Turkish Journal of Biology

Volume 34 | Number 1 Article 2

1-1-2010

The effect of 4-chloroindole-3-acetic acid on some growth parameters in mung bean under cadmium stress

MOHAMMAD MAZID

BARKET ALI

SHAMSUL HAYAT

AQIL AHMAD

Recommended Citation

MAZID, MOHAMMAD; ALI, BARKET; HAYAT, SHAMSUL; and AHMAD, AQIL (2010) "The effect of 4-chloroindole-3-acetic acid on some growth parameters in mung bean under cadmium stress," *Turkish Journal of Biology*: Vol. 34: No. 1, Article 2. https://doi.org/10.3906/biy-0805-9

Available at: https://journals.tubitak.gov.tr/biology/vol34/iss1/2

Turk J Biol
34 (2010) 9-13
© TÜBİTAK
doi:10.3906/biy-0805-9

The effect of 4-chloroindole-3-acetic acid on some growth parameters in mung bean under cadmium stress

Mohammad MAZID[1], Barket ALI[2], Shamsul HAYAT[1,*], Aqil AHMAD[3]

[1]Plant Physiology & Biochemistry Division, Department of Botany, Aligarh Muslim University, Aligarh 202 002 - INDIA

[2]Department of Botany, Government College for Women, M.A. Road, Srinagar, Kashmir- INDIA

[3]Department of Applied Sciences, Higher College of Technology, Al-Khuwair - SULTANATE OF OMAN

Received: 13.05.2008

Abstract: Seedlings of mung bean (*Vigna radiata* L. Wilczek) cv. T-44 grown on a sand culture were exposed to cadmium (Cd) (100 µM) on day 10 of growth. These seedlings were subsequently sprayed with deionized water (control) or 4-chloroindole-3-acetic acid (4-Cl-IAA) (10^{-6} M) on day 15 and were sampled 30 days after sowing to assess growth and metabolite content. Growth (length, and fresh and dry mass of roots and shoots) and the level of metabolites (chlorophyll, nitrogen, phosphorus, total sugar, and soluble protein) in the leaves significantly decrease in response to Cd stress; however, the 4-Cl-IAA (10^{-6} M) treatment, both in the absence and presence of Cd, significantly increased the parameters studied. The response generated in the plants grown under stress-free conditions was more prominent, as compared in those exposed to Cd.

Key words: Chloroindole, cadmium, chlorophyll, growth, nitrogen, phosphorus, soluble protein, *Vigna radiata*

Kadmiyum stresi altında Mung (Çin Yaban Fasulyesi) Fasulyesi'nin bazı büyüme parametreleri üzerine 4-kloroindol-3-asetik asidin etkileri

Özet: Kum kültüründe yetişen T-44 kültürel varyete ait Mung Fasulyesi fideleri, 10 günlük büyüme evresinde kadmiyuma (100 µM) maruz bırakıldı. Sonra bu fideler, değişik metabolitlerin içeriklerini ve büyümeyi değerlendirmek için, ekim işleminden sonra 30 veya 15 gün süren evrelerde 4-kloroindol-3-asetik asit (4-Cl-IAA) (10^{-6} M) veya deiyonize su (kontrol) ile muamele edildi. Kadmiyum(Cd) stresine karşı, yapraklarda büyüme (Uzunluk, kök ve gövdenin yaş ve kuru kütlesi) ve metabolitlerin seviyesi (Klorofil, nitrojen, fosfor, total şeker ve çözünür protein) önemli ölçüde bir azalma gösterdi. Bununla birlikte, 4-Cl-IAA (10^{-6} M) muamelesi, kadmiyum varlığında veya yokluğunda, her iki durumda çalışılan parametreleri önemli ölçüde yükseltti.

