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Phytochemical Changes in Green Gram (*Vigna radiata*) under Cobalt Stress

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Abstract: The effect of cobalt treatment on Antioxidant enzyme activities of *Vigna radiata* (L.) Wilczek, were studied. The plants were raised in earthen pots containing soils amended with different concentrations of cobalt (50, 100, 150, 200 and 250 mg kg^{-1}). Biochemicals like reducing, non-reducing, total sugar, starch, amino acid and protein content; antioxidant enzymes like catalase (CAT), peroxidase (POX) and polyphenol oxidase (PPO) activities were analysed on 30 days after sowing (DAS). All the biochemical constituents and antioxidant enzyme activities have beneficial value at 50 mg kg^{-1} cobalt level in the soil, when compared with control. Further increase in the cobalt level (100-200 mg kg^{-1}) in the soil have a negative effect on these parameters.

Key words: Antioxidants, Biochemical, Cobalt, *Vigna radiata*

INTRODUCTION

Cobalt is not classified as an essential element for plants, however, it is usually described as "beneficial". This trace element can be a contaminant in soils due to agricultural additives or metal refineries [1]. Certain plant species have the ability to extract metals (such as cobalt) from soils, thus, cleaning the environment. Cobalt is known to cause irreversible damage to a number of vital metabolic constituents and plant cell and cell membrane. While it has been known for many years that cobalt is an essential element for humans, animals and prokaryotes, a physiological function for this element in higher plants has not been identified. The cobalt-containing vitamin B_{12} does not occur in plants. Whereas normal cobalt concentrations in plants are cited to be as low as 0.1-10 μg g^{-1} dry weight, its beneficial role as a trace element has been described [1]. Trace elements are necessary for the normal metabolic functions of the plant, but at higher concentrations, these metals are toxic and may severely interfere with physiological and biochemical functions [2-4].

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 [5]. Plants under stress produces 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 [6]. ROS scavenging depends on the detoxification mechanism provided by an integrated system of non-enzymatic reduced molecules like ascorbate and glutathione and enzymatic antioxidants [7]. 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. [8]. The pathways include the water-water cycle in chloroplasts and the ascorbate-glutathione cycle [9]. Antioxidant mechanisms may provide a strategy to enhance metal tolerance in plants.

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The present investigation was executed with an objective to study the effects of cobalt treatment of *Vigna radiata* L. with specific emphasize on biochemical constituents and antioxidant enzymes activities which are the defense mechanism to any type of abiotic stress.

MATERIALS AND METHODS

The seeds of green gram (*Vigna radiata* (L.) Wilczek) were obtained from Tamil Nadu Rice Research Institute, 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. Plants were grown in pots in untreated soil (control) and in soil to which cobalt had been applied (50, 100, 150, 200 and 250 mg kg^{-1} soil). The inner surfaces of pots were lined with polythene sheet. Each pot contained 3 kg of air-dried soil. The cobalt as finely powdered ($CoCl_2$) was applied to the surface soil and thoroughly mixed with the soil. Ten 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 six times. The plant samples were collected on 30 days after sowing (DAS) for the analyse of various biochemical constituents and antioxidant enzyme activities.

The biochemical analysis such as total sugar [10], starch [11], amino acid [12] and protein [13] were carried out in fresh samples.

Catalase (CAT) (EC 1.11.1.6) activity was measured according the method of Chandlee and Scandalios [14] with small modification. 0.5 g of frozen plant material was homogenized in a prechilled pestle and mortar with 5 ml of ice cold 50 mM sodium phosphate buffer (pH 7.5) containing 1 mM phenylmethylsulfonyl fluoride (PMSF). The extract was centrifuged at 4°C for 20 min at 12,500 rpm. 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) was assayed by the method of Kumar and Khan [15]. 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 was assayed by the method of Kumar and Khan [15]. 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 purpurogallin formed was read at 495 nm. To the blank 2.5 N H_2SO_4 was added of the zero time at 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.

Each treatment was analysed with at least seven replicates and a standard deviation (SD) was calculated and data are expressed in $\overline{X}$ ± SD of seven replicates.

RESULTS AND DISCUSSION

Results on the effect of different concentration of cobalt on reducing, non-reducing, total sugar and starch content of green gram leaves are given in Table 1. There was a gradual decrease in the sugar and starch content with increasing cobalt level. The maximum decrease of sugar and starch content was recorded at 250 mg kg^{-1} cobalt level in the soil on 30 DAS.

