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Role of Gibberellic Acid in Abolishing the Detrimental Effects of Cd and Pb on Broad Bean and Lupin Plants

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Abstract: This work describes the changes in photosynthetic pigments, soluble carbohydrates, proteins and activities of certain enzymes of both broad bean and lupin plants in response to heavy metals (Cd or Pb), gibberellic acid (GA_3) and their interactions. Significant reduction in contents of chlorophylls, soluble carbohydrates, soluble proteins were recorded in tested plants due to the treatment with either Cd or Pb. Application of GA_3, in most cases, decreased contents of chlorophylls and soluble carbohydrates in absence and presence of either Cd or Pb, while contents of soluble proteins were markedly increased. All applied treatments greatly affected the activities of amylases, proteases, catalases and peroxidases in both broad bean and lupin plants. Generally, it could be concluded that GA_3 have (to some extent) a beneficial regulatory role in plants grown under both Cd and Pb polluted conditions.

Key words: Broad been, lupin, gibberellic acid, lead, cadmium.

INTRODUCTION

Cadmium and lead are emitted from metal working and manufacturing industries and from care. The problem in Egypt is that most industrial areas are located close to the cultivated lands. Moreover, roads and high ways carry a vast number of motor vehicles running through the cultivated areas in Egypt. Consequently, these heavy metals, once taken up by plants, inter human food chains[19,22]. The uptake of trace elements from aerial sources through the leaves may have a significant impact on plant contamination especially of elements such as Cd and Pb[31].

Metal tolerance is a phenomenon often noted, but the understanding of the physiological and biochemical aspects of these fascinating aspects of plant metabolism has not progressed a pace. The use of growth regulators is promising for enhancing and improving the production of economic food crops. Recently, these substances could not only regulate plant growth and development but also increase plant resistance to various environmental stress conditions[14,12]. The present study was undertaken to investigate the effects of heavy metals (Cd or Pb), GA_3 and their interactions on certain metabolic activities of broad bean and lupin plants.

MATERIALS AND METHODS

Seeds of broad bean "*Vicia faba*" (Giza-716) & lupin "*Lupinus albus*" (Giza-1) were obtained from Agricultural Research Centre, Ministry of Agriculture, Giza, Egypt. Uniform broad bean and lupin seeds were planted in Botanical garden, Botany and Microbiology Dept., Fac. of Sci., Al-Azhar Univ., in two plots containing 12 ridges (6 for each plot). The seeds were sown on one side of the ridge, with 20 cm apart between the hills. The developed plants were irrigated when ever required. Each of the two experimented plants was divided into 6 groups representing the foliar treatments. water (control), 20 ppm Cd as cadmium chloride, 100 ppm Pb as lead acetate, 100 ppm GA_3, 20 ppm Cd + 100 ppm GA_3 and 100 ppm Pb + 100 ppm GA_3. The plants were treated twice with the above mentioned treatments. In cases of the combined treatment, GA_3 was sprayed firstly followed by heavy metal with 24 h interval. The first treatment was made when the age of plants was 33 days; while the second treatment was made the age of plants was 65 days. The plant samples were collected for analysis when the plants were 72 days old. At the end of the growth season, analysis of the seeds yielded from the different treatments and the control were measured. Contents of chlorophylls were estimated using the method of Vernon and Selly [33]. Contents of soluble carbohydrates were measured according to the method of Umbriet *et al.* [32]. Contents of soluble proteins were estimated according to the methods of lowery *et al.* [20]. Activates of amylases, were determined using the method of Afifi *et al.* [3]. Proteases activates were estimated using the method of Ong and Guacher [24]. Catalases activity was measured according to the method of Chen *et al* [7]. Peroxidase activity was assayed according to the method of Bergmeyer[5]. Statistical analysis of the obtained results was done using (L.S.D.) according to Snedecor and Cochran [30].

