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Biochemical Variations in Ground Nut under Cobalt Applications

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Abstract: In the present investigation, the effect of cobalt (Co) stress on biochemical contents of *Arachis hypogaea* was studied. Biochemicals like total sugar, starch, amino acid and protein contents were analysed. Biochemicals have beneficial value at 50 mg kg^{-1} Co level in the soil, when compared with untreated control plants. Further increase in the Co level (100-200 mg kg^{-1}) in the soil has a negative effect on these parameters. From these results it is clear that biochemical contents of *A. hypogaea* have a positive response upon low concentrations of cobalt in the soil.

Key words: *Arachis hypogaea,* Total sugar, Starch, Amino acid, Protein

INTRODUCTION

With the development of industries, mining activities, application of wastewater and sewage sludge on land, heavy metal pollution of soils is increasingly becoming a serious environmental problem [1]. The concentration of heavy metals in air, water and soil leads to many hazardous effects to living organisms. Excessive metal concentrations in contaminated soils can result in decreased soil microbial activity and soil fertility and yield losses [2]. Accumulation of trace elements, especially heavy metals, in the soil has potential to restrict the soil's function, cause toxicity to plants and contaminate the food chain [3].

The heavy metals can create a major ecological crisis since they are non-degradable and often accumulate by plant parts, biologically magnified through trophic levels and causing a deleterious biological effect. As a response to the toxic action of metals various protective mechanisms have been elaborated in plants [4-6].

Cobalt (Co) as a trace element can be a contaminant in soils due to agricultural additives or metal refineries [7]. Certain plant species have the ability to extract metals (such as Co) from soils, thus, cleaning the environment. Co are known to cause irreversible damage to a number of vital metabolic constituents and plant cell and cell membrane. While it has been known for may years that Co is an essential element for humans, animals and prokaryotes, a physiological function for this element in higher plants has not been identified.

The present investigation was executed with an objective to study the effects of Co stress on growth and biochemical constituents of *Arachis hypogaea* L. with specific emphasize on antioxidant 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

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surface of pots was 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 biochemical constituents.

Biochemical Analysis

Estimation of Total Sugars [8]: Plant samples were treated with 80 per cent boiling ethanol for taking extractions (5ml extract representing 1g of tissue). Five readings for each sample were taken. One ml of ethanol extract taken in the test tubes was evaporated in a water bath. To the residue, 1 ml of distilled water and 1ml of 1N sulphuric acid were added and incubated at 49°C for 30 minutes. The solution was neutralised with 1N sodium hydroxide using methyl red indicator. One ml of Nelson's reagent was added to each test tube prepared by mixing reagent A and reagent B in 25:1 ratio (Reagent A: 25g sodium carbonate, 25g sodium potassium tartarate, 20g sodium bicarbonate and 200g anhydrous sodium sulphate in 1000 ml: Reagent B: 15g cupric sulphate in 100 ml of distilled water with 2 drops of concentrated sulphuric acid). The test tubes were heated for 20 minutes in a boiling water bath, cooled and 1ml of arsenomolybdate reagent (25g ammonium molybdate, 21 ml concentrated sulphuric acid, 5g sodium arsenate dissolved in 475 ml of distilled water and incubated at 37°C in a water bath for 48 hours) was added. The solution was thoroughly mixed and diluted to 25 ml and measured at 495 nm in a spectrophotometer. The reducing sugar contents of unknown samples were calculated from glucose standard.

Estimation of Protein [9]: Fresh tissue weighting 0.5g was macerated in 20 per cent trichloroacetic acid using mortar and pestle. The homogenate was then centrifuged at 600 xg for 30 minutes and the supernatant was discarded. To the pellet, 5 ml of 0.1N NaOH was added and centrifuged for 30 minutes. The supernatant was saved for the estimation of protein.

To 0.5 ml of protein extract, 5 ml of copper reagent (C) was added (Reagent C: mixture of reagent A and B at 50:1 ratio; reagent A: 2 per cent Na_2CO_3 in 0.1N NaOH; reagent B: equal volume of 1 per cent $CuSO_4$ and two per cent of sodium potassium tartarate). The tubes were shaken well and allowed to stand in dark for 10 minutes at room temperature. Properly diluted Folin-ciocalteau reagent (0.5 ml) was added to this solution and mixed thoroughly. The absorbance was read at 660nm in a spectrophotometer against an appropriate blank. Bovin serum albumin was used as the standard.

