

Studies of Aluminum (Al_2O_3) Stress with enzymatic parameters of *Vigna radiata*, L. seedling

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Abstract

Experimental Study of Aluminum (Al_2O_3) Stress on enzyme activities of Catalase (CAT), Peroxidase (POD) and Polyphenol Oxidase (PPO) of *Vigna radiata*, L. seedlings was conducted with the Al treatment and concentrations being 200, 400, 600, 800 and 1000 mg/L for 7 days. The enzymatic antioxidants like Catalase, POD and PPO are responsible for reactive oxygen species (ROS) scavenging in plants. The Catalase enzyme activity in roots were elevated with the increase in Aluminum stress where as Shoots did not respond. However, Peroxidase (POD) and Poly Phenol Oxidase (PPO) showed an increasing trend which was statistically significant. This indicated that, the response of the seedlings to overcome the Al stress by showing more enzyme activity. The shoots of the seedlings showed more POD and PPO activity in comparison to roots. This was due to the retention of more Al in roots than shoots of the seedlings. The Al tolerance of mung seedlings was due to increased antioxidant enzymes activities in the storage organs (Root and Shoot) of the seedlings. Aluminum toxicity triggered alterations in the antioxidant and physiological status of growing mung seedlings.

Key words: Aluminum, Mung, enzymes, Seedlings, stress.

1. Introduction

Aluminum (Al) is one of the most abundant elements of the planet and exposure to this metal can cause oxidative stress and lead to various signs of toxicity in plants. The toxic effect of aluminum is the

major cause of decreased crop productivity. Aluminum does not affect the seed germination but helps in new root development and seedling establishment (Nosko et al., 1988). Root growth inhibition was detected 2–4 days after the initiation of seed germination (Bennet et al., 1991). Several reviews on Al toxicity are available (Haug, 1984; Taylor, 1988; Rengel, 1992); here we limit our discussion to the sites of Al toxicity in higher plants. Al ions are taken up by plants mostly through the root system, and only small amounts penetrate the leaves. Most authors now agree that generally the active metal up-take processes involve ion-specific carriers with energy expenditure but a specific Al carrier has not yet been found.

The most easily affected region of Al toxicity is the root in plant. The Al toxicity is due to the inhibition of root growth. Al rapidly accumulates in the plasma membrane of sensitive plants affecting processes of root growth. Plasma membrane is rich in phospholipids, representing sensitive target of the Al phytotoxicity. Al can alter the function of plasma membrane by interacting with the lipid thus inducing lipid peroxidation. Al can bind principally to phospholipids within the membrane. Several reports have been described the Al mediated interference with membrane lipid, as a result of which there is an increase in the highly toxic reactive oxygen free radicals. Decrease activities of K^+ , Mg^+ and ATPase of plasma membrane were scored due to Al stress. It is proved that exposure to Al could affect production of reactive oxygen species (ROS) in plants because Al stress causes peroxidation of lipids in the plasma

membrane, the effect that could be due to ROS Long-term treatment of green gram (*Vigna radiata*) with Al resulted in greatly increased levels of peroxide and lipid peroxidation in the leaves. The imposition of biotic and abiotic stresses can give rise to further increases in ROS levels. Metals, including Al, are known to act as catalysts in ROS production and to induce oxidative damage in plants. Large number of swollen mitochondria with many vacuoles, structural disturbances of the plasma membrane, and pre-apoptotic nuclear structures were some of the characteristic features of Al treated tobacco cells, confirming that Al signaling follows the mitochondrial pathway of cell death.

Thus, the objective of the present study was to analyze the effects of aluminum on the activity of antioxidant enzymes such as Catalase (CAT - E.C. 1.11.1.6), Poly Phenol Oxidase (POD - E.C.1.11.1.x) and Peroxidase (PPO - E.C.1.10.3.2) levels in *Vigna radiata* L. Seedlings were grown at different concentrations of Aluminum ranging from 200 to 1000 mg/L for 10 days.

2. Materials and Methods

2.1 : Test Chemical & Concentration:

The test chemical, Aluminum oxide (Al_2O_3) was used in the seedling stress study was of AR grade and the concentrations selected were 200,400,600,800 and 1000 mg/L of test chemical. The concentrations were chosen basing on our earlier LC 50 study. (Mohapatra et al, 2015a)

Experiments were conducted in petriplates (6") with cotton and blotting paper soaked with different concentrations of Aluminum oxide (Al_2O_3). 15 healthy seeds were used to each petriplate to study the(%) percentage of germination after 24 to 72 hours.

