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Effect of Cadmium and Paclobutrazol on Anabolic Capacities and Electrophoteric Protein Patterns in Peanut (*Arachis hypogeae*) Plant

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Abstract: This study was mainly concerned with some Cd-sensitive anabolic capacities and the correlated effects of paclobutrazol (PP333) as a protectant against cadmium toxicity (stress) in peanut *Arachis hypogeae*. Vegetative growth parameters, chlorophyll and carbohydrate contents showed high stability against Cd stress. However, higher cadmium levels resulted in some inhibitory effects. Paclobutrazol (50 and 100 ppm) resulted in synergistic inhibitory effects on carbohydrates content at high Cd levels. Cadmium and /or Paclobutrazol treatments induced quantitative and qualitative changes in the protein PAGE profile of peanut plants. Polypeptides of varying molecular weights were newly synthesized, while the synthesis of the others was stimulated by Cd and Paclobutrazol. The total number of protein bands was, in general, decreased by Cd treatments, but increased at the high level of Cd and Paclobutrazol combination.

Key words: Peanut, Cd-stress, paclobutrazol, protein profile

Introduction

Cadmium is one of the most toxic heavy metals in our modern environment. It is an ever increasing industrial pollutant particularly in areas associated with the smelting of Zn and heavy road traffic (Ernst, 1980; Lagerwerff and Specht, 1970; Varma and Katz, 1978).

A 50% decrease in yield was found in some field crops with tissue concentration as low as 2-9 ppm Cd (Page *et al.*, 1972). The high sensitivity of plants to Cd is thought to be due to inhibitory effects on ATPase activity and consequent decline in respiratory and photosynthetic processes (Lee *et al.*, 1976). However, little is known about the interactions between Cd as an inhibitory environmental pollutant and the different protein patterns in plants.

During the last decades, Cd has been enriched in agrosystems by atmospheric deposition (Watton *et al.*, 1986), application of sewage sludge and mineral P fertilizers, and concern has grown with regard to contamination of the food chain and decrease in soil fertility (Babich and Stotzky, 1978; Jackson and Alloway, 1992).

Trizole plant growth regulators, which inhibit gibberellin biosynthesis, appear to protect plants against various types of stress, including drought, atmospheric sulfur dioxide and high temperature (Davis *et al.*, 1988; Fletcher and Hofstra, 1985; Fletcher, 1989). Recent studies on paclobutrazol (ICI PP333) reported that paclobutrazol treatments increased leaf chlorophyll content (Kim and Kwak, 1991; Zhao *et al.*, 1992 and Xu-Hong-Yuan *et al.*, 1995), increased photosynthesis (Zhao *et al.*, 1992) and improved field establishment after visible wilting (Latimar, 1991). In wheat, it delayed permanent wilting and decreased wilting coefficient (Xu-Hong-Yuan *et al.*, 1995). In susceptible cotton cultivar (Giza 85), paclobutrazol was efficient in increasing their drought tolerance (Beltagi and El-Meleigy, 2000).

The present investigation has been mainly concerned with Cd sensitive anabolic capacities to Cd stress in peanut plants with an emphasis on the electrophoretic protein patterns that are either stimulated by Cd toxicity or paclobutrazol as a protecting treatment against Cd injury.

Materials and Methods

Plant material: The experimental plant used in this experiment was pure strain (Giza 5) of peanut (*Arachis hypogeae)*. Seeds were brought from the Agricultural Research Center (ARC), Giza, Egypt. This work was carried out at Botany Department, Faculty of Science, Suez Canal University, Ismailia, Egypt during June 2000.

Plantation: Plastic pots (15 cm) were equally filled with pre-sieved garden soil (sandy loam). About 6.0 cm deep, healthy peanut seeds were soaked. All pots were gently watered up to saturation, and then kept in the open air and irrigated regularly every other day until treatments.

Cadmium and /or paclobutrazol treatment: After 30 days from seed soaking, the planted pots were randomly divided into equal groups (3 pots each). One group was sprayed with distilled water (control); while the rest of the groups (11 groups) were treated with either Cd (5, 10 or 100 ppm) or paclobutrazol (50 or 100 ppm) or a combination of both (Table 1). Chemical compound treatments were sprayed foliary on both surfaces of the plant leaves until dripping. Plants were kept in open garden of Botany Department and irrigated regularly for two more weeks.

Measurements and sampling: Vegetative growth parameters (shoot and root lengths, fresh and dry weights) were taken to the six-weeks-old plants. Fresh plant samples were taken for chlorophyll and carotenoids and dry samples for carbohydrate analysis. The rest of the plant samples were kept under freezing for protein electrophoresis. All parameters were statistically analyzed by a multiple comparison procedure at 5% significant level ($p = 0.05$) using t-test and mean separation by least significant difference (LSD), (Steel and Torrie, 1980).

