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Effect of Soil Applied Cobalt on Activities of Antioxidant Enzymes in *Arachis hypogaea*

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Abstract: The present investigation was executed with an objective to study the effects of Co stress in *Arachis hypogaea* L. with special emphasis on antioxidant enzymes activities which are the defense mechanism to any type of abiotic stress. In this we have analysed the effect of cobalt (Co) stress on antioxidant enzyme activities (catalase, peroxidase and polyphenol oxidase) of *Arachis hypogaea* L. were studied. The antioxidant enzymes like catalase, peroxidase and polyphenol oxidase activities were highly altered due to the abiotic stress resulted from cobalt stress. The antioxidant enzyme activities have beneficial value at 50 mg kg^{-1} Co level in the soil, when compared with control. Further increase in the Co level (100-200 mg kg^{-1}) in the soil have a negative effect on these parameters. From these results it is clear that Antioxidant potentials acts as a protective mechanism in *A. hypogaea* under soil cobalt stress.

Key words: *Arachis hypogaea,* Antioxidants, Cobalt, Catalase, Peroxidase, Polyphenol oxidase

INTRODUCTION

Metals play an integral role in the life processes of microorganisms. Some metals, such as calcium, cobalt, copper, iron, potassium, magnesium, manganese, sodium, nickel and zinc, are essential, serve as micronutrients and are used for redox-processes; to stabilize molecules through electrostatic interactions; as components of various enzymes; and for regulation of osmotic pressure [1]. Many other metals have no biological role (e.g. silver, aluminium, cadmium, gold, lead and mercury) and are nonessential and potentially toxic to microorganisms. Toxicity of nonessential metals occurs through the displacement of essential metals from their native binding sites or through ligand interactions [2,3]. In addition, at high levels, both essential and nonessential metals can damage cell membranes; alter enzyme specificity; disrupt cellular functions; and damage the stucture of DNA.

In abiotic stress, metal response will results in the production of reactive oxygen species (ROS) which leads to the activation of defense mechanisms in terms of antioxidant enzymes. Generation of ROS such as superoxide, H_2O_2 and hydroxyl molecules cause rapid cell damage by triggering off a chain reaction [4]. Plants under stress produce some defence mechanisms to protect themselves from the harmful effect of oxidative stress. ROS scavenging is one among the common defense response against abiotic stresses [5]. ROS scavenging depends on the detoxification mechanism provided by an integrated system of non-enzymatic reduced molecules like ascorbate and glutathione and enzymatic antioxidants [6]. The major ROS scavenging activities includes complex non-enzymatic (ascorbate, glutathione, α-tocopherol) and enzymatic antioxidants like catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR), superoxide dismutase (SOD), polyphenol oxidase (PPO), peroxidase (POX) etc. [7]. The pathways include the water-water cycle in chloroplasts and the ascorbate-glutathione cycle [8]. Antioxidant mechanisms may provide a strategy to enhance metal tolerance in plants [9,10].

The present investigation was executed with an objective to study the effects of Co stress in *Arachis hypogaea* L. with special emphasis on antioxidant

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enzymes activities which are the defense mechanism to any type of abiotic stress.

MATERIALS AND METHODS

Plant Materials and Cultivation: The seeds of groundnut (*Arachis hypogaea* L.) were obtained from Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India and surface sterilized with 0.1% $HgCl_2$ solution for 5 min with frequent shaking and then thoroughly washed with deionised water. The experiments were conducted during January-April, 2005. Plants were grown in pots in untreated soil (control) and in soil to which Co had been applied (50, 100, 150, 200 and 250 mg kg^{-1} soil). The inner surface of pots were lined with polythene sheet. Each pot contained 3 kg of air-dried soil. The Co as finely powdered ($CoCl_2$) was applied to the surface soil and thoroughly mixed with the soil. Five seeds were sown in each pot. All the pots were watered to field capacity daily. Plants were thinned to a maximum three per pot, after a week of germination. Each treatment including control was replicated five times. The plant samples were collected on 30 days after sowing (DAS) for the measurement of various antioxidant enzyme activities.

Antioxidant Enzymes

Catalase (Cat) (EC 1.11.1.6) Activity: Catalase (CAT) (EC 1.11.1.6) activity was measured according the method of Chandlee and Scandalios [11] with small modification. 0.5 g of frozen plant material was homogenized in a prechilled pestle and mortar with 5ml of ice cold 50 mM sodium phosphate buffer (pH 7.5) containing 1 mM phenyl methyl sulfonyl fluoride (PMSF). The extract was centrifuged at 4°C for 20 min at 12,500 xg. The supernatant was used for enzyme assay. The assay mixture contained 2.6 mL of 50 mM potassium phosphate buffer (pH 7.0), 400 µL of 15 mM H_2O_2 and 40 µL of enzyme extract. The decomposition of H_2O_2 was followed by the decline in absorbance at 240 nm.