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RESULTS AND DISCUSSION

Photosynthetic Pigments: Results presented in Table (1) showed that, in broad bean plants, contents of chlorophyll (a) as well as total chlorophyll (a + b) were, mostly, decreased due to the application of GA_3, however, contents of chlorophyll (b) were significantly increased. In lupin plants, treatment with GA_3 caused, generally, significant increases in the contents of chlorophyll (a), (b) and total chlorophyll (a + b) contents.

Increases in photosynthetic pigments that observed in the present work in response to GA_3 agreed to those observed by El-Khateeb [10] working on broad bean and soybean, Abd El-Hamid *et al.*[1] working on sugar beet, Abdel-Hameid [2] working on safflower plants and Picazo *et al.* [25] working on rice plants. The present study showed that, contents of chlorophyll (a), (b) and their contents in both broad bean and lupin plants were significantly reduced in response to the application of either Cd or Pb. Similar results were reported by several other investigators [23,11,1,21,4,29].

With respect to the combined treatments of GA_3 with either Cd or Pb, the obtained results showed different responses as regards the contents of chlorophylls of the two tested plants. In broad bean plants, application of GA_3 was found to be, mostly, ineffective in overcoming the adverse effects of both Cd or Pb regarding the contents of chlorophyll (a), (b) and total (a + b) chlorophyll contents. However, in lupin plants treated with either Cd or Pb, application of GA_3 caused, generally, slightly increment in the chlorophyll (a), (b) and total chlorophyll contents. These results are in accordance with other investigators; Abd El-Hamid *et al.*[1] found that, in sugar beet plants, treatment with GA_3 declined the adverse effects of either Cd or Pb as regards the level of different photosynthetic pigments. Also, Picazo *et al.* [25]. found that, the net photosynthesis rates recovered transitorily in Cd-treated rice plants after the addition of GA_3 (14 µm).

Carbohydrates: Table (2) showed that, contents of soluble carbohydrates in shoots as well as in the yielded seeds of broad bean plants were, highly significantly decreased in response to the application of GA_3. However, opposite trends were recorded in lupin plants, where as contents of soluble carbohydrates in shoots and also, in the yielded seeds were markedly increased in response to the treatment with GA_3. In this regard Abdel-Hameid[2]. reported that, spraying safflower plants with GA_3 caused highly significant increases in the contents of total soluble carbohydrates in all parts (leaves, stems, roots and yielded seeds). Also, Biaecka and Kepezynski [6]. found that, application of GA_3 increased the contents of total soluble carbohydrates of *Amaranthus caudatus* plants.

In the present study, it was found that, contents of total soluble carbohydrates in shoots, roots and yielded seeds of both broad bean and lupin plants were highly significantly decreased in response to the application of Cd or Pb. These results are in agreement with those obtained by several other investigators [28,11,1,8,21,4,25,27].

With respect to the combined treatments, results of the present work (Table 2) revealed that, in both broad bean and lupin plants, application of GA_3 with either Cd or Pb resulted in, mostly, significant decreases as regards the contents of total soluble carbohydrates in shoots, roots as well as in the yielded seeds. These indicate that, application of GA_3 was not effective in overcoming the adverse effects of Cd and Pb as regards the contents of total soluble carbohydrates of broad bean and lupin plants. On the contrary, the obtained results, Abd El-Hamid *et al.* [1] reported that, the use of growth regulators, especially GA_3 seemed to have pronounced effect on the level of sugars fractions of sugar beet plants and could be used for minimizing harmful effects of Cd or Pb. Also, Picazo *et al.* [25] found that, GA_3 increased the sugar content in roots, second and third leaves and also, modified the carbohydrate distribution pattern of rice plants grown under the application of Cd or Ni.