Anahtar sözcükler: Kloroindol, kadmiyum, klorofil, büyüme, nitrojen, fosfor, çözünür protein, *Vigna radiata*

Introduction

Soil contamination with heavy metals is becoming a major problem worldwide, leading to losses in agricultural productivity and hazardous effects as they enter the food chain. Nonetheless, their availability to plants via soil is determined by natural processes, especially lithogenic and pedogenic, as well as by anthropogenic factors. Heavy metals that have a direct impact on plants include Fe, Cu, Mn, Co, Ni, Cd, Pb, and Cr. Among them, Cd is extremely toxic, to both plants and animals (1). Symptoms of toxicity observed in plants in the presence of excessive quantities of metals in general, and Cd in particular, may be due to a wide range of interactions at the cellular level (2). Cd exerts its toxic effect by damaging cell membranes and inactivating enzymes by interfering with the sulfhydryl (-SH) groups of soluble proteins or disrupting their structure (3). Other effects of Cd include growth retardation, inhibition of photosynthesis, altered stomatal function and water relations, efflux of ions, and generation of free radicals (1).

A search for other chemicals with auxin-like activity in plants resulted in the identification of some other naturally occurring auxins. These include indole-3-butyric acid, phenyl acetic acid, and 4-chloroindole-3-acetic acid (4-Cl-IAA) (4). 4-Cl-IAA occurs in plants that belong to the family Fabaceae (5) and in the seeds of *Pinus sylvestris* (6). The strong auxin-like activity of this chloro-substituted auxin has been demonstrated in a number of bioassays (5,7,8). It is reported to stimulate rooting and ethylene production in leafy pea cuttings (9). It favors seed germination under stress-free conditions (10), as well as under stress conditions (11) and photosynthesis (12).

The present study aimed to assess the role of 4-Cl-IAA in plants under Cd stress and to explore remedial measures to counter Cd toxicity.

Materials and methods

Seeds of mung bean (*Vigna radiata* L. Wilczek) cv. T-44 were obtained from National Seed Corporation, Ltd., New Delhi. The healthy seeds were surface sterilized with 5% hypochlorite solution, followed by repeated washing with running tap water, and then with double distilled water (DDW) 3 times. The surface sterilized seeds were sown in sterilized sand moistened with DDW, which was repeatedly washed prior to transferring to plastic pots (10 cm in diameter). The seedlings were supplied with nutrient solution daily, from day 3 of growth onwards. Then 7 days after sowing (DAS) the seedlings were treated with Cd (0 or 100 μM) and nutrient solution. These seedlings were subsequently sprayed 15 days after sowing with DDW (control) or 10^{-6} M of 4-Cl-IAA. The concentrations of Cd and 4-Cl-IAA were selected on the basis of our earlier findings (11). The seedlings were grown in a plant growth chamber under controlled environmental conditions. The day/night temperature, humidity, and photoperiod were maintained at 25/20 °C, 75%, and 12 h, respectively. The plants were sampled 15 days after foliar spray treatment (30 days after sowing) to assess the various parameters.

The plants were gently removed from their pots and their roots were dipped in a bucket filled with water to remove adhering sand particles. Root and shoot length were measured using a meter scale, followed by separating and subsequently weighing them to record their fresh weights. The roots and shoots were then transferred to an oven set to 80 °C for 48 h, and then were weighed to determine their dry mass.

Chlorophyll in the intact leaves was measured using a SPAD chlorophyll meter (SPAD, Konica Minolta Sensing, Inc, Japan). Nitrogen and phosphorus were extracted by digesting dried leaf material with H_2SO_4. Nitrogen and phosphorus content in the aliquot was estimated spectrophotometrically (Spectronic 20D, Milton Roy, USA) at 525 and 620 nm, following the methods of Lindner (13), and Fiske and Subba Row (14), respectively. Total sugar was extracted using H_2SO_4 (15) and was estimated according to Dubois et al. (16). Total soluble protein was extracted with trichloroacetic acid and the content was estimated using Folin phenol reagent, according to Lowry et al. (17).

The data were obtained in triplicate and were subjected to statistical analysis using SPSS. The significance of the treatments was tested at the 5% level and the standard error of the replicates was also calculated.

Results

The treatment significantly affected all the growth characteristics studied (Table 1). The presence of

Table 1. The effect of 4-Cl-IAA (10^{-6} M) on Cd (100 μM)-induced changes in shoot and root length (cm), and fresh and dry mass (g) per plant in 30-day-old *Vigna radiata* plants.