Peroxidase (Pox; EC 1.11.1.7) Activity: Peroxidase (POX; EC 1.11.1.7) was assayed by the method of Kumar and Khan [12]. Assay mixture of POX contained 2 mL of 0.1 M phosphate buffer (pH 6.8), 1 mL of 0.01 M pyrogallol, 1 mL of 0.005 M H_2O_2 and 0.5 mL of enzyme extract. The solution was incubated for 5 min at 25 °C after which the reaction was terminated by adding 1 mL of 2.5 N H_2SO_4. The amount of purpurogallin formed was determined by measuring the absorbance at 420 nm against a blank prepared by adding the extract after the addition of 2.5 N H_2SO_4 at zero time. The activity was expressed in unit mg^{-1} protein. One unit (U) is defined as the change in the absorbance by 0.1 min^{-1} mg^{-1} protein.

Polyphenol Oxidase (PPO; EC 1.10.3.1) Activity: Polyphenol oxidase (PPO; EC 1.10.3.1) activity was assayed by the method of Kumar and Khan [12]. Assay mixture for PPO contained 2 mL of 0.1 M phosphate buffer (pH 6.0), 1 mL of 0.1 M catechol and 0.5 mL of enzyme extract. This was incubated for 5 min at 25°C, after which the reaction was stopped by adding 1 mL of 2.5 N H_2SO_4. The absorbancy of the benzoquinone formed was read at 495 nm. To the blank 2.5 N H_2SO_4 was added of the zero time of the same assay mixture. PPO activity is expressed in U mg^{-1} protein (U = Change in 0.1 absorbance min^{-1} mg^{-1} protein). The enzyme protein was estimated by the method of Bradford [13] for expressing all the enzyme activities.

Statistical Analysis: Statistical analysis was performed using one way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT). The values are mean ± SD for six samples in each group. P values ≤ 0.05 were considered as significant.

RESULTS AND DISCUSSION

The leaf CAT activity was high in 50 mg kg^{-1} and it was low in 250 mg kg^{-1}. The increase in metal concentration decreased the CAT activity. POX and PPO activities in leaves were high in 250 mg kg^{-1} and it was low in 50 mg kg^{-1} (Fig. 1). These enzymatic studies showed that the increase in metal concentration there was an increase in POX and PPO activities. CAT activity decreased with increasing concentration of Co (100-250 mg kg^{-1}) than the control and low level of Co (50 mg kg^{-1}) treated *A. hypogaea* plants. POX and PPO activities increased (except 50 mg kg^{-1}) with an increase in Co level in the soil. This can be compared with earlier reports such as Seliga [14] and Savour *et al.* [15].

To be able to endure oxidative damage under conditions which favours increased oxidative stress such as high/low temperatures, water deficit and salinity etc., plants must possess efficient antioxidant system [16]. Plants posses antioxidant systems in the form of enzymes such as SOD, APX, CAT and metabolites viz., ascorbic acid, glutathione, α-tocopherol, carotenoid, flavonoids, etc. [17]. These antioxidant enzymes and metabolites are reported to increase under various environmental stresses

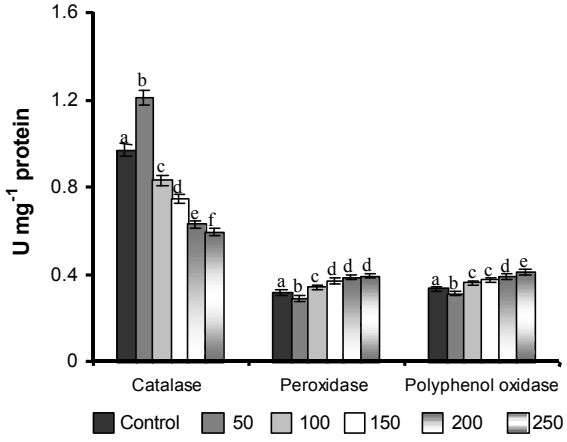

Fig. 1: Cobalt induced changes in antioxidant enzyme activities of *A. hypogaea*. Values are given as mean±SD of six experiments in each group. Bar values are not sharing a common superscript (a,b,c,d,e,f) differ significantly at $P \leq 0.05$ (DMRT).

[18] as well as comparatively higher activity has been reported in fungicide, triadimefon [5] and salt treatments [6,7] in medicinal plants, suggesting that higher antioxidant enzymes activity have a role in imparting tolerance against any type of environmental stresses.

Co treatment at all levels tested (except 50 mg kg^{-1}) decreased the various growth parameter such as root ad shoot length, number of nodules, total leaf area and dry weight of root and shoot; biochemical (pigment, sugar, starch, amino acid and protein) contents of leaves; antioxidant enzyme (CAT) activity of *A. hypogaea* plants. However the antioxidant enzymes (POX and PPO) increased with an increase in Co level in the soil. From the present investigation it can be concluded that the 50 mg kg^{-1} level of Co in the soil is beneficial for the growth of *A. hypogaea* plants.

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