Cobalt at 50 mg kg^{-1} soil level increased the reducing, non-reducing, total sugar and starch contents of green gram leaves. Further increase in cobalt level decreased the reducing, non-reducing, total sugar and starch contents. This was strengthened by the findings of Smarakoon and Rauser [16]; Mahadeswaraswamy *et al*. [17]; Greger and Lindberg [18]; Jayakumar and Vijayarengan [4].

The results of amino acid and protein content of cobalt treated green gram plants are given in Table 1. The amino acid and protein content of green gram leaves decreased appreciably with increasing concentration of applied cobalt in the soil. Maximum amino acid and protein content was recorded at 50 mg kg^{-1} cobalt level in the soil and minimum amino acid and protein content was observed at 250 mg kg^{-1} cobalt level in the soil on 30 DAS. The decrease in amino acid and protein content in excess of cobalt treated green gram is similar to the reported by Kastori *et al.* [19] and Bhattacharjee and Mukherjee [20].

Table 1: Effect of cobalt on biochemical (mg kg^{-1} FW) changes in *Vigna radiata*

Cobalt (mg kg^{-1})	Reducing sugar	Non-reducing sugar	Total sugar	Starch	Amino acid	Protein
Control	2.183±0.065	2.763±0.082	4.946±0.148	4.432±0.132	2.847±0.085	5.317±0.159
50	2.898±0.086	3.115±0.093	6.013±0.180	5.869±0.176	3.215±0.096	6.263±0.187
100	1.967±0.059	2.469±0.074	4.436±0.133	4.265±0.127	2.683±0.080	4.635±0.139
150	1.634±0.049	2.133±0.063	3.767±0.113	3.241±0.097	2.465±0.073	3.794±0.113
200	1.103±0.033	1.217±0.036	2.320±0.069	2.837±0.085	2.141±0.064	2.832±0.084
250	0.784±0.023	0.946±0.028	1.730±0.051	2.165±0.064	1.943±0.058	2.119±0.063

Values are given as mean ± SD of seven experiments in each group

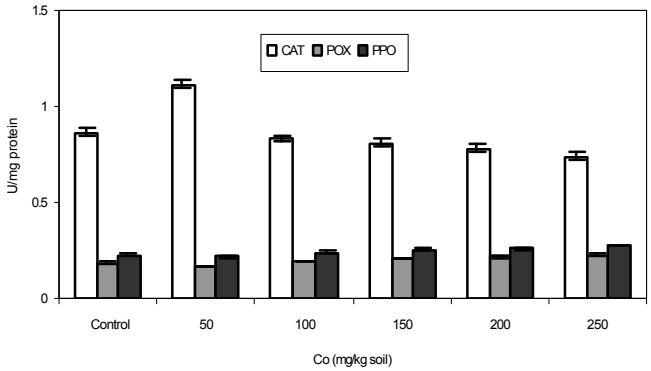

Fig. 1: Effect of cobalt on catalase (CAT), peroxidase (POX) and polyphenol oxidase (PPO) activities of Vigna radiata (Values are given as mean ± SD of seven experiments in each group)

CAT activity decreased with increasing concentration of Cobalt (100-250 mg kg^{-1}) than the control and low level of Cobalt (50 mg kg^{-1}) treated *Vigna radiata* plants. POX and PPO activities increased (except 50 mg kg^{-1}) with an increase in Cobalt level in the soil. This can be compared with earlier reports such as Seliga [21], Savour *et al*. [22] and Chen *et al*. [23].

To be able to endure oxidative damage under conditions, which favours increased oxidative stress such as high/low temperatures, water deficit, salinity etc., plants must possess efficient antioxidant system [24]. 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. [25]. These antioxidant enzymes and metabolites are reported to increase under various environmental stresses [26] as well as comparatively higher activity has been reported in fungicide, triadimefon [27] and salt treatments [28] in medicinal plants, suggesting that higher antioxidant enzymes activity have a role in imparting tolerance against any type of environmental stresses.

CONCLUSION

Cobalt treatment at all levels tested (except 50 mg kg^{-1}) decreased the biochemical constituents and antioxidant enzyme (CAT) activity of *Vigna radiata* plants. However the antioxidant enzymes (POX and PPO) increased with an increase in cobalt level in the soil. From the present investigation it can be concluded that the 50 mg kg^{-1} level of cobalt in the soil is beneficial for the growth of *Vigna radiata* plants.

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