Treatment	Shoot length	Root length	Shoot fresh mass	Root fresh mass	Shoot dry mass	Root dry mass
Control	11.80 ± 0.53	12.2 ± 0.44	0.200 ± 0.84	0.050 ± 0.008	0.53 ± 0.43	0.012 ± 0.006
4-Cl-IAA	19.03 ± 0.87	25.8 ± 1.06	0.289 ± 1.05	0.083 ± 0.010	0.71 ± 0.31	0.023 ± 0.005
Cd	8.25 ± 0.46	7.5 ± 0.35	0.115 ± 0.72	0.023 ± 0.004	0.34 ± 0.15	0.010 ± 0.001
Cd + 4-Cl-IAA	14.43 ± 0.69	20.3 ± 0.89	0.153 ± 0.80	0.034 ± 0.006	0.46 ± 0.28	0.016 ± 0.005
LSD at 5%	1.5	2.3	0.051	0.006	0.08	0.002

cadmium in the nutrient medium was deleterious to plant growth, decreasing shoot length, root length, fresh mass of shoots and roots, and dry mass of shoots and roots to levels 30.0%, 38.5%, 42.5%, 54.0%, 35.8%, and 16.6%, below those of the control, respectively. Nevertheless, 4-Cl-IAA (10^{-6} M) alone significantly increased all of the above parameters. The negative effects of cadmium were also neutralized by the hormone, resulting in values comparable with those of the control.

In the absence of cadmium stress, the foliar spray with 4-Cl-IAA (10^{-6} M) significantly increased SPAD chlorophyll values (Table 2). Moreover, the plants treated with cadmium alone had the least SPAD chlorophyll; however, the follow-up treatment of these stressed plants with 4-Cl-IAA increased the level of SPAD chlorophyll, to a limited extent.

It is also evident from Table 2 that the Cd treatment caused a decrease in both nitrogen and phosphorus content in the leaves; however, 4-Cl-IAA treatment, even in the presence of Cd, increased both of these parameters. In the case of nitrogen the values were very close to those of the controls, but phosphorus content was still less than that of the controls.

The level of soluble protein and total sugar in the leaves followed a pattern similar to that of the other parameters (Table 2). Plants treated with cadmium (100 μM) and nutrient medium had soluble protein and total sugar content below that of the controls. The use of 4-Cl-IAA (10^{-6} M) together with Cd improved the values of both of these parameters. This combined treatment increased the values to a level comparable with that of the control treatment.

Discussion

In the present study the exposure of mung bean (*Vigna radiata* L. Wilczek) plants to Cd significantly retarded plant growth due to inhibited cellular expansion and elongation (1). In cells Cd becomes associated with the cell wall and middle lamellae, which increases the cross linking of pectins and results in inhibition of cell growth (18).

Cd is also reported to inhibit the synthesis of δ-aminolevulinic acid and the protochlorophyllide reductase complex (19), key steps in chlorophyll synthesis. This could have resulted in a decrease in the level of SPAD chlorophyll (Table 2). Earlier studies also reported a decrease in the level of chlorophyll in

Table 2. The effect of 4-Cl-IAA (10^{-6} M) on Cd (100 μM)-induced changes in chlorophyll, leaf nitrogen and phosphorus content (%), and leaf carbohydrate and protein content (%) in 30-day-old *Vigna radiata* plants.

Treatment	SPAD chlorophyll	Nitrogen content	Phosphorus content	Total sugar	Soluble protein content
Control	25.4 ± 1.06	3.78 ± 0.26	0.20 ± 0.10	7.57 ± 0.027	10.24 ± 0.087
4-Cl-IAA	28.3 ± 1.95	4.30 ± 0.31	0.26 ± 0.14	11.15 ± 0.036	13.13 ± 2.25
Cd	19.6 ± 0.85	3.45 ± 0.20	0.12 ± 0.002	8.37 ± 0.022	9.81 ± 0.09
Cd + 4-Cl-IAA	20.3 ± 2.13	2.61 ± 0.07	0.14 ± 0.005	10.48 ± 0.032	11.28 ± 0.82
LSD at 5%	2.8	0.54	0.05	1.66	1.73

sunflower (20) and barley (21); however, in the present study 4-Cl-IAA increased the level of SPAD chlorophyll in mung plants subjected to Cd stress (Table 2).

