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Alterations in sugar metabolism of *Glycine max* with cobalt applications

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Abstract
Exogenous Cobalt was given to Soybean (*Glycine max*) plants in pot culture by soil drenching method in order to study the effects of Co stress in the sugar metabolism. For estimating the changes in the sugar metabolism, reducing and non-reducing sugars, total sugar, and starch contents were extracted and estimated from both control and treated plant parts (from roots and shoot). The results showed the low concentrations of cobalt (50 mg kg^{-1} Co level) in the soil enhanced these parameters while higher concentration (Co level (100-200 mg kg^{-1}) in the soil) didn't show any significant effect.

1. Introduction

Mineral rock weathering and anthropogenic sources provide two of the main types of metal inputs to soils. The anthropogenic sources of metal contamination can be divided to five main groups: metalliferous mining and smelting (arsenic, cadmium, lead and mercury); industry (arsenic, cadmium, chromium, cobolt, copper, mercury, nickel, zinc); atmospheric deposition (arsenic, cadmium, chromium, copper, lead, mercury, uranium); agriculture (arsenic, cadmium, copper, lead, selenium, uranium, zinc); and waste disposal (arsenic, cadmium, chromium, copper, lead, mercury, zinc) [1-5]. In Finland, most cases of soil metal contamination have been caused by waste treatment plants, sawmills, wood impregnation plants, shooting ranges, garages and scrap yards [6].

Metals play an integral role in the life processes of microorganisms. Some metals, such as calcium, cobalt, chromium, 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 [7-9]. 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 [10]. 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 [7-9].

Cobalt usually occurs in association with other metals such as copper, nickel, manganese and arsenic. All soils contain some amount of cobalt. The average concentration of cobalt in soils throughout the world is 8 ppm. Cobalt concentrations in soils around Ontario mine sites have been reported as high as 6,450 ppm.

The present investigation has been carried out to find out the effect of cobalt as a heavy metal pollutant on sugar metabolism of soybean (*Glycine max* (L.) Merr.) cultivar CO-1.

2. Materials and Methods

Soybean (*Glycine max* (L.) Merr.) cultivar CO-1 belongs to the family Fabaceae was selected for the study. Seeds with uniform size, colour and weight were chosen for experimental purpose. Seeds were surface sterilized with 0.1 per cent mercuric chloride solution and washed thoroughly with tap water and then with distilled water.

The 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 a 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 pots were watered to field capacity daily. Plants were thinned to a maximum of six per pot, after a week of germination. Each treatment including the control was replicated five times.

The plant samples were collected at thirty days interval, upto harvest stage viz., 30, 60 and 90^{th} day for the measurement of various morphological growth parameters. The reducing sugar, total sugar, and starch contents of the plants were estimated at all the three sampling periods. Six plants from each replicate of a pot were analysed for their various parameters and the average was calculated. These mean values were used for statistical analysis.

2.1. Estimation of non-reducing sugars [11]

Non-reducing sugars present in the ethanol extracts (extraction as in reducing sugar) were hydrolysed with sulphuric acid to reducing sugars. Reducing sugars present in the hydrolysates were estimated following Nelson's method. The difference between the total sugars and the reducing sugars estimated without hydrolysis corresponds to the non-reducing sugars.

One ml of extract was taken in a test tube and evaporated to dryness on a water bath for 15 minutes. To the residue, 1 ml of distilled water and 1 ml of 0.1 N sulphuric acids were added. The mixture was hydrolysed by incubating at 49°C for 30 minutes in a thermostat. The solution was neutralized with 0.1 N NaOH (5 ml) methyl red as indicator. To this 1 ml of reagent C (copper reagent) was added and heated for 20 minutes cooled and 1 ml of arsenomolybdate reagent was added. The content was made up to 25 ml and the absorbance read at 495 nm. Reagent blank was prepared with 1ml of distilled water.

2.2. Estimation of total sugars [11]

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.

2.3. Estimation of starch [12]

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).

3. Results

3.1. Reducing sugar

Reducing sugar content of root of soybean plants under cobalt stress is represented in Table 1. Reducing sugar content of root was maximum at 50 mg kg^{-1} soil level (3.131). Minimum reducing sugar content of soybean root was recorded at 250 mg kg^{-1} soil level (1.649).

