International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Impact Factor (2012): 3.358

Influence of Cadmium Metal Toxicity on Hormones Profiling of *Brassica juncea* L. Plants

Dhriti Kapoor[1], Renu Bhardwaj[2]

[1]Department of Botanical and Environmental Sciences, Guru Nanak Dev University, Amritsar, 143005, Punjab, India

[2]Professor, Department of Botanical and Environmental Sciences, Guru Nanak Dev University, Amritsar, 143005, Punjab, India

Abstract: ***Heavy metals are major environmental pollutants, which may prove toxic to living organisms by entering through food chain. Toxicity of heavy metals produce oxidative burst by generation of reactive oxygen species. Plants possess various growth regulators, which help in scavenging the free radicals and thus protect the plants from various types of environmental stresses. In the present study, Brassica juncea plants subjected to different concentrations (0, 0.2, 0.4 and 0.6mM) of cadmium (Cd) metal to observe the hormones profiling. LC/MS study revealed the increased expression of different plant growth regulators like polyamines, brassinosteroids, auxins, abscisic acid, gibberellic acid and jasmonic acid under metal stressed conditions. In conclusion, endogenous level of plant growth regulators got activated due to heavy metal stress, which protects the plants by scavenging free radicals.***

Keywords: Cd toxicity, oxidative stress, *Brassica juncea*, plant growth regulators, LC/MS.

1. Introduction

Heavy metal pollution has been exasperated in environment by industrial revolution and anthropogenic activities and it has created a foremost threat to mankind by its incorporation to the food chain that leads to the degradation of ecosystem [1]. Some heavy metals are basically essential for the cellular functions, but their doses beyond tolerable limits produce the reactive oxygen species (ROS). Among heavy metals, Cd is considered as most toxic to plants and animals. It is present as free hydrated ions or may be complexed by organic or inorganic ligands. Excessive concentrations of Cd cause retardation in growth and leaf chlorosis in plants. Water balance, photosynthetic apparatus and stomatal opening are disturbed by Cd toxicity. Activities of different metabolic enzymes are also altered due to this metal [2].

In response to heavy metal stress, endogenous plant hormones contribute in combating their deleterious effects. Plant growth regulators like brassinosteroids, polyamines auxins etc. have significant effects on vegetative and reproductive development and stress tolerance [3]. They promote cell elongation, fission and photosynthetic capacity at cellular level [4]. Plant hormones are reported to play key role as a stress protectant [5].

Brassica juncea possesses economic as well as medicinal values and is considered to be a hyperaccumulator of heavy metals [6]. Keeping this point, the present study was designed to examine the defence mechanism of 90 days old *Brassica juncea* plants in terms of expression of plant hormones under Cd stress conditions.

2. Materials and Methods

Field experiment was conducted in Botanical Garden of Guru Nanak Dev University, Amritsar. 20 X 20 feet area was taken for the experimentation and soil: manure in a ratio of 3:1 was added into it (Fig 3.2). Uniform sized seeds were surface sterilized with 0.01% $HgCl_2$ for 1 minute and then washed with distilled water for 3-4 times. The surface sterilized seeds were sowed in different blocks of field, in which different treatments of Cd metal was given (0, 0.2, 0.4 and 0.6 mM Cd). Plants were then harvested after 90-days of germination.

2.1.1 LC/MS Analysis of Plant Hormones

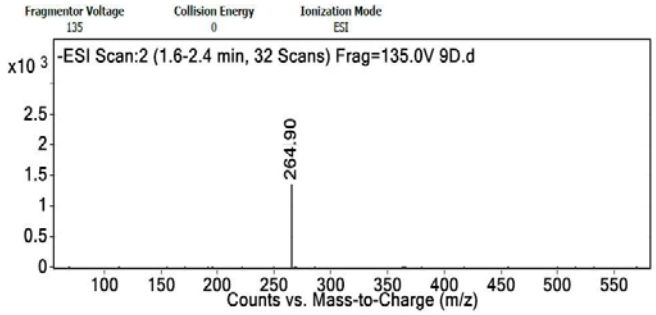
Plant samples were subjected to LC/MS in order to identify the presence of plant hormones like brassinosteroids, polyamines, auxins, abscisic acid, jasmonic acid, salicylic acid and gibberellic acid.