Estimation of Starch [10]: The ethanol insoluble residues taken from ethanol extraction were dried at 60°C for 4 hours in an oven. To 200 mg of the powdered residue, 3 ml of 6 N HCl was added and autoclave at 100°C for an hour. The flask was cooled and volume was raised to 25 ml with distilled water. One ml of aliquot was drawn and neutralized with 1 N NaOH and sugar was estimated by Nelson's method (Nelson 1944).

Estimation of Total Free Amino Acids [11]: One ml ethanol extract was taken in 25 ml test tubes and neutralized with 0.1N sodium hydroxide using methyl red indicator. One ml of ninhydrin reagent was added (800 mg stannous chloride in 500 ml citrate buffer, pH 5.0, 20g ninhydrin in 500 ml methyl cellosolve; both solutions were mixed). The contents were boiled in a water bath for 20 minutes, 5 ml of diluent solution (distilled water and *n*-propanol mixed in equal volume) was added, cooled and diluted to 25 ml with distilled water. The absorbance was measured at 570 nm in a spectrophotometer. The standard graph was prepared using leucine.

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

Total sugar, amino acid, starch and protein contents (Fig. 1) of *A. hypogaea* were increased at 50 mg kg^{-1} soil level of Co and decreased further with an increase in the Co level (100-250 mg kg^{-1}). Sugar and starch content of plants showed a decreasing trend with progressive increase in Co level in the soil, however, 50 mg kg^{-1} Co level produced positive effect on the total sugar and starch content which is in consonance with the findings of Vijayarengan and Dhanavel [12] in blackgram. The accumulation of total sugar and starch decreased with increase in Co level. Response is similar to that response by Greger & Lindberg [13] in sugar beets. Considerable change in the "Physiological effect"

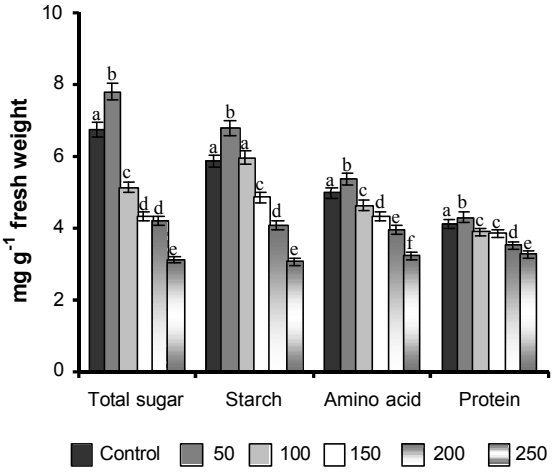

Fig. 1: Cobalt induced changes in biochemical parameters of *A. hypogaea*. Values are given as mean ± SD of six experiments in each group. Bar values are not sharing a common superscript (a,b,c,d,e,f) differ significantly at $P \leq 0.05$ (DMRT)

has been observed in crops grown in soil contaminated with even moderate level of metals [14]. In order to obtain a better understanding the basis of "Physiological effect" the effect of Co on chlorophyll, sugar, starch, amino acid and protein contents have been reported.

Co level above 50 mg kg^{-1} significantly reduced the amino acid and protein contents in leaves of *A. hypogaea* plants. Nitrogen is a precursor for the synthesis of amino acids [15], since the nitrogen content of the metal treated plants was found reduced, ultimately amino acids and protein contents of plants were also reduced [6] because there was only limited availability of nitrogen for the synthesis of amino acid.

Co at 50 mg kg^{-1} soil level increased the amino acid and protein contents of *A. hypogaea* plants. Nag *et al.* [16] observed similar trends due to heavy metals like copper, zinc, mercury, lead and cadmium in rice. Further increase in Co level decreased the amino acid and protein contents. This was strengthened by the findings of Stiborova *et al.* [17] in copper and zinc, cadmium, mercury and lead.

Co treatment at all levels tested (except 50 mg kg^{-1}) decreased the various biochemical parameters such as sugar, starch, amino acid and protein contents of leaves. 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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