Reducing sugar content of shoot of soybean plants under cobalt stress is represented in Table 1. Reducing sugar content of shoot was maximum at 50 mg kg^{-1} soil level (3.367). Minimum reducing sugar content of soybean shoot was recorded at 250 mg kg^{-1} soil level (1.953).

3.2. Non-reducing sugar in Root

Non-reducing sugar content of root of soybean plants under cobalt stress is represented in Table 2. Non-reducing sugar content of root was maximum at 50 mg kg^{-1} soil level (3.917). Minimum non-reducing sugar content of soybean root was recorded at 250 mg kg^{-1} soil level (2.317).

Table 1. Effect of cobalt on reducing sugar content (mg g^{-1} fresh weight) of *Glycine max* (L.) Merr.

Cobalt added in the soil (mg kg^{-1})	ROOT			SHOOT		
	Sampling days					
	30	60	90	30	60	90
Control	1.865	2.117	2.862	2.065	2.335	3.142
50	1.937 (+3.860)	2.362 (+11.57)	3.131 (+9.399)	2.237 (+8.329)	2.612 (+11.86)	3.367 (+7.116)
100	1.613 (-13.51)	1.842 (-12.99)	2.532 (-11.53)	1.815 (-12.10)	2.064 (-11.60)	2.835 (-9.770)
150	1.347 (-27.77)	1.611 (-23.90)	2.273 (-20.58)	1.562 (-24.35)	1.890 (-19.05)	2.592 (-17.50)
200	1.198 (-35.76)	1.472 (-30.46)	2.039 (-28.75)	1.376 (-33.36)	1.683 (-27.92)	2.274 (-27.62)
250	0.967 (-48.15)	1.143 (46.00)	1.649 (-42.38)	1.143 (-44.64)	1.371 (-41.28)	1.953 (-37.84)

Table 2. Effect of cobalt on non - reducing sugar content (mg g^{-1} fresh weight) of *Glycine max* (L.) Merr.

Cobalt added in the soil (mg kg^{-1})	ROOT			SHOOT		
	Sampling days					
	30	60	90	30	60	90
Control	2.132	2.865	3.632	2.352	3.112	3.865
50	2.617 (+22.74)	3.273 (+14.24)	3.917 (+7.846)	2.641 (+12.28)	3.541 (+13.78)	4.259 (+10.19)
100	1.931 (-9.427)	2.634 (-8.062)	3.212 (-11.56)	2.198 (-6.547)	2.9212 (-6.137)	3.585 (-7.244)
150	1.743 (-18.24)	2.471 (-13.75)	2.867 (-21.06)	1.963 (-16.53)	2.715 (-12.75)	3.221 (-16.66)
200	1.517 (-28.84)	2.198 (-23.28)	2.638 (-27.36)	1.741 (-25.97)	2.368 (-23.90)	2.976 (-23.00)
250	1.362 (-36.11)	1.843 (-36.67)	2.317 (-36.20)	1.594 (-32.22)	2.103 (-32.42)	2.631 (-31.92)

(Per cent over control values are given in parentheses)

Non-reducing sugar content of shoot of soybean plants under cobalt stress is represented in Table 2. Non-reducing sugar content of shoot was maximum at 50 mg kg^{-1} soil level (4.259). Minimum non-reducing sugar content of soybean shoot was recorded at 250 mg kg^{-1} soil level (2.631).

3.3. Total sugar

Total sugar content of root of soybean plants under cobalt stress is represented in Table 3. Sugar content of root was maximum at 50 mg kg^{-1} soil level (7.048). Minimum sugar content of soybean root was recorded at 250 mg kg^{-1} soil level (4.166).

Total sugar content of shoot of soybean plants under cobalt stress is represented in Table 3. Sugar content of shoot was maximum at 50 mg kg^{-1} soil level (7.629). Minimum sugar content of soybean shoot was recorded at 250 mg kg^{-1} soil level (4.929).

