Botany Research International 2 (3): 153-156, 2009
ISSN 2221-3635

Effect of Different Concentrations of Cobalt on Pigment Contents of Soybean

K. Jayakumar, Cheruth Abdul Jaleel and P. Vijayarengan

Department of Botany, Annamalai University, Annamalainagar 608 002, Tamilnadu, India

Abstract: A pot culture experiment was conducted to estimate the effect of cobalt (0, 50, 100, 150, 200 and 250 mg kg^{-1} soil) on pigment accumulation of soybean. Pot culture experiments were conducted. Periodical observations were made to record. From the results, it is observed that, cobalt at lower concentrations have some beneficial values on soybean.

Key words: Soybean · *Glycine max* · Cobalt · Pigments

INTRODUCTION

Heavy metals are widely distributed in the environment, in soils, in plants and animals in most of their tissues. Pollution of the environment by heavy metals is normally associated with human activity. Metallic elements are an intrinsic component of the environment. Heavy metals are included in the main category of environmental pollutants as they can remain in the environment for long periods; their accumulation is potentially hazardous to humans, animals and plants [1-5]. We have witnessed in the last few decades a dramatic, worrisome increase in contamination of the environment, including soil, air and water. It would appear that humans are the only ones to blame, because anthropogenic activities are the main source of the pollution that is causing the contamination [3-5]. Moreover, there are numerous reports indicating that accidents, such as the contamination of the soil in mining areas, spills of toxic metals into rivers and eventual contamination of water, among other possible events, have been responsible for the deterioration of the environment and have affected people's lives in several ways. It is important to mention that such disasters have not solely been observed in developing countries, but throughout the world. It is quite obvious from the studies carried out along the years that heavy metals have adverse effects on plants and their productivity, although some metals are essential for plant growth in small quantities [2-6]. Furthermore, these metals can enter the plant system, accumulate and later may enter the food chain and cause harm to humans and animals [5-7]. The accumulation of metals in plants is particularly important because some species have been characterized as hyperaccumulators. They may be used, as along with specifically designed transgenic plants, in phytoremediation [6-9]. Moreover, the use of bio-remediation techniques has been replacing whenever possible the traditional engineering approaches.

Such a situation has led to investigations on a wide range of aspects related to heavy metals. For instance, there has been intensive research on metals in soil related to plant nutrition, general effects on plant metabolism, tolerance-susceptibility and environmental effects as well as on how contaminated areas can be reclaimed [10-11].

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 [12-14]. Cobalt is known to cause irreversible damage to a number of vital metabolic constituents and plant cell and cell membrane. A pot culture experiment was conducted to estimate the effect of cobalt (0, 50, 100, 150, 200 and 250 mg kg^{-1}soil) on photosynthetic pigments of soybean.

MATERIALS AND METHODS

The *Glycine max* (L.) Merr. seeds used in the experiments were obtained from the Tamil Nadu Agricultural University, Coimbatore, India. 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. Soybean (*Glycine max* (L.) Merr.) were grown in pots in

Corresponding Author: Dr. P. Vijayarengan, Department of Botany, Annamalai University, Annamalainagar 608 002, Tamilnadu, India

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 photosynthetic pigments. Six plants from each replicate of a pot was analysed for it's various parameters and the average was calculated. These mean values were used for statistical analysis.

Estimation of Chlorophyll [15]: Hundred milligram of fresh leaf was ground in a mortar and pestle with 20 ml of 80% acetone. The homogenate was centrifuged at 3000 rpm for 15 minutes. The supernatant was saved. The pellet was reextracted with 5 ml of 80% acetone each time, until it become colourless. All the supernatants were pooled and utilized for chlorophyll determination. Absorbance was measured at 645nm and 663nm in spectrophotometer. The chlorophyll content was determined by using Arnon formula.

RESULTS AND DISCUSSION

Effect of cobalt on the pigment content of soybean was represented in Table 1-3. Pigments, such as chlorophyll-a (Table 1), chlorophyll-b (Table 2) and total chlorophyll (Table 3) content of soybean leaves increased at lower concentration (50 mg kg^{-1}) and decreased further with an increase in the cobalt level (100-250 mg kg^{-1}). Total chlorophyll content of the control is (1.249) and increased at 50 mg kg^{-1} (1.423) and decreased at 250 mg kg^{-1} respectively. Heavy metal pollution causes potential ecological risk. The base of phytoremediation, a promising method for cleaning of soil and water, is pollutant uptake or bounding by plants. Initially much more interest was focused on hyper-accumulator plants than those taken in non-accumulator plants [16], but later, as an alternative to substitution of the endemic, hardly cultivable plants accumulator plants have been used; possibly coupled with treatment of soil [17]; either considering factors to increase the metal availability and plant uptake. Other possibility to decrease available concentration of pollutants is stabilisation. Phyto-stabilisation can gain results from either physical or chemical effects of plants and of chemicals, such beringit, lime or clay minerals [18-19].