Leaf nitrogen (N) and phosphorus (P) content in plants treated with Cd was lower than in the control plants (Table 2). The level of N in plants is regulated by the coordinative action of a number of enzymes, including nitrogenase, nitrate reductase, nitrite reductase, glutamine synthetase, and glutamate synthase (22). The activity of all these enzymes is inhibited by the presence of Cd (23,24). In addition, Cd stress also induces alterations in membrane permeability and ATPase activity (25), restricting the uptake of mineral nutrients by roots (26). As such, a decrease in both N and P content was observed (Table 2). The level of other nutrients, namely potassium, calcium, and manganese, exhibited a similar pattern in response to Cd stress (27).

Plant hormones, such as IAA and IBA (28), 4-Cl-IAA (12), and kinetin (29) increased the activity of nitrate reductase and nitrate uptake (30). 4-Cl-IAA also modifies membrane permeability (31), which, in the present study, might have facilitated the uptake of N and P, resulting in the elevation of their levels, even in the plants subjected to Cd stress (Table 2).

The toxic effect of Cd on plants is expressed in the form of the inhibition of the activity of various enzymes involved in metabolism (24), which consequently lowers the level of the amino acid pool (32). This is one of the possible reasons for the observed decrease in soluble protein content in the Cd-treated plants in the present study (Table 2). The presence of Cd results in stomatal closure and thus alters the activity of the enzymes involved in photosynthesis, including rubisco and carbonic anhydrase (1), and consequently decreases the net photosynthesis rate (26) and total sugar level (Table 2). A decrease in soluble and insoluble saccharides content in Cd-stressed sun flower has been previously observed (20).

As auxin is involved in the process of transcription and/or translation, it favors an increase in soluble protein content, which may be an expression of elevated nitrogen (Table 2). Additional proof of the role of auxins comes from the increase in the activity of various enzymes (33), which is quite prominent in cases of 4-CI-IAA treatment (Table 2). Moreover, the damaging effect of Cd (Table 2) is also partly overcome by 4-Cl-IAA. The increase in total sugar content (Table 2) observed in the present study was the direct outcome of the improved rate of photosynthesis, which could have elevated the level of total sugars (12) in the plants sprayed with 4-Cl-IAA.

It may be deduced from the present study's results that Cd stress inhibited all the metabolic and growth parameters studied; however, subsequent treatment with 4-Cl-IAA countered the toxicity generated by Cd, although to a limited extent. Therefore, it is proposed that 4-Cl-IAA might play an important role in countering the effects of Cd stress.

Corresponding author:

Shamsul HAYAT

Plant Physiology Section,

Department of Botany,

Aligarh Muslim University,

Aligarh 202 002 - INDIA

hayat_68@yahoo.co.in

References

1. Prasad MNV. Cd toxicity and tolerance in vascular plants. Environ Exp Bot 35: 525-545, 1995.
2. Hall JL. Cellular mechanisms for heavy metal detoxification and tolerance. J Expt Bot 53: 1-11, 2002.
3. Van Assche F, Clijisters H. Effect of metals on enzyme activity in plant. Plant Cell Environ. 13, 195-206, 1990.
4. Normanly J. Auxin metabolism. Physiol Plant 100: 431-442, 1997.
5. Engvild KC. The chloroindole auxins of pea, strong plant growth5 hormones or Endogenous Herbicides? Riso National Laboratory, Denmark, 1994.
6. Ernstsen A, Sandberg G. Identification of 4-chloroindole-3-acetic acid and indole-3-aldehyde in seeds of *Pinus sylvestris*. Physiol Plant 68: 511-518, 1986.
7. Reinecke DM. 4-Chloro-indole-3-acetic acid and plant growth. Plant Growth Regul 27: 3-13, 1999.

8. Ali B, Hayat S, Fariduddin Q, et al. 4-chloroindole-3-acetic acid: Metabolism and bioactivity in plants, In Hemataranjan, A. (ed.) Advances in plant physiology, 9, 369-375, Scientific Publishers, Jodhpur, 2006.

9. Ahmad A, Andersen AS, Engvild KC. Rooting, growth and ethylene evolution of pea cuttings in response to chloroindole auxins. Physiol Plant 69: 137-140, 1987.

10. Ahmad A, Hayat S, Fariduddin Q et al. Germination and - amylase activity in the grains of wheat, treated with chloroindole acetic acids. Seed Technol 23: 88-91, 2001.

