Modification of cadmium toxicity in pea seedlings by kinetin

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ABSTRACT

The effect of foliar application of kinetin on the growth and some physiological processes of pea plants growing in soil supplemented with 25 or 50μM Cd were studied. Cadmium treatment inhibited the growth rate, chlorophyll (Chl) content, net photosynthesis (P_N), content of soluble sugars and free amino acids of either shoots or roots. The application of kinetin (kin) enhanced the growth rate, Chl content, P_N, soluble sugars and free amino acids content of shoots and roots; dark respiration rate (R_D), contents of soluble protein and proline were increased by cadmium treatment. The addition of kinetin to Cd-stressed plant reduced R_D, soluble protein and proline content. Considerable variations in the content of Na^+, K^+, Ca^{2+} and Mg^{2+} were induced by Cd-treatments. Foliar application of kinetin exhibited a favorable effect on the accumulation of some ions and antagonized or ameliorated the inhibitory effect of Cd stress on some others.

Keywords: growth; chlorophyll; net photosynthesis; dark respiration; cadmium; pea; kinetin

Cadmium (Cd) is dispersed in natural environment through human activities as well as natural rock mineralization processes (Sanita di Toppi and Gabrielli 1999). Major inputs of Cd into agricultural soils are due to the application of phosphoric fertilizers (Mc Lauglin et al. 2000). Plants easily absorb Cd from soil and transport it to shoots, whence it reaches human nutrition through food chains, thus representing a health hazard.

Cadmium induces complex changes in plants at genetical, biochemical and physiological levels, leading to phytotoxicity, whose most obvious symptoms are: reduction of tissue and organ growth, leaf chlorosis, and leaf and root necrosis (Hernandez and Cooke 1997). Among other changes, Cd affects mineral nutrition (Ouzonidou et al. 1997) and chlorophyll metabolism and photosynthesis (Baryla et al. 2001), and thus these parameters may be used as indicators of its phytotoxicity (Ernst et al. 2000).

Plant growth regulators may be a part of a signal transduction chain, or their presence may stimulate reactions that are signals and/or causative agents for stress responses. Cytokinins are a class of phytohormones that stimulate water uptake, increase cell division, promote organ development and lead to the regeneration and proliferation of shoots (Letham and Palni 1983). Cytokinin-induced accumulation of RNA and protein for CAM-specific phosphoenolpyruvate carboxylase isoforms in the absence of salt stress was observed (Thomas et al. 1992). Many investigators use plant growth regulators to alleviate severe effects of stress. Kinetin is thought to be promising in that respect. The effect of kinetin on chlorophyll content, growth and on some metabolic processes was demonstrated by Kaur et al. (1998).

In the present work we have studied the effects of exogenous kinetin application on growth criteria and some metabolites of pea plants grown at two toxic levels of Cd (25 and 50μM).

MATERIAL AND METHODS

Pea (*Pisum sativum* L.) seeds were obtained from the Crop Institute, Agricultural Research Center, Taiz, Yemen. Seeds were sterilized with sodium hypochlorite solution (5%) for five minutes, and washed thoroughly with distilled water before use. Ten seeds were transferred into polyethylene pots (10 cm in diameter and 13.5 cm high); three pots for each treatment, each filled with 2 kg soil (sand-clay 2:1) and watered twice with the Hoagland solution. The moisture content of the soil was never allowed to fall below field capacity.

