD activities in roots treated with 500 µM Al were noted to be high significantly ($P< 0.05$) in comparison with the control (Figure 5). Both the pearl millet varieties treated with Al showed higher POD activity in the roots.

Plasma membrane is a major target for the action of Al ions. Interaction of Al with the membrane lipids leads the increasing high production of ROS. Cakmak and Horst (1991) found the highest lipid peroxidation in the root tips (<2 cm) of soybean at a longer duration of Al exposure. Major ROS-scavenging enzymes in seedlings include SOD, APX and CAT (Willekens, 1997; Bowler *et al.*, 1992; Mittler, 2002). The balance between SOD and APX or CAT activities in cells is crucial for determining the steady-state level of superoxide radicals and hydrogen peroxide (Bowler *et al.*, 1992). Most previous researches have shown adverse effects of Al on plant growth and enhancement of ROS. They attributed this to the existence of Al in acidic conditions as polyvalent cations which bind strongly to the negative charges in the cell (Poschenrieder *et al.*, 2008; Rbia *et al.*, 2011; Zhang *et al.*, 2010). Previous study showed, however, that most of the supplied Al was absorbed by tobacco cells in MS medium, pH 5.5 (Shokuhi and Ghanati, 2007). Reactive oxygen species production is closely related to the response of plants to heavy metals (Nagajyoti *et al.*, 2010).

AKNOWLEDGEMENT

The authors wish to acknowledge the support of Seed Bank, ICRISAT for providing the seeds of Pearl millet varieties.

REFERENCES

Anthon GE and Barrett DM (2004). Comparison of three colorimetric reagents in the determination of methanol with alcohol oxidase. Application to the assay of pectin methylesterase, *Journal of Agricultural and Food Chemistry* **52** 3749–3753.

Akeson MA, Munns DN and Burau RG (1989). Adsorption of Al^{3+} to phosphatidylcholine vesicles. *Biochimica et Biophysica Acta* **986** 33–40.

Alamgir ANM and Akhter S (2009). Effects of aluminium (Al^{3+}) on seed germination and Seedling growth of wheat (*Triticum aestivum* L.). *Bangladesh Journal of Botany* **38**(1) 1-6.

Baligar VC (1995). Aluminum effects on plant and nutrient uptake parameters of soil and solution grown sorghum genotypes usda-ars-appalachian soil & water conservation research laboratory, Beckley. *Journal of Plant Nutrition* **11** 2325-2338.

Barcelo J and Poschenrieder C (2002). Fast root growth responses, root exudates and internal detoxification as clues to the mechanisms of aluminum toxicity and resistance: a review. *Environment and Experimental Botany* **48** 75-92.

Boscolo PRS, Menossi M and Jorge RA (2003). Aluminum-induced oxidative stress in maize. *Photochemistry* **62** 181-189.

Bowler C, Camp VW, Montagu V and Inzé D (1992). Superoxide dismutase and stress tolerance. *Annual review of Plant Physiology Plant Molecular Biology* **43** 83-116, **83** 463-468.

Cakmak I and Horst HJ (2001). Effect of aluminium on lipid peroxidation, superoxide dismutase, catalase, and peroxidase activities in root tips of soybean (*Glycine max*). *Physiologia Plantarum* **83** 463–468.

Deleers M, Servais JP and Wulfert E (1986). Neurotoxic cations induce membrane rigidification and membrane fusion atmicromolar concentrations. *Biochimica et Biophysica Acta* **855** 271–276.

Delhaize E, Ryan RP and Ma FJ (2001). Aluminum tolerance in plants and the complexing role of organic acids. *Trends Plant Sciences* **6** 273-278.

Eswaran H, Reich P and Beinroth F (1997). Global distribution of soils with acidity In: Plant-Soil Interactions at low pH. *Brazilian Soil Science Society* 159-164.