Table 1: Effect of cobalt on chlorophyll 'a' content (mg g^{-1} fresh weight) of *Glycine max* (L.) Merr

Cobalt added in the soil (mg kg^{-1})	Sampling days 30	60	90
Control	0.513	0.578	0.613
50	0.607 (+18.32)	0.693 (+19.89)	0.732 (+19.41)
100	0.482 (-6.042)	0.545 (-5.709)	0.592 (- 3.425)
150	0.405 (-24.05)	0.437 (-24.39)	0.489 (- 20.22)
200	0.317 (-38.20)	0.369 (-36.15)	0.385 (- 37.19)
250	0.231 (-54.97)	0.282 (- 51.21)	0.314 (- 48.77)

(Per cent over control values are given in parentheses)

Comparison of significant effectsF test

Cobalt levels** Sampling days**

Table 2: Effect of cobalt on chlorophyll 'b' content (mg g^{-1} fresh weight) of *Glycine max* (L.) Merr

Cobalt added in the soil (mg kg^{-1})	Sampling days 30	60	90
Control	0.386	0.511	0.636
50	0.430 (+11.39)	0.587 (+14.87)	0.691 (+8.647)
100	0.347 (-10.10)	0.471 (-7.827)	0.589 (- 7.389)
150	0.312 (-19.17)	0.392 (-23.28)	0.517 (- 18.71)
200	0.281 (-27.20)	0.334 (-34.63)	0.430 (- 32.38)
250	0.267 (-30.82)	0.293 (- 42.66)	0.369 (- 41.98)

(Per cent over control values are given in parentheses)

Comparison of significant effectsF test

Cobalt levels** Sampling days**

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

Cobalt added in the soil (mg kg^{-1})	Sampling days 30	60	90
Control	0.899	1.089	1.249
50	1.037 (+15.35)	1.326 (+21.76)	1.423 (+13.93)
100	0.829 (-7.786)	1.016 (-6.703)	1.181 (- 5.444)
150	0.717 (-20.24)	0.799 (-26.62)	1.006 (- 19.45)
200	0.598 (-33.48)	0.683 (-37.28)	0.823 (- 34.10)
250	0.476 (-47.05)	0.565 (- 48.11)	0.683 (- 45.31)

(Per cent over control values are given in parentheses)

Comparison of significant effectsF test

Cobalt levels** Sampling days**

The photosynthetic pigments such as chlorophyll-a, chlorophyll-b and total chlorophyll contents of soybean decreased with increasing cobalt level in the soil. Similar changes in the content by various metal treatments were recorded [20,21]. The chlorophyll content of leaves varied greatly with the zinc level in the soil indicating the pronounced effect on chlorophyll biosynthesis. The increased chlorophyll content at lower level of cobalt was obviously due to better growth.

The mechanism of heavy metals on photosynthetic pigments may be owed to three reasons: 1. Heavy metals enter frond chloroplast and may be over-accumulated in local causing oxidative stress which will cause damages like peroxidation of chloroplast membranes [22]. Also they can directly destroy the structure and function of chloroplast by binding with -SH group of enzyme and over all chlorophyll biosynthesis through Mg2þ, Fe2þ or Zn2þ [23]. Heavy metal ions inhibit uptake and transportation of other metal elements such as Mn, Zn and Fe by antagonistic effects and therefore the fronds lose the capacity of synthesis of pigments [24]. Heavy metals may activate pigment enzyme and accelerate the decomposition of pigment.

The excess cobalt treatment brought about by a marked depression in photosynthetic pigments in soybean plants. It might be due to excess supply of cobalt resulting in interference with the synthesis of chlorophyll. The formation of chlorophyll pigments depends on the adequate supply of iron [19-21] which has suggested protoporphyrin is a precursor for chlorophyll synthesis. The excess supply of cobalt seems to prevent the incorporation of iron in protoporphyrin molecule resulting in the reduction of chlorophyll pigment. This was strengthened by the fact that excessive amounts of a range of heavy metals such as copper. Impaired chlorophyll development by heavy metals may be due to the interference of protein; the treatments presumably blocked the synthesis and activities of enzyme proteins responsible for chlorophyll biosynthesis.