This was achieved by checking the masses of pots twice a day. Ten-day old seedlings were irrigated with the Hoagland solution containing 0 (control), 25 and 50μM cadmium as $CdCl_2$ .2 H_2O for a period of 15 days. The control plants were watered with the Hoagland solution only. Cd-treated plants received 10 or 20μM kin in the Hoagland solution last 10 days before harvesting. At the end of the experimental period (35 days) fresh shoots and roots were dried in an aerated oven at 70°C while being weighed until a constant dry mass was reached. The contents of chlorophyll a and chlorophyll b were determined by the method of Moran (1982). Net photosynthetic rate (oxygen evolution) and dark respiration (oxygen consumption) were determined manometrically using leaf disks (diameter 16 mm) kept at 25°C, irradiance of 12 W/m^2 (40 W GEF lamps) using the Warburg buffer No. 2961 type VL 85 (Umbreit et al. 1959); soluble sugars were determined according to the method of Fales (1951); soluble protein was determined according to Lowry et al. (1951); proline content was determined following the procedure of Bates et al. (1973); total free amino acids were estimated by the Lee and Takahashi (1966) method; sodium and potassium were determined by using the flame photometer (Williams and Twine 1960); and calcium and magnesium by the versene titration method.

Statistical analysis. Statistical analysis was carried out by one-way ANOVA using Student's *t*-test to test the significance of differences between means. Means were considered significantly different at 5% or 1% level of probability.

RESULTS AND DISCUSSION

Fresh weight and dry mass production of Cd-treated plants decreased as Cd level raised in the nutrient medium (Figures 1 and 2). The inhibitory effects of Cd stress on the plant growth are recorded in many plant species (Wu et al. 2004, Drazic and Mihailovic 2005). Such a reduction in

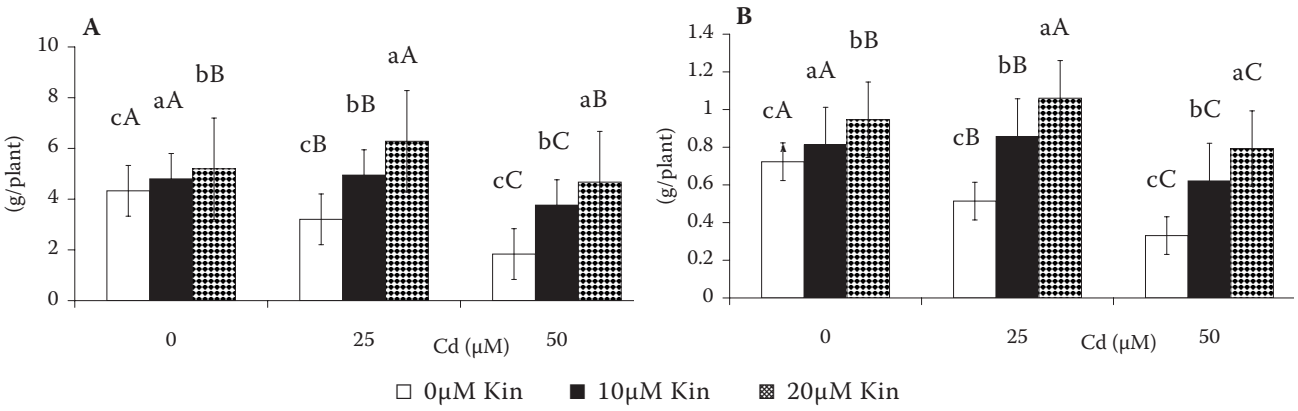

Figure 1. Fresh matter (A) and dry matter (B) of shoots of *Pisum sativum* plant supplemented with different levels of cadmium or cadmium plus kinetin (10 and 20μM); values in parentheses represent ± SD (n = 3). Means which are not significantly different (P = 0.05) are followed by the same letter