The control set was kept with Al_2O_3 free environment. In each concentration of Al_2O_3 , three replicates were taken to find out the % of germination of seeds. The seed germinator (Remi,C-6) was used in experimentation with $25 \pm 2^\circ$ C temperature, 90% humidity and 12 hours light cycle exposure.

2.2 Test Organism: The prime pulse seed *Vigna radiata*, L. var.PDM 139, Samart commonly used in eastern state of India, particularly Odisha State has

been chosen for study. Healthy seeds of *Vigna radiata*, L. were obtained from OUAT Extension Centre,Ratnapur Ganjam for the experimentation.

2.3 Parameters Evaluated: The seedling parameters studied were root length, shoot length. R/S ratio of the seedlings after treatment and seedling growth period of 7 days. In enzyme studies, Catalase, Peroxidase (POD) and Polyphenyl Oxidase (PPO) of shoot and root of 7 day old seedlings were analyzed (Kar and Mishra.1976).The catalase enzyme activity was expressed in terms of $\mu\text{mol H}_2\text{O}_2$ released $\text{min}^{-1} \text{g}^{-1}$ fresh wt., POD activity in Absorbency Units ($A_{420\text{nm}}$) and PPO activity in Absorbency Units ($A_{420\text{nm}}$)

3. Results and Discussions

The results obtained in the enzymatic study with varied Al stress to 10days old *Vigna radiata*,L. Seedlings were given in Fig. No.1 to 3. The Catalase enzyme activity in roots were elevated with the increase in Aluminum stress where as Shoots did not respond. However, Peroxidase (POD) and Poly Phenol Oxidase (PPO) showed an increasing trend which was statistically significant. This indicated that, the response of the seedling to overcome the Al stress was by showing more enzyme activity. The shoots of the seedlings showed more POD and PPO activity in comparison to roots. This was due to the retention of more Al in roots than shoots of the *Vigna radiata*, L. seedlings .The Al tolerance of seedlings was due to increased antioxidant enzymes activity in the storage organs (Root and Shoot) of the seedlings.

The common responses of shoots to Al include: cellular and ultra structural changes in leaves, increased rates of diffusion resistance, reduction of stomatal aperture, decreased Photosynthetic activity leading to chlorosis and necrosis of leaves, total decrease in leaf number and size, and a decrease in shoot bio-mass (Thornton et al., 1986).

The root apex (root cap, meristem, and elongation zone) accumulates more Al and attracts greater physical damage than the mature root tissues In general; many plant species are resistant or can be tolerant to certain amounts of metals. This is probably achieved through trapping

of these metals with metal-binding proteins. Many of the biochemical effects of Al on plants is probably associated with the alteration of root membrane structure and function.

Inhibition of root and shoot growth is a visible symptom of Al toxicity (Mohapatra et al., 2015b, 2016). The earliest symptoms concern roots. Shoots in contrast to the situation observed for Mn toxicity are less affected (Chang et al., 1999) Young seedlings are more susceptible than older plants. Al apparently does not interfere with seed germination, but does impair the growth of new roots and seedling establishment (Nosko et al., 1988). This review discusses re-cent information on aluminum toxicity with an emphasis on plant response to Al stress. Molecular and biochemical studies suggest that abiotic stress signaling in plants involves receptor-coupled phospho-relay, phospho-inositol induced Ca^{2+} changes, mitogen- activated protein kinase cascades and transcriptional activation of stress-responsive genes (Xiong and Zhu, 2001). Al tends to bind to the phosphate or carboxyl groups rather than to .SH groups characteristic for chelatins (Gunsé et al., 1997). However, Snowden et al. (1995) and Wu et al. (2000) suggested that plant metallo thionein-like protein and phyto chelatins may play a role in Al tolerance. Although aluminum has been shown to be as nontoxic metal, the molecular mechanism of Al toxicity to plants is not well understood. Al is a complicated ion in terms of chemical form and exerts a divergent biological function.

4. Conclusions

An experimental study of Aluminum (Al_2O_3) Stress on enzyme activities of Catalase (CAT), Peroxidase (POD) and Polyphenol Oxidase (PPO) of *Vigna radiata* ,L. seedlings was conducted .The Catalase enzyme activity in roots were elevated with the increase in Aluminum stress where as Shoots did not respond. However, Peroxidase (POD) and Poly Phenol Oxidase (PPO) showed an increasing trend which was statistically significant. This indicated that, the response of the seedlings to overcome the Al stress by showing more enzyme activity. The shoots of the seedlings showed more POD and PPO

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