Estimation of photosynthetic pigments: Chlorophyll a, chlorophyll b and carotenoids were estimated in the fresh plant leaves according to the procedure of Metzner *et al.* (1965). Fifty-mg fresh leaves were homogenized in 25 ml of 80% acetone and the homogenate was then centrifuged. The concentration of pigments was determined as mg/g using spectrophotometer.

Estimation of total carbohydrates: Total carbohydrate contents were extracted according to Smith *et al.* (1964) and estimated colorimetrically by the phenol-sulphuric acid method (Dubois *et al.*, 1951). The results were expressed as g/100g dry weight.

Table 1: Vegetative growth parameters, chlorophyll (a), chlorophyll (b), carotenoid, and carbohydrate contents of peanut (*Arachis hypogeae*) plants as affected by cadmium (Cd^{2+}) and /or paclobutrazol (ppm) treatments.

Treatment (ppm)	Shoot length (cm)	Root length (cm)	Fresh wt. (g)	Dry wt. (g)	Chl.a (µg/g)	Chl.b (µg/g)	Carotenoids (µg/g)	Total carbohydrate (mg/g)
Control	21.73	13.83	8.33	0.933	65.22	27.17	18.17	203.93
50 pacl.	12.50	13.67	4.53	0.667	47.36	21.48	11.98	206.05
100 pacl.	13.60	10.70	7.33	1.000	66.63	43.08	10.69	194.50
5 Cd	19.67	14.00	7.27	0.833	78.38	30.04	20.68	187.97
5 Cd + 50 pacl	14.00	12.83	6.23	0.667	59.32	25.71	16.90	177.49
5 Cd + 100 pacl.	13.33	10.87	5.30	0.767	73.12	38.98	16.90	199.96
10 Cd	16.33	10.60	6.77	0.767	59.63	32.20	13.45	196.57
10 Cd + 50 pacl	23.93	13.03	8.00	0.800	62.26	30.62	15.53	189.03
10 Cd + 100 pacl.	14.63	10.67	5.13	0.567	54.04	72.56	15.06	192.39
50 Cd	12.33	9.87	6.33	0.967	62.78	26.16	17.79	204.8
50 Cd + 50 pacl.	13.97	10.67	6.20	0.633	43.58	16.69	13.93	77.80
50 Cd + 100 pacl.	11.63	11.67	6.00	0.667	56.38	23.27	15.91	70.67
LSD (P = 0.05)	8.295	3.602	2.430	0.365	20.80	14.220	5.26	72.81

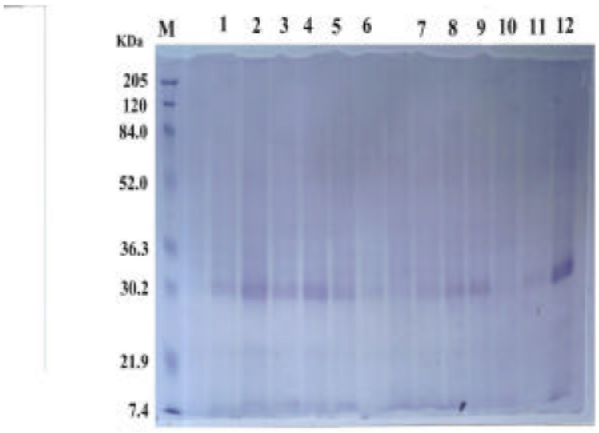

Fig. 1: Electrophotograph of sodium dodecyl sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) of the total proteins of peanut (*Arachis hypogeae*) plants. 1, untreated (control) plants (Track 1); 2 and 3, plants treated with 50 and 100 ppm paclobutrazol (Tracks 2 and 3); 4, 7 and 10, plants treated with 5, 50 and 100 ppm Cd (Tracks 4, 7 and 10); 5 and 6, plants treated with 5 ppm Cd + 50 ppm paclobutrazol and 5 ppm Cd + 100 ppm paclobutrazol (Tracks 5 and 6); 8 and 9, plants treated with 10ppm Cd + 50ppm paclobutrazol and 10ppm Cd + 100ppm paclobutrazol (Tracks 8 and 9); 11 and 12, plants treated with 50 ppm Cd + 50 ppm paclobutrazol and 50 ppm Cd + 100 paclobutrazol (Tracks 11 and 12), respectively.