Foy CD (1992). Soil chemical factors limiting plant root growth. In*: Advances in Soil Sciences: Limitations to Plant Root Growth* **19**, edited by Hatfield JL and Stewart BA (Springer Verlag, New York) 97-149.
Haider I, Shamsi, Wei K, Jilani G and Zhang G (2007). Interactions of cadmium and aluminum toxicity in their effect on growth and physiological parameters in soybean. *Journal of Zhejiang University Science B* **8** 181–188.
Horst WJ, Asher CJ, Cakmak I, Szulkiewica P and Wissemeier AH (1992). Short-term responses of soybean roots to Aluminum. *Journal of Plant Physiology* **140** 174–178.
Matustumoto H (2000). Cell biology of aluminum toxicity and tolerance in higherplants. *International Review of Cytology* **200** 1-46.
Meng QM, Zou J, Zou JH, Jiang WS and Liu DH (2007). Effect of Cu^{2+} concentration on growth, antioxidant enzyme activity and malindialdehyde content in garlic (Allium sativum L.). *Acta Biologica* Cracoviensia Series *Botanica* **49** 95-101.
Mimmo T, Marzadori C, Francioso O, Deiana S and Gessa CE (2003). Effects of aluminum sorption on calciumepolygalacturonate network used as soil e root interface model. *Biopolymers* **70** 655-661.
Mimmo T, Marzadori C, Montecchio D and Gessa D (2005). Characterisation of Ca-Alepectate gels by thermal analysis and FTeIR spectroscopy. *Carbohydrate Research* **340** 2510-2519.
Mimmo, T, Marzadori C and Gessa CE (2009). Does the degree of pectin esterification influence aluminium sorption by the root apoplast?. *Plant Soil* **314** 159-168.
Mittler R (2002). Oxidative stress, antioxidants and stress tolerance. *Trends Plant Science* **7** 405-410.
Nagajyoti PC, Lee KD and Sreekanth TVM (2010). Heavy metals occurrence and toxicity for plants. *Environmental Chemistry Letters* **8** 199-216.
Nasr N, Carapetian J, Heidari R, Asri Rezaei S, Abbaspour N and Darvishzadeh R (2011). Differential effects of aluminium on the seedling parameters of wheat. *African Journal of Biotechnology* **10** 3345-3353.
Poschenrieder CH, Gunsé B, Corrales I and Barceló J (2008). A glance into aluminum toxicity and resistance in plants. *Science Total Environment* **400** 356-368.
Qin R, Jiao Y, Zhang S, Jiang W and Liu D (2010). Effects of aluminum on nucleoli in root tip cells and selected physiological and biochemical characters in *Allium cepa*. *BMC Plant Biology* **10** 225.
Rbia O, Horchani F, Smida I, Mejri M and Aschi-Smiti S (2011). Aluminium phytotoxicity and plant acclimation to acidic soils. *International Journal of Agricultural Research* **6** 194-208.
Shen Z, Wang J and Guan H (1993). Effect of aluminium and calcium on growth of wheat seedlings and germination of seeds. *Journal of Plant Nutrition* **11** 2135-2148.
Shokuhi KH and Ghanati F (2007). Aluminum-induced growth inhibition and changes in cell wall components of tobacco cells. *Journal of Science Teacher Training Universal* **7** 855-864.
Silva S, Pinto G, Dias MC, Correia CM, Moutinho-Pereira J, Pinto-Carnide O and Santos C (2012). Aluminium long-term stress differently affects photosynthesis in rye genotypes. *Plant Physiology and Biochemistry* **54** 105-112.
Tamas L, Huttova J, Mistrik I, Simonovicova M and Siroka B (2006). Aluminum-induced drought and oxidative stress in barley roots. *Journal of Plant Physiology* **163** 781-784.
Uexküll HRV and Mutert E (1995). Global extent, development and economic impact of acid soils. In: *Plant-Soil interactions at low pH. Principles and Management, edited by* Date RA (Natural Journal of Kluwer, Academic Publishers, Netherlands) 5-19.
Vierstra RD and Ubiquitin (1987). A key component in the degradation of plant proteins. *Physiolgia Plantarum* **70** 103-106.
Vitorello VA, Capaldi FRC and Stefanuto VA (2005). Recent advances in aluminum toxicity and resistance in higher plants. *Brazilian Journal of Plant Physiology* **17** 129-143.
Willekens H (1997). Catalase is a sink for H_2O_2 and is indispensable for stress defense in C-3 plants. *Embo Journal* **16** 4806-4816.
Xie CX and Yokel RA (1996). Aluminum facilitation of iron-mediated lipid peroxidation is dependent on substrate, pH, and aluminum and iron concentrations. *Archiologia Biochemistry and Biophysics* **327** 222–226.

Xiong ZT and Wang H (2005). Copper toxicity and bioaccumulation in Chinese cabbage (*Brassica pekinensis* Rupr.). *Environmental Toxicology* **20** 188-194.

Yamamoto Y, Koayashi Y and Matsumoto H (2001). Lipid peroxidation is an early symptom triggered by aluminum, but no the primary cause of elongation inhibition in pea roots. *Plant Physiology* **125** 199-208.

Yang JL, Li YY, Zhang YJ, Zhang SS, Wu YR, Wu P and Zheng SJ (2008). Cell Wall Polysaccharides are Specifically Involved in the Exclusion of Aluminum from the Rice Root Apex. *Plant Physiology* **146** 602–611.

Yang YH and Chen SM (2001). Physiological effects of aluminum/calcium ratios on aluminum toxicity of mungbean seedling growth. *Journal of Plant Nutrition* **24** 585-597**.**

Zhang B, Wang X, Li X, Ni Y and Li H (2010). Aluminum uptake and disease resistance in *Nicotiana rustica* leaves. *Ecotoxicology and Environmental Safety* **73** 655-66.

Zhang HY, Jiang YN, He ZhY and Ma M (2005). Cadmium accumulation and oxidative burst in Garlic (*Allium sativum*). *Journal of Plant Physiology* **162** 977-984.