2.1.2 *Sample Preparation*

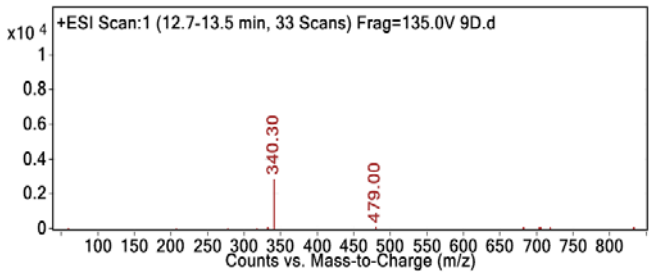
5 g of fresh plant sample was homogenized in 40mL of 80%methanol. Mixture was vortexed and centrifuged. 0.2mL of mixture was diluted to 4mL with 80% methanol and filtered by filter papers of 0.22 micron pore size. 2 μL of sample was injected for LC/MS study. Total run time of sample required in positive mode was 16 minutes and 6 minutes in negative mode. Agilent 1100 LC has been coupled with Brukermakemass spectrometer model Esquire 3000. PDA detector was used in the instrument for detecting compounds. Temperature of column was 40^0C. The solvent system includes solvent A (water with 0.5% formic acid) and solvent B (methanol).

3. Results

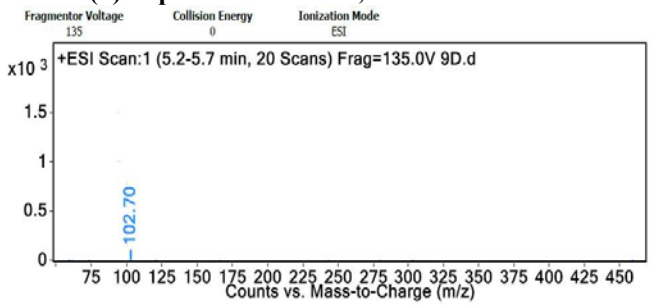
In 90 days old plants (Table 1), cadaverine, papaverine, dolicholide, 24-epibrassinolide, abscisic acid and jasmonic acid were recognized (Fig 1(a-e)). Indole 3- acetic acid and putrescine were expressed in 0.2mM Cd stressed plants (Fig 2(f-g)), spermidine in 0.4mM Cd treated plants in comparison to control plants (Fig 3(h)). Gibberellic acid and castasterone got activated in 0.6mM Cd treatment with respected to control (Fig 4(i-j)).



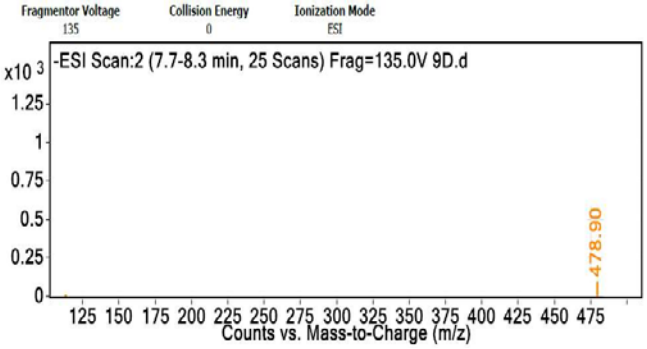
(a). ABA – 264.90



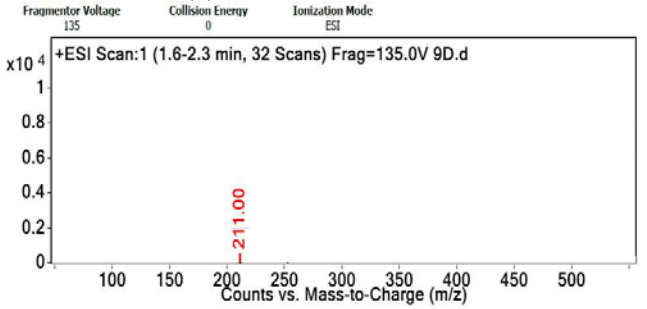
(b) Papaverine – 340.30, 24-EBL – 479.00