Protein contents: Results of the present work, Table (3) revealed that, in both broad bean and lupin plants, treatment with GA_3, in absence and presence of either Cd or Pb, resulted in, mostly, highly significant increases in protein contents in shoots, roots and also, in the yielded seeds. These results are in harmony with those obtained by Khalaf *et al.* [17] on soybean, common bean and cowpea plants where they found that, contents of proteins increased as a result of GA_3 application. Abdel-Hameid [2] reported that, the contents of soluble proteins of the different parts of safflower plants were, mostly, highly significantly increased in response to the treatment with GA_3. On the contrary, Gaber *et al.* [13] found that, application of GA_3 decreased the contents of soluble proteins in the yielded seeds of *Vica faba* plants.

On the other hand, the obtained results showed that, application of Cd or Pb caused significant decreases as regards the contents of soluble proteins in shoots, roots as well as in the yielded seeds of both broad bean and lupin plants. These results are in a coincident with several others [16,8,21,27].

Table 1: Effect of Cadmium, Lead and GA3 and their interaction on the chlorophyll contents (mg/g fresh weight) of broad bean and lupin plants .Each value is mean of 3 replicates ± standard error of means.

Broad bean plant			
Treatment	Chlorophyll (a)	Chlorophyll (b)	Total (a+b)
control	6.34 ± 0.05	3.95 ± 0.02	10.89 ± 0.04
Cd	5.43 ± 0.02 **	3.14 ± 0.07 **	8.57 ± 0.09 **
Pb	5.01 ± 0.02 **	2.92 ± 0.02 **	7.93 ± 0.11 **
GA_3	5.46 ± 0.01 **	4.51 ± 0.12 *	9.97 ± 0.01 **
Cd + GA_3	5.26 ± 0.04 **	3.32 ± 0.02 **	8.58 ± 0.07 **
Pb+ GA_3	4.44 ± 0.04 **	2.91 ± 0.01 **	7.34 ± 0.05 **
Lupin plants			
Treatments	Chlorophyll (a)	Chlorophyll (b)	Total (a + b)
Control	8.76 ± 0.15	3.59 ± 0.30	12.35 ± 0.15
Cd	6.49 ± 0.17 **	3.11 ± 0.02	9.60 ± 0.12 **
Pb	7.15± 0.03 **	3.91 ± 0.11	11.0 ± 0.05 **
GA_3	9.90 ± 0.13 *	4.67 ± 0.06 *	14.57 ± 0.07 **
Cd + GA_3	8.07± 0.05 *	4.03 ± 0.10	12.10 ± 0.07
Pb + GA_3	9.17 ± 0.07 **	4.12 ± 0.02	13.29 ± 0.17**

** *Significant at 5% confidence level; ** Significant at 1% confidence level.

Table 2: Effect of Cd, Pb, GA_3 and their interactions on the total soluble carbohydrates contents (mg/g dry weight) of broad bean and lupin plants. Each value is the mean of 3 replicates ± standard error of means.

Broad bean plants			
Treatment	shoots	Roots	Seeds
control	64.13 ± 0.37	51.21 ± 0.82	80.36 ± 0.27
Cd	49.36 ± 0.11 **	40.36 ± 0.91 **	59.52 ± 0.36 **
Pb	54.21 ±0.19 **	41.09 ± 0.31 **	55.36 ± 0.18 **
GA_3	61.20 ± 0.19 **	59.11 ± 0.17 **	68.45 ± 0.19 **
Cd + GA_3	55.18 ± 0.23 **	47.38 ± 0.09 *	61.24 ± 0.18 **
Pb+ GA_3	60.11 ± 1.01 *	50.31 ± 0.14	63.27 ± 0.19 **
Lupin plants			
Treatment	Shoots	Roots	Seeds
control	74.24 ± 0.72	54.01 ± 0.37	79.36 ± 0.13
Cd	60.73 ± 0.31 **	40.07 ± 0.28 **	51.28 ± 0.34 **
Pb	59.30 ± 0.57 **	49.29 ± 0.18 **	55.34 ±0.41 **
GA_3	79.01 ± 0.07 **	51.26 ± 0.29 *	91.21 ± 0.27 **
Cd + GA_3	63.52 ± 0.67 **	55.28 ± 0.37	60.78 ± 0.08 **
Pb+ GA_3	61.36 ± 0.27 **	50.84 ± 0.39 *	59.14 ± 0.24**

***Significant at 5% confidence level; ** Significant at 1% confidence level.

Table 3: Effect of Cd, Pb, GA_3 and their interaction on the total- soluble protein contents (mg/g dry weight) of broad bean plants. Each value is mean of 3 replicates ± standard error of means.