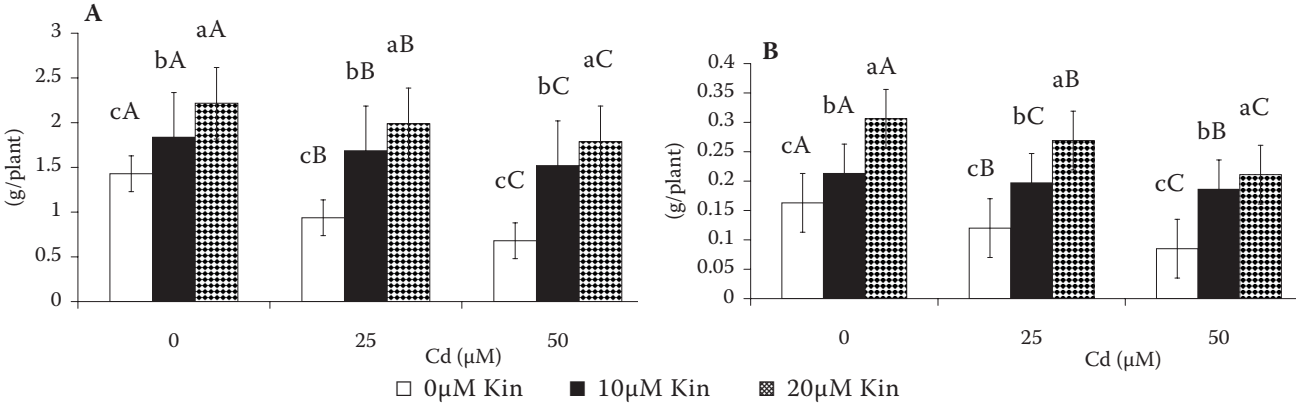

Figure 2. Fresh matter (A) and dry matter (B) of roots of *Pisum sativum* plant supplemented with different levels of cadmium or cadmium plus kinetin (10 and 20μM); values in parentheses represent ± SD (n = 3). Means which are not significantly different (P = 0.05) are followed by the same letter

the growth of Cd stressed plants could be ascribed mainly to inhibited cell division and/or cell enlargement (Davies et al. 1991). Foliar application of kinetin with Cd counteracted the inhibitory effects of Cd stress on the growth. These results are in agreement with those recorded by Gadallah and El-Enany (1999).

Table 1 shows that the chlorophyll a and b contents of Cd-treated pea plants significantly decreased below control levels. The decrease in Chl a and Chl b induced by Cd treatments was as follow: 27% and 52% at 25μM and 28% and 56% at 50μM Cd, respectively. The decrease in leaf Chl content with increasing Cd-concentration was in accordance with the results of Drazic and Mihailovic (2005). Cadmium may have inhibited the production of Chl by affecting both the synthesis of 5-aminolevulinic acid and the protochlorophyllide reductase (Stobart et al. 1985). The application of kinetin at 10 or 20μM significantly increased Chl a and Chl b in both control and Cd-treated plants (Table 1). The ameliorative effects of kinetin at 10 and 20μM on Cd-induced reduction in Chl a and Chl b were 85%, 78%, 139% and 162% at 25μM Cd and 115%, 108%, 188% and 194% at 50μM Cd, respectively; these results are in agreement with Gadallah and El-Enany (1999). The enhancement of Chl content by kinetin may be due to the effects of kinetin on both Chl synthesis and degradation (Sabater and Rodriquez 1978).

The data in Table 1 reveal that the net photosynthetic rate was reduced by cadmium treatment. The maximum reduction in photosynthesis was 12% and 58% of control plants at 25μM and 50μM Cd, respectively. A similar conclusion was made by Jing et al. (2005). Supplemented kinetin (10 or 20μM) significantly enhanced net photosynthetic rate at low and high levels of Cd. The maximum increase in net photosynthetic rate was 55%, 67%, 78% and 93% of 25μM and 50μM Cd-treated plants at 10μM and 20μM kin, respectively. Kaur et al. (1998) reported a similar increase in the photosynthetic rate of chickpea plants in response to the kinetin treatment.

Data presented in Table 1 show that the dark respiration rate significantly increased with increasing Cd concentrations. Moreover, kinetin treatment caused a significant decrease in the dark respiration rate below that of the treated samples. A similar increase in respiration rate under Cd-stress was observed by Iqbal and Khudsar (2000).