(c) Cadaverine – 102.70

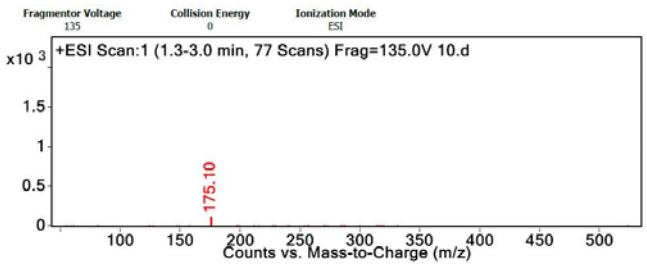


(d) Dolicholide – 478.90

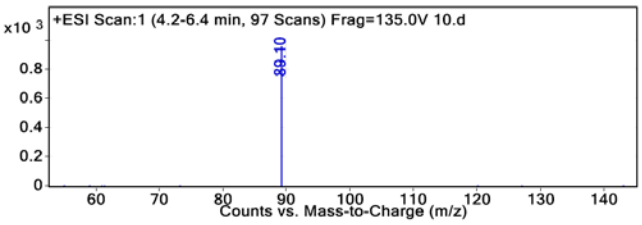


(e) JA – 211.00

Figure 1: (a-e). Hormones Profiling in 90-days old plants of *Brassica juncea*

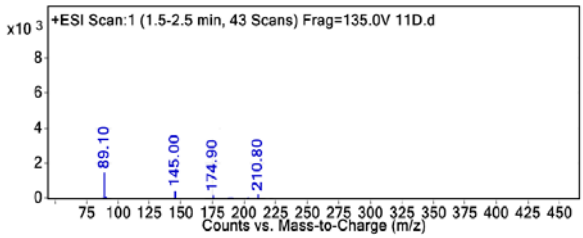


(f) IAA – 175.10



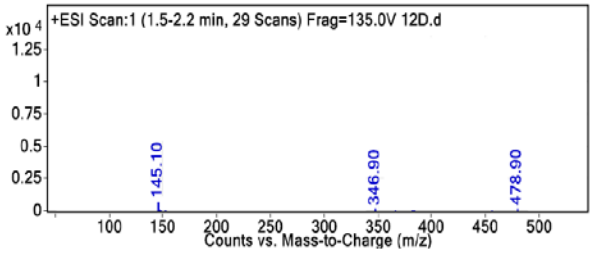
(g) Putrescine – 89.10

Figure 2: (f-g). Effect of Cd metal on Hormones Profiling in 90-days old plants of *Brassica juncea* treated with 0.2mM Cd

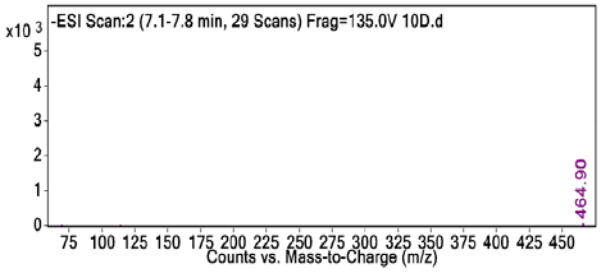


(h) Putrescine – 89.10, Spermidine – 145.00, IAA – 174.90, JA – 210.80

Figure 3: (h). Effect of Cd metal on Hormones Profiling in 90-days old plants of *Brassica juncea* treated with 0.4mM Cd



(i) Spermidine – 145.10, GA – 346.32, Dolicholide – 478.90



(j) Castasterone – 464.90

Figure 4: (i-j). Effect of Cd metal on Hormones Profiling in 90-days old plants of *Brassica juncea* treated with 0.6mM Cd

Table 1: Effect of Cd Metal on the Hormones Profiling in 90-days old Plants of *Brassica juncea*

S. No.	*Hormones*	*CN*	*0.2mM Cd*	*0.4mM Cd*	*0.6m M Cd*
1.	Papaverine	+	+	+	+
2.	Dolicholide	+	+	-	+
3.	ABA	+	+	+	+
4.	Putrescine	-	+	+	-
5.	24-EBL	+	-	-	-
6.	JA	+	+	+	-
7.	IAA	-	+	+	-
8.	Cadaverine	+	+	-	+
9.	Castasterone	-	-	-	-
10.	GA	-	-	-	+
11.	Spermidine	-	-	+	+

4. Discussion

Heavy metal stress has become a major focus due to the increased environmental pollution. Metals often cause lethal biological effects as they are non-biodegradable in nature [7]. Formation of oxidants/free radicals is the primary response of plants exposed to stress. Reduced forms of atmospheric oxygen (O_2) like superoxide radicals ($O_2^{\cdot-}$), hydroxyl radical ($HO^{\cdot}$) etc. are the intermediates of ROS. These intermediates are generated due to transfer of electrons [8]. Defence mechanism in plants involves the presence of plant growth regulators (PGRs). PGRs like auxins, abscisic acid, brassinosteroids and polyamines regulate metabolic processes related to plant growth and development and they have also been found to work as stress protect ants by scavenging the reactive oxygen species [9]. These hormones contribute in the activation of antioxidative defence system of plants subjected to stress and thus help in overwhelming the stress [10, 11]. Similarly, in present study, hormones were much expressed in metal treated plants. These results are in coherence with the findings of Groppa et al. [12, 13] where biosynthesis of putrescine was increased under Cu and Cd stress in sunflower discs. The increased expression of putrescine was due to enhanced activities of ornithine decarboxylase (ODC) and arginine decarboxylase (ADC) enzymes, which triggers the synthesis of hormone. Similarly, significant rise in the endogenous levels of ABA was recorded in the seeds of chick pea exposed to Zn and Pb stress [14]. The present work was also in accordance with the observations of Munzuroglu et al. [15], where Hg, Cu, and Cd toxicity caused noteworthy enhancement in the ABA in wheat seeds.