Table 3. Effect of cobalt on total sugar content (mg g^{-1} fresh weight) of *Glycine max* (L.) Merr.

Cobalt added in the soil (mg kg^{-1})	ROOT			SHOOT		
	Sampling days					
	30	60	90	30	60	90
Control	3.997	4.982	6.494	4.417	5.447	7.007
50	4.554 (+13.93)	5.635 (+13.10)	7.048 (+8.530)	4.878 (+10.43)	6.153 (+12.96)	7.626 (+8.834)
100	3.544 (-11.33)	4.476 (-9.427)	5.849 (-9.932)	4.013 (-9.146)	4.985 (-8.481)	6.420 (-8.377)
150	3.090 (-22.69)	4.082 (-18.06)	5.288 (-18.57)	3.525 (-20.19)	4.605 (-15.45)	5.813 (-17.04)
200	2.624 (-34.35)	3.670 (-26.33)	4.777 (-26.43)	3.117 (-29.43)	4.051 (-25.62)	5.20 (-25.07)
250	2.329 (-41.73)	2.986 (-40.06)	4.166 (-35.84)	2.737 (-38.03)	3.474 (-36.22)	4.929 (-29.65)

(Per cent over control values are given in parentheses)

3.4. Starch

Starch content of root of soybean plants is presented in Table 4. Starch content of root of soybean plants increased at 50 mg kg^{-1} soil level (4.863) and decreased further with an increase in cobalt level in the soil. Minimum starch content of soybean root was observed at 250 mg kg^{-1} soil level (2.294).

Starch content of shoot of soybean plants is presented in Table 4. Starch content of shoot of soybean plants increased at 50 mg kg-1 soil level (6.119) and decreased further with an increase in cobalt level in the soil. Minimum starch content of soybean shoot was observed at 250 mg kg-1 soil level (3.974).

4. Discussion

Sugars (reducing, non-reducing and total sugar) and starch content of soybean plants showed a decreasing trend with progressive increase in cobalt level in the soil. However, 50 mg kg^{-1} cobalt level produced positive effect on the reducing, non-reducing, total sugar and starch contents, which is in consonance with the findings [13]. The accumulation of reducing, non-reducing, total sugar and starch decreased with increase in cobalt level. The response is similar to early reports [14-16].

Table 4. Effect of cobalt on starch content (mg g^{-1} fresh weight) of *Glycine max* (L.) Merr.

Cobalt added in the soil (mg kg^{-1})	ROOT			SHOOT		
	Sampling days					
	30	60	90	30	60	90
Control	3.540	3.934	4.217	4.103	5.312	5.631
50	4.103 (+15.90)	4.423 (+12.43)	4.863 (+15.31)	4.561 (+11.16)	5.873 (+10.56)	6.119 (+8.666)
100	3.121 (-11.83)	3.481 (-11.51)	3.814 (-9.556)	3.318 (-19.13)	4.213 (-20.68)	4.497 (-20.13)
150	2.761 (-22.00)	3.063 (-22.14)	3.367 (-20.15)	3.214 (-21.66)	4.092 (-22.96)	4.268 (-24.20)
200	2.354 (-33.50)	2.613 (-33.57)	2.858 (-32.22)	2.932 (-28.54)	3.938 (-25.86)	4.118 (-27.01)
250	1.985 (-43.92)	2.186 (-44.43)	2.294 (-45.60)	2.727 (-33.33)	3.768 (-29.06)	3.974 (-29.42)

(Per cent over control values are given in parentheses)

Heavy metals taken up by vegetables grown with wastewater tend to remain elements in the roots, and only a fraction of the heavy elements absorbed is translocated to the tops, part of which reaches the fruit and showed a marked variation in their soluble starch and sugar contents [17]. The accumulation of heavy metals in different parts of the plant body might be due to the tendency of different parts of the plant to accumulate certain amounts of metals which in turn alters the soluble carbohydrate concentrations [18].

Translocation of heavy metals in tomato is dependent upon the carbohydrate partitioning, which is under control by the effects of heavy metals [19]. From this point of view, it is quite clear that, plants under cobalt treatment might be largely affected in terms of their soluble carbohydrate (starch and sugar) concentration.

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