The data in Tables 2 and 3 reveal that the content of soluble sugars decreased in both shoots and roots of Cd-treated plants as compared with control. This reduction in soluble sugars may be attributed to the inhibition of photosynthetic activity (Huang et al. 1974). Also, Gadallah (1995) indicated a decrease in soluble sugars under toxic concentrations of Cd. Kinetin treatment caused a significant increase in the content of soluble sugars. In this regard, the content of soluble sugars was also increased in sunflower plants in relation to kinetin (Gadallah 1995).

The content of soluble proteins in shoots and roots increased significantly as cadmium level increased in pots cultures (Tables 2 and 3), which is in accordance with the findings of Ali et al. (1998). The combination of kinetin with cadmium treatment lowered soluble protein content in plants shoots and roots at 10 and 20μM kin. The same results were obtained by Yadav et al. (1997).

The proline contents progressively accumulated in both shoots and roots of pea plants treated with 25 and 50μM Cd (Tables 2 and 3). These results are in agreement with earlier findings of Shaw and Rout (2002); they also concluded that proline is believed to protect plant tissues against heavy metal stress by acting as N-storage compound, osmotically active solute and protectant for enzymes, other macromolecules and cellular structures (Shah and Dubey 1997, Munoz et al. 1998). It is also observed that kinetin lowered proline contents in Cd- treated plants. In this respect, Gadallah and El-Enany (1999) found the same result in the case of heavy metals in *Lupinus termis* plants.

The free amino acid accumulation showed a marked decrease as the Cd level in the pot experiments increased (Tables 2 and 3). These results are in agreement with Chiu et al. (1998). The application of kinetin did not alleviate the inhibitory effects of Cd-stress on amino acid contents but it induced a significant stimulatory effect. This is in compliance with the report of Yadav et al. (1997).

The Na^+/K^+ ratio of shoots and roots of *Pisum sativum* plants was significantly increased as Cd level rose to 50μM (Table 4). The application of kinetin significantly reduced Na^+/K^+ ratio in both shoots and roots. Table 4 also indicates that Ca^{2+}/Mg^{2+} ratio raised more significantly in roots than in shoots. However, the addition of kinetin minimized the Ca^{2+}/Mg^{2+} ratio in shoots and roots. These results are in accordance with some of the results of Ouariti et al. (1997) and Lagriffoul et al. (1998) who used other plant tissues. The alterations in distribution and accumulation of mono-and divalent cations in different organs of Cd-stressed

Table 1. Chlorophyll a and b content, net photosynthetic (P_N) and dark respiration (D_R) rates of *Pisum sativum* plants supplemented with different levels of cadmium or cadmium plus kinetin (10 and 20μM)

Parameter	Kin (μM)	Cd (μM)			LSD (%)	
		0	25	50	5	1
Chl a (mg/g fm)	0	0.711^{cA}	0.522^{cB}	0.343^{cC}	0.007	0.013
	10	0.796^{bC}	0.965^{bA}	0.821^{bB}	0.012	0.022
	20	0.814^{aC}	1.122^{aA}	0.987^{aB}	0.010	0.019
LSD	5%	0.017	0.012	0.009		
	1%	0.013	0.024	0.016		
Chl b (mg/g fm)	0	0.652^{cA}	0.468^{cB}	0.287^{cC}	0.013	0.025
	10	0.686^{bC}	0.832^{bA}	0.751^{bB}	0.010	0.019
	20	0.732^{aC}	0.974^{aA}	0.843^{aB}	0.012	0.022
LSD	5%	0.013	0.010	0.014		
	1%	0.023	0.018	0.026		
P_N (μmol O_2/g dm/s)	0	313.267^{cA}	274.878^{cB}	130.842^{cC}	10.785	19.798
	10	373.493^{bB}	425.399^{bA}	232.676^{bC}	16.107	29.567
	20	388.443^{aB}	458.147^{aA}	252.091^{aC}	11.555	21.211
LSD	5%	9.378	17.458	9.496		
	1%	17.216	32.047	17.431		
D_R (μ-mol O_2/g dm/s)	0	71.872^{aC}	173.276^{aB}	368.023^{aA}	7.055	12.951
	10	61.823^{bC}	96.224^{bB}	167.389^{bA}	3.509	6.441
	20	54.420^{cC}	86.428^{cB}	141.223^{cA}	4.122	7.567
LSD	5%	3.279	6.213	5.944		
	1%	6.020	11.404	10.911		