Broad bean plants			
Treatment	Shoots	Roots	Seed
control	101.12 ± 0.28	70.26 ± 0.22	126.21 ± 0.20
Cd	70.11 ± 0.26 **	57.53 ± 0.27 **	89.71 ± 0.17 **
Pb	62.07 ± 0.75 **	63.11 ± 0.94 **	93.76 ± 0.12 **
GA_3	120.26 ±0.19 **	83.35 ± 0.07 **	145.26 ± 0.16 **
Cd + GA_3	118.29 ±0.24 **	76.08 ± 0.55 **	136.32 ± 0.28 **
Pb+ GA_3	86.18 ± 0.91 **	79.28 ± 1.02 **	140.19 ± 0.23 **
Lupin plants			
Treatment	Shoots	Roots	Seeds
control	86.11 ± 0.29	72.21 ± 0.07	138.21 ± 0.21
Cd	61.31 ± 0.62 **	60.11± 0.56 **	81.37 ± 0.12 **
Pb	60.71 ± 0.15 **	57.04± 0.28 **	64.34 ± 0.17 **
GA_3	110.91±0.94 **	77.02 ± 0.18 **	153.32 ± 0.17 **
Cd + GA_3	90.43 ± 1.01 *	70.06± 0.17 **	150.25 ± 0.16 **
Pb+ GA_3	96.19 ± 0.06 **	68.21± 0.58 **	157.55 ± 0.21**

**Significant at 5% confidence level ; ** Significant at 1% confidence level.

Enzymes Activities: Data presented in Table (4) revealed that, in broad bean plants, treatment with GA_3 caused increases in the activity of catalase enzymes, while activities of amylase, protease and peroxidase were insignificantly affected. However, in lupin plants activities of amylases, catalases and peroxidases were increased while, activities of proteases were insignificantly affected. The stimulatory effects of GA_3 as regards the activities of some enzymes were recorded by other investigators, Abdel-Hameid [2] found that, amylolytic and proteolytic activities were significantly stimulated in safflower plants in response to the treatment with GA_3. Also, El-Fouly *et al.* [9] found that, GA_3 (25, 50 and 100 ppm) increased amylase activity in broad bean plants.

Different responses, in the present work, were recorded as regards the studied enzymes activities of broad bean and lupin plants in response to the application of either Cd or Pb. In broad beans, treatment with Cd caused significant increases in the activities of amylases, catalases and peroxidases, while activities of proteases were insignificantly affected. Treatment with Pb significantly increased amylases activities, while activities of proteases, catalases and peroxidases were, generally, decreased.

In lupin plants, treatment with Cd increased catalytic activity, but activities of amylases, proteases and peroxidases were, in most cases, insignificantly affected. Under the application of Pb, catalytic activity was increased; proteolytic activity was decreased while activities of amylases and peroxidases were, mostly, insignificantly affected.

Concerning the effect of both Cd and Pb on enzyme activity, Kumar and Banerji (1992) found that, in *Cicer arietinum*, activities of peroxidases and proteases were decreased while amylase activity was increased in response to the application of Cd and Pb. They found also that, the activities of all the studied enzymes were decreased in wheat plants grown under the same treatment with Cd or Pb. Malecka *et al.* [22] reported that, lead causes two types of unfavorable processes in biological system. Firstly, lead inactivates several enzymes by bindings with their SH-groups. Secondly, lead ions, similarly as those of other heavy metals, can intensify the processes of reactive oxygen species production leading to oxidative stress. Przymusinski *et al.*[26] reported that, activity of peroxidases in every tested organ of Pb–treated lupin plant. Also, Shaaban [29] working on *Phaseolus vulgaris* plants, found that, treatment with Cd (25 μM) increased the activities of catalase, superoxidase dismutase and ascorbate peroxidase enzymes. However, Kasim [15] found that, Cd and Cu induced reduction in the activities of catalases, peroxidases in 25-day-old broad bean plants.