5. Conclusions

Cd is one of the most toxic heavy metals, which increases the production of ROS. Metabolic activities are disturbed by Cd stress. Defence mechanisms of *Brassica juncea* plants like hormonal level got activated to combat the stress. Thus, the plants' possess defensive strategies like plant growth regulators, which provide protection to plants from oxidative stress generated by Cd.

6. Future Scope of Study

The present study will help to plant breeders in developing stress-tolerant varieties by understanding the physiological mechanisms of plants in response to various environmental stresses.

References

[1] R. Ramasubbu and C. Prabha, "Effect of HeavyMetals on Growth and Biochemical Components of Plants," LAP Lambert Academic Publishing, 2012.

[2] G. Flora, D. Gupta, and A. Tiwari, "Toxicity of lead: a review with recent updates," Interdisciplinary Toxicology, 5, pp. 47-58, 2012.

[3] H. Ryu, H. Cho, K. Kim, and I. Hwang, "Phosphorylation dependent nucleocytoplasmic shuttling of BES1 is a key regulatory event in brassinosteroid signalling," Molecular Cells, 29, pp. 283- 290, 2010.

[4] J.Q.Yu, L.F. Huang, W.H. Hu, Y.H. Zhou, W.H. Mao, S.F. Ye, and S. Nogues, "A role for brassinosteroids in regulation of photosynthesis in *Cucumis sativus*," Journal of Experimental Botany, 55, pp. 1135-1143, 2004.

[5] G. Kartal, A. Temel, E. Arican, and N. Gozukirmizi, "Effects of brassinosteroids on barley root growth, antioxidant system and cell division," Plant Growth Regulation, 58, pp. 261-267, 2009.

[6] K. Shekhawat, S. S. Rathore, O. P. Premi, B. K. Kandpal, and J. S. Chauhan, "Advances in agronomic management of indian mustard (*Brassica juncea* (L.) Czernj. Cosson): an overview," International Journal of Agronomy, Article ID408284, 14 pages, 2012.

[7] C. A. Jaleel, K. Jayakumar, Z. C. Xing, and M. M. Azooz, "Antioxidant potentials protect Vigna radiata (L.) Wilczek plants from soil cobalt stress and improve growth and pigment composition," Plant Omics, 2, pp. 120-126, 2009.

[8] N. la Rocca, C. Andreoli, G. M. Giacometti, N. Rascio, and I. Moro, "Responses of the antarctic microalga *Koliella Antarctica* (Trebouxiophyceae, Chlorophyta) to cadmium contamination," Photosynthetica, 47, pp. 471-479, 2009.

[9] P. Sharma and R. Bhardwaj, "Effect of 24-epibrssinolide on seed germination, seedling growth and heavy metal uptake in *Brassica juncea* L.," General and Applied Plant Physiology, 33, pp. 59-73, 2007.

[10] L. L. Haubrick and S. M. Assmann, "Brassinosteroids and plant function: Some clues, more puzzles," Plant, Cell and Environment, 29, pp. 446-457, 2006.

[11] A. Verma, C. P. Malik, and V. K. Gupta, "*In vitro* effects of brassinosteroids on the growth and antioxidant enzyme activities in groundnut," ISRN Agronomy, Article ID 356485, 8 pages, 2012.

[12] M. D. Groppa, M. L. Tomaro, and M. P. Benavides, "Polyamines and heavy metal stress: the antioxidant behavior of spermine in cadmium- and copper-treated wheat leaves," BioMetals, 20, pp. 185-195, 2007.

[13] M. D. Groppa, M. S. Zawoznik, M. L. Tomaro, and M. P. Benavides, "Inhibition of root growth and polyamine metabolism in sunflower (*Helianthus annuus*) seedlings under cadmium and copper stress,"

Biological Trace Element Research, 126, pp. 246-256, 2008.

[14] ¨ O. Atici, G. A˘gar, and P. Battal, "Changes in phytohormone contents in chickpea seeds germinating under lead or zinc stress," Biologia Plantarum, 49, pp. 215-222, 2005.

[15] O.Munzuroglu, F.K. Zengin, and Z. Yahyagil, "The abscisic acid levels of wheat (*Triticum aestivum* L. cv. cakmak 79) seeds that were germinated under heavy metal (Hg++, Cd++, Cu++) stress," Gazi University Journal of Science, 21, pp. 1-7, 2008.

Author Profile

Dhriti Kapoor is Ph.D. Scholar at Department of Botanical and Environmental Sciences at Guru Nanak Dev University, Amritsar.

Dr. Renu Bhardwaj is Professor Department of Botanical and Environmental Sciences at Guru Nanak Dev University, Amritsar.