11. Ali B, Rani I, Hayat S et al. Effect of 4-Cl-indole-3-acetic acid on the seed germination of *Cicer arietinum* exposed to Cd. Acta Bot Croat 66: 57-65, 2007.

12. Ahmad A, Hayat S, Fariduddin Q, et al. Photosynthetic efficiency of plants of *Brassica juncea*, treated with chlorosubstituted auxins. Photosynthetica 39: 565-568, 2001.

13. Lindner RC. Rapid analytical method for some of the more common inorganic constituents of plant tissue. Plant Physiol 19: 76-89, 1944.

14. Fiske CH, Subba Row Y. The calorimetric determination of phosphorus. J Biol Chem 66: 373-400, 1925.

15. Yih RY, Clark HE. Total sugar and soluble protein content of boron deficient tomato root tips in relation to anatomy growth. Plant Physiol 40: 312-315, 1965.

16. Dubois M, Gills K, Hamilton JK et al. Calorimetric method for determination of sugars and related substances. Ann Chem 28: 350-356, 1956.

17. Lowry OH, Rosenbrough NJ, Farr AL et al. Soluble protein measurement with the Folin phenol reagent. J Biol Chem 193, 265-275, 1955.

18. Poschenreider C, Guinse B, Barcelo J. Influence of Cd on water relations, stomatal resistance, and abscisic acid content in expanding bean leaves. Plant Physiol 90: 1365-1371, 1989.

19. Stobart AK, Griffits W, Bukhari IA et al. The effect of Cd^{2+} on the biosynthesis of chlorophyll in leaves of barley. Physiol Plant 63: 293-298, 1985.

20. Gadallah MAA. Interactive effect of heavy metals and temperature on the growth and chlorophyll, saccharides and soluble nitrogen contents in *Phaseolus* plants. Biol Plant 36: 373-382, 1995.

21. Vassilev A, Berora M, Zlateve Z. Influence of Cd^{2+} on growth chlorophyll content and water relations in young barley plants. Biol Plant 41: 601-606, 1998.

22. Hopkins WJ. Introduction to plant physiology, John Wiley & Sons Inc. New York, 1995.

23. Chugh LK, Gupta KV, Sawhney SK. Effects of Cd on enzymes of nitrogen metabolism in pea seedlings. Photochem 3: 395-400, 1992.

24. Gouia H, Ghorbel M, Meyer C. Effects of Cd on activity of nitrate reductase and on the other enzymes of nitrate assimilation pathway in bean. Plant Physiol Biochem 38: 629-638, 2000.

25. Obata H, Inoue N, Umebayashi M. Effect of Cd on plasma membrane ATPase from plants roots differing in tolerance to Cd. Soil Sci Plant Nutr 42: 361-366, 1996.

26. Barcelo J, Poschenreider C. Plant water relations as effected by heavy metal stress: A review. J Plant Nutr 13, 1-37, 1990.

27. Heranandez LE, Carpena-Ruiz R, Garate A. Alterations in the mineral nutrition of pea seedlings exposed to Cd. J Plant Nutr 19: 1581-1598, 1996.

28. Hussain A. Response of *Pisum sativum* L. to exogenously applied plant growth regulators. PhD Thesis, Aligarh Muslim University, Aligarh, 2000.

29. Fariduddin Q, Ahmad A, Hayat S. Photosynthetic response of *Vigna radiata* to pre-sowing seed treatment with 28-Homobrassinolide. Photosynthetica 41: 307-310, 2003.

30. Mai YY, Lin JM, Zeng XL et al. Effect of homobrassinolide on the activity of nitrate reductase in rice seedlings. Plant Physiol Commun 2: 50-52, 1989.

31. Karcz W, Burdach Z. A comparison of the effect of IAA and 4-Cl-IAA on growth, proton secretion and membrane potential in maize coleoptiles segments. J Expt Bot 53: 1089-1093, 2002.

32. El-Shintinawy F, El-Ansary A. Differential effect of Cd^{2+} and Ni^{2+} on amino acid metabolism in soybean seedlings. Biol Plant 43: 79-84, 2000.

33. Ali B, Hayat S, Hasan SA et al. A comparative effect of IAA and 4-Cl-IAA on growth, nodulation and nitrogen fixation in *Vigna radiata* L. Wilczek. Acta Physiol Plant 30: 35-41, 2008.