It has been found that, in the present investigation that the combined treatment of (Cd + GA_3) caused, in broad bean plants, significant increase in the amylolytic activity, while activities of proteases, catalases and peroxidases were significantly decreased. In lupin plant,

Table 4: Effect of Cd, Pb, GA_3 and their interactions on the enzyme activity of broad bean and lupin plants. Each value is mean of 3 replicates ± standard error of means.

		Broad bean plants		
Treatments	Amylase (mg/ g. F.wt)	Protease (mg/ g. F.wt)	Catalase (µg/ g. F.wt)	Peroxidase (mg/ g. F.wt)
Control	0.12 ± 0.01	2.20 ± 0.33	0.17 ± 0.03	0.65 ± 0.03
Cd	0.27 ± 0.02 **	2.41 ± 0.12	0.48 ± 0.05 **	1.33 ± 0.04 **
Pb	0.29 ± 0.02 **	0.47 ± 0.02 **	0.04 ± 0.02 *	0.12 ± 0.07 **
GA_3	0.11 ± 0.02	1.14 ± 0.20	0.58 ± 0.03 **	0.19 ± 0.03 **
Cd + GA_3	0.67 ± 0.05 **	0.42 ± 0.01 **	0.03 ± 0.01 *	0.03 ± 0.02 **
Pb + GA_3	0.42 ± 0.03 **	2.37 ± 0.11	0.51 ± 0.06 **	1.79 ± 0.07 **
		Lupin plants		
Treatments	Amylase (mg/ g. F.wt)	Protease (mg/ g. F.wt)	Catalase (µg/ g. F.wt)	Peroxidase (mg/ g. F.wt)
Control	0.07 ± 0.02	0.75 ± 0.02	0.15 ± 0.04	1.44 ± 0.43
Cd	0.10 ± 0.03	0.67 ± 0.15	0.31 ± 0.01 *	1.42 ± 0.55
Pb	0.08 ± 0.02	0.34 ± 0.02**	0.34 ± 0.04 *	1.44 ± 0.17
GA_3	0.36 ± 0.02 **	0.97 ± 0.10	0.30 ± 0.02 *	3.61 ± 0.38 *
Cd + GA_3	0.44 ± 0.02 **	0.45 ± 0.11	0.70 ± 0.06 **	1.52 ± 0.68
Pb + GA_3	0.21 ± 0.03 *	0.32 ± 0.08**	0.42 ± 0.03 **	1.11 ± 0.33

*Significant at 5% confidence level; **Significant at 1% confidence level.

the same treatment (Cd + GA_3) significantly increased activities of amylases and catalases while, activities of proteases and peroxidases were, generally, insignificantly affected.

The obtained results revealed also that, treatment with (Pb + GA_3) caused in broad bean plants, significant increases as regards the activities of amylases, catalases and peroxidases while, activities of proteases were insignificantly affected especially at the second stage of growth. In lupin plants, treatment with (Pb + GA_3) caused significant increases in the activities of amylases and catalases, significant decrease in proteolytic activity and insignificant changes in the activities of peroxidases. It could be deduced that, the increases observed as regards amylases activities of the two tested plants in response to the combined treatments of GA_3 with either Cd or Pb may be related to the lower levels of carbohydrates resulted by the same treatments. Also, plants that have higher levels of proteins revealed lower activity of proteases. This indicates that, close relationships may be found between the levels of both carbohydrates and proteins on one side and activities of either amylases or proteases on the other side.

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