REFERENCE

1. Abdul Jaleel, C., K. Jayakumar, Zhao Chang-Xing and Muhammad Iqbal, 2009. Low Concentration of Cobalt Increases Growth, Biochemical Constituents, Mineral Status and Yield in *Zea Mays*. Journal of Scientific Research, 1: 128-137.
2. Jayakumar, K., P. Vijayarengan, Zhao Chang-Xing and C. Abdul Jaleel, 2008. Soil applied cobalt alters the nodulation, leg-haemoglobin content and antioxidant status of *Glycine max* (L.) Merr. Colloids and Surfaces B: Biointerfaces, 67(2): 272-275.
3. Webber, J., 1981. Trace metals in agriculture. In: Lepp NW, editor. Effect of heavy metal pollution on plants: Metals in the environment, vol. 11. London and New Jersey: Applied Sci. Publ, pp: 15-184.
4. Baker, A.J.M., S.P. McGrath, C.M.D. Sidoli and R.D. Reeves, 1994. The possibility of in situ heavy metal decontamination of polluted soils using crops of metal-accumulating plants. Resource, Conservation and Recycling, 11: 41-49.
5. Deram, A., D. Petit, B. Robinson, R.R. Brooks and P. Gregg and Ch. vanHalluvyn 2000. Natural and induced heavy-metal accumulation by *Arrhenatherum elatius*: Implications for phytoremediation. Commun Soil Sci Plant Anal., 31: 413-421.
6. Lehoczky, É., P. Marth, I. Szabados and A. Szomolányi, 2000. The cadmium uptake by lettuce on contaminated soils as influenced by liming. Commun Soil Sci. Anal., 31: 2433-2438.
7. Jayakumar, K., C. Abdul Jaleel and M.M. Azooz, 2008. Impact of cobalt on germination and seedling growth of *Eleusine coracana* L. and *Oryza sativa* L. under hydroponic culture. Global Journal of Molecular Sciences, 3(1): 18-20.
8. Jayakumar, K., C. Abdul Jaleel and P. Vijayarengan, 2007. Changes in growth, biochemical constituents and antioxidant potentials in radish (*Raphanus sativus* L.) under cobalt stress. Turkish Journal of Biology, 31: 127-136.
9. Jayakumar, K., C. Abdul Jaleel, M.M. Azooz. Mineral constituent variations under cobalt treatment in *Vigna mungo* (L.) Hepper. Global Journal of Molecular Sciences, 3(1): 32-34.
10. Jayakumar, K., Zhao Chang-Xing, M.M. Azooz and C. Abdul Jaleel, 2009. Antioxidant potentials protect *Vigna radiata* (L.) Wilczek plants from soil cobalt stress and improve growth and pigment composition. Plant Omics Journal, 2/3: 120-126.
11. Németh, T., E. Molnár, J. Csillag, K. Bujtás, A. Lukács and G. Pártay, 1993. Fate and Plant Uptake of Heavy Metals in Soil-Plant Systems Studied on Soil Monoliths. Agrokémia és Talajtan, 42: 195-207.
12. Simon, L., 2001. Effects of natural zeolite and bentonite on the phytoavalability of heavy metals in chicory. In Environmental Restoration of Metals Contamined Soil ed., Iskander, IK Chapter 13, Lewis Publ, Boca Raton, pp: 261-271.
13. Haavisto, T., 2002. Contaminated sites in Finland-Overview, 2001. The Finnish environment series, Helsinki, Finland (in press).
14. Nies, D.H., 1999. Microbial heavy-metal resistance. Appl. Microbiol. Biotechnol., 51: 730-750.

15. Bruins, M.R., S. Kapil and F.W. Oehme, 2000. Microbial resistance to metals in the environment. Ecotoxicol and Environ Safety, 45: 198-207.
16. Barcelo, J. and C. Poschenrieder, 2002. Fast root growth responses, root exudates and internal detoxification as clues to the mechanisms of aluminium toxicity and resistance: a review. Environ. Exp. Bot., 48: 75-92.
17. Ciamporova, M., 2002. Morphological and structural responses of plant roots to aluminium at organs, tissue and cellular levels. Biol. Plant, 45: 161-171.
18. Exley, C., 2004. The pro-oxidant activity of aluminium. Free Radic. Biol. Med., 36: 380-387.
19. Jain, M., G. Mathur, S. Koul and N.B. Sarin, 2001. Ameliorative effects of proline on salt stress-induced lipid peroxidation in cell lines of groundnut (*Arachis hypogea* L.). Plant Cell Rep., 20: 463-468.
20. Clemens, S., 2001. Molecular mechanisms of plant metal tolerance and homeostasis. Planta, 212(4): 475-486.
21. Clemens, S., 2001. Molecular mechanisms of plant metal tolerance and homeostasis, Planta, 212: 475-486.
22. Clemens, M.G. Palmgren and U. Kramer, 2002. A long way ahead: understanding and engineering plant metal accumulation, Trends Plant Sci., 7: 309-315.
23. Baker, A.J.M., 1981. Accumulators and excluders. Strategies in the response of plants to heavy metals, J. Plant Nutr., 3: 643-654.
24. Cobbett, C.S., 2000. Phytochelatins and their roles in heavy metal detoxification, Plant Physiol., 123: 825-832.