Protein electrophoresis

Extraction of total proteins: Total protein extracts were prepared by extracting appropriate weight from the frozen plant material with 0.125 M tris/borate, pH 8.9. All the obtained extracts were kept at 4°C for 24 hr and then centrifuged at 10,000 rpm for 20 min. The supernatants were used for electrophoresis.

Gel electrophoresis: SDS polyacrylamide gel electrophoresis (PAGE) was carried out with gel slabs according to the method of Laemmili (1970). Protein subunit bands were stained with coomassie blue R-250 by standard techniques. The gel was scanned using Jel-Pro-Analyzer ver. 3.3 (Media Cybermetics, 93-97).

Results and Discussion

The statistical analysis (LSD, P=0.05) of the vegetative growth parameters, photosynthetic pigments and carbohydrate content (Table 1) showed considerable inhibitory effects for cadmium treatments. However, some of the parameters were not effected by Cd due to the high tolerance of peanut plants to many stressful conditions (Antonivics, 1975). The shoot and root lengths of peanut plants were inhibited only by the highest Cd treatment (50 ppm). The decrease in growth of ryegrass plant height might be due to an accumulation of heavy metals in leaves (Bonnet *et al.*, 2000). Both the fresh and dry weights of peanut plants were not affected by any Cd treatment (5, 10 or 50 ppm). On the other hand, the investigated metabolic capacities (photosynthetic pigments and carbohydrate contents) did not significantly respond to Cd treatments. There are two tolerance mechanisms in plant against heavy metal toxicity, external and internal. Internal tolerance mechanism is immobilizes and compartmentalizes or formation of heavy metal-citrate or oxalate complex, a non-phytotoxic form (Shao *et al.*, 1998). Another mechanism for heavy metal detoxification in plants is the chelation of the metal by a ligand, in some cases, the subsequent compartmentalization of the ligand-metal complex and chelation by organic acids such as citrate and malate (Christopher, 2000 and Metch *et al.*, 2000). Paclobutrazol treatments alone resulted in some inhibitory effects to vegetative growth parameters, chlorophyll b and carotenoids. In consistent with our findings, Cd significantly decreased root dry weight, total chlorophyll and carotenoid contents in *Brassica napus* (Larsson *et al.*, 1998). In wheat, the inhibited growth by increased Cd concentrations was attributed to indirect effects of Cd on the contents of essential nutrients or structural damages of chloroplasts (Quzounidou *et al.*, 1997). At the highest level of Cd (50 ppm), the application of high concentrations (50 or 100 ppm) of paclobutrazol resulted in synergistic inhibitory effect on carbohydrate contents (Table 1). In sunflower, Pankovi *et al.* (2000) reported on decreased photosynthetic rates at high Cd concentrations. In stress-tolerant crops only Trizole compounds were not effective in inducing chilling tolerance in chill-tolerant sweet potato (Beltagi, 1995) or in drought-tolerant cotton plants (Beltagi and El-Meleigy, 2000).

In the present study, the gel electrophoretic technique revealed both quantitative (band intensity and relative mobility) and qualitative (disappearance of some bands or the appearance of new characteristic bands) changes in SDS-PAGE protein patterns of peanut plant in response to either cadmium or paclobutrazol or their combinations (Table 2, Figs.1 and 2). In general, the number of protein bands decreased (9 bands) in response to higher Cd treatments (10 and 50 ppm). These bands increased (12 and 14) by paclobutrazol (50 and 100 ppm). Peanut plants formed individual proteins and protein complexes each of which is composed of more than one molecular weights (KDa). The newly formed protein functions in the detoxification of excess Cd and determine resistance to Cd toxicity (El-Enany and Abd-

Fig. 2: Scans of the tracks in Fig. 1. A, scan of the track M (mol. Wt. Marders); B, scan of track 1 (control); C and D, sans of tracks 2 and 3 (50 and 100 ppm paclobutrazol); D, G and J, scans of tracks 4,7 and 10 (5, 50 and 100 ppm Cd^{2+}); E and F, scans of tracks E and F (5 ppm Cd^{2+} + 50 ppm, and 100ppm paclobutrazol); H and I, scans of tracks 8 and 9 (10 ppm Cd^{2+} + 50 ppm, and 100 ppm paclobutrazol); K and L, scans of tracks 11 and 12 (50 ppm Cd^{2+} + 50 ppm, and 100 ppm paclobutrazol); respectively.

Table 2: Comparative analysis of relative concentrations, molecular weight, and mobility rate (Rm) of proteins in *Arachis hypogeae* plants treated with different combinations of cadmium (Cd^{2+}) and paclobutrazol (PP333). These bands were separated using SDS-PAGE technique.

Band No.	Treatment and Band %												Rm	M.Wt. (KDa)
	1	2	3	4	5	6	7	8	9	10	11	12		
1	2.73	3.21	-	4.8	3.05	4.35	-	3.24	1.83	-	-	-	2.20	75.73
2	-	-	5.42	3.92	4.07	8.05	3.22	1.90	4.07	-	-	2.09	0.22	70.06
3	-	-	-	6.39	6.56	7.06	-	4.8	2.74	-	2.88	2.25	0.32	40.30
4	-	-	-	-	-	-	-	-	-	-	5.78	3.21	0.33	39.01
5	2.5	3.17	1.34	5.82	5.08	2.21	7.69	3.53	3.04	4.96	2.56	1.84	0.34	37.02
6	-	-	-	-	-	-	-	-	-	-	-	0.93	0.40	34.44
7	1.78	1.37	2.39	1.63	1.14	-	0.85	0.97	1.81	0.49	0.63	0.65	0.46	32.49
8	-	-	-	-	-	-	-	1.03	0.76	0.33	0.14	-	0.50	31.28
9	1.19	1.27	0.76	0.47	0.86	1.95	0.69	0.61	0.78	0.41	0.20	0.38	0.54	39.54
10	2.83	0.65	0.43	0.93	2.25	2.17	1.95	1.22	1.68	0.39	0.42	0.33	0.57	26.89
11	7.79	10.1	7.16	9.67	8.34	5.27	7.34	5.60	12.0	1.07	3.44	14.1	0.62	23.52
12	8.01	7.10	6.33	11.4	8.53	3.98	5.59	4.77	9.36	1.04	2.02	13.6	0.63	22.49
13	4.42	1.67	1.15	1.87	1.45	2.92	2.21	0.93	1.65	1.01	1.24	3.66	0.84	7.52
14	1.43	0.55	-	0.68	-	-	-	-	-	-	1.34	0.54	0.86	7.05
15	-	-	1.01	-	-	-	-	-	-	-	-	-	0.90	5.45
16	1.71	-	-	-	-	-	-	-	-	-	1.38	2.42	0.94	4.46
17	26.1	9.5	15.4	11.9	17.2	-	18.4	17.9	4.6	4.73	-	6.4	0.98	3.68
Total no. of protein bands	11	10	10	12	11	9	9	12	12	9	12	14		

Treatments:
1 = untreated (control) plants; 2 and 3 = 50 and 100 ppm paclobutrazol; 4, 7 and 10 = 5, 50 and 100 ppm Cd; 5 and 6 = 5 ppm Cd + 50 ppm paclobutrazol and 5 ppm Cd + 100 ppm paclobutrazol; 8 and 9 = 10 ppm Cd + 50 ppm paclobutrazol and 10 ppm Cd + 100 ppm paclobutrazol; 11 and 12 = 50 ppm Cd + 50 ppm paclobutrazol and 50 ppm Cd + 100 ppm paclobutrazol.

Allah, 1995).

The highest concentration (50-ppm) of Cd completely inhibited the synthesis of a 75 KDa polypeptide even in the presence of 100-ppm paclobutrazol. Lower Cd concentration (10- ppm) also inhibited the synthesis of this protein, but paclobutrazol treatments (50 and 100 ppm) were able to alleviate the Cd-stress effects and this protein was kept formed. This finding indicates the role of paclobutrazol in the protection of peanut plants at the level of protein synthesis. The other polypeptides participated in forming the Cd stress protein complex have the molecular weights 70, 40, 39, 34 and 31 KDa. Only one molecular weight of about 5 KDa was formed in response to 100- ppm paclobutrazol.

The synergistic behavior of two more polypeptides of molecular weights 23 and 22 KDa were observed in response to Cd and paclobutrazol treatments (Table 2). The amounts of these proteins were induced, firstly, by Cd treatments, and secondly, by paclobutrazol. Their synthesis increased as the concentrations of both Cd and paclobutrazol increased. These proteins could be declared among the proteins and peptides involved in the strategy of peanut plants to avoid and even sequester the toxicity of Cd stress. Thus, resistance of peanut plants are highly correlated with the stimulation and/or synthesis of certain polypeptides of specific molecular weights that have been known as metal-binding polypeptides or Cd-binding proteins (Jackson *et al*., 1985; Rauser, 1984; Reese and Wagner, 1987; El-Enany and Abd-Allah, 1995).

From the results of this experiment, it can be concluded that, the peanut plant able to grow in atmosphere, contaminated with Cd without exhibiting toxicity symptoms and application of paclobutrazol with Cd stimulate the synthesis of new polypeptides.

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