Means which are not significantly different (P = 0.05) are followed by the same letter

Table 2. Soluble sugars, soluble protein, proline and amino acids content of *Pisum sativum* plant shoots supplemented with different levels of cadmium or cadmium plus kinetin (10 and 20μM)

Parameter	Kin (μM)	Cd (μM)			LSD (%)	
		0	25	50	5	1
Soluble sugars (mg/g dm)	0	20.387^{cA}	13.642^{cB}	9.909^{cC}	3.051	5.601
	10	42.522^{aA}	30.871^{bC}	38.383^{bB}	1.737	3.189
	20	36.756^{bC}	46.102^{aB}	50.677^{aA}	2.494	4.579
LSD	5%	3.911	1.158	1.516		
	1%	7.179	2.127	2.784		
Soluble protein (mg/g dm)	0	60.871^{aC}	72.622^{aB}	80.776^{aA}	1.523	2.796
	10	50.672^{bA}	46.648^{bB}	40.178^{bC}	3.630	6.664
	20	43.680^{cA}	37.283^{cB}	30.135^{cC}	3.421	6.279
LSD	5%	2.896	2.305	3.676		
	1%	5.316	4.231	6.747		
Proline (mg/g dm)	0	2.455^{aA}	3.134^{aB}	4.821^{aC}	0.014	0.026
	10	2.052^{bB}	1.964^{bC}	2.133^{bA}	0.029	0.053
	20	1.617^{cB}	1.459^{cC}	1.835^{cA}	0.22	0.040
LSD	5%	0.023	0.026	0.018		
	1%	0.043	0.047	0.033		
Amino acids (mg/g dm)	0	30.334^{cA}	22.258^{cB}	19.823^{cC}	1.539	2.826
	10	38.713^{bC}	45.671^{bB}	51.322^{bA}	0.314	0.577
	20	40.285^{aC}	54.324^{aB}	60.784^{aA}	0.081	0.149
LSD	5%	0.306	1.278	0.861		
	1%	0.562	2.347	1.580		

Means which are not significantly different (P = 0.05) are followed by the same letter

Table 3. Soluble sugars, soluble protein, proline and amino acids content of *Pisum sativum* plant roots supplemented with different levels of cadmium or cadmium plus kinetin (10 and 20μM)

Parameter	Kin (μM)	Cd (μM)			LSD (%)	
		0	25	50	5	1
Soluble sugars (mg/g dm)	0	11.294^{cA}	9.727^{cB}	7.797^{cC}	0.465	0.854
	10	13.573^{bC}	15.804^{bB}	17.354^{aA}	0.245	0.450
	20	14.486^{aC}	18.699^{aA}	16.538^{bB}	0.426	0.782
LSD	5%	0.294	0.549	0.429		
	1%	0.539	1.008	0.787		
Soluble protein (mg/g dm)	0	41.907^{aC}	52.292^{aB}	61.126^{aA}	2.305	4.228
	10	25.667^{bC}	30.899^{bB}	36.749^{bA}	2.575	4.728
	20	23.072^{cC}	27.892^{cB}	33.320^{cA}	1.919	3.523
LSD	5%	1.824	2.046	2.847		
	1%	3.349	3.757	5.226		
Proline (mg/g dm)	0	1.521^{aC}	2.129^{aB}	3.616^{aA}	0.186	0.341
	10	0.789^{bC}	0.988^{bB}	1.228^{bA}	0.020	0.038
	20	1.123^{cA}	0.684^{cB}	0.885^{cC}	0.006	0.011
LSD	5%	0.110	0.042	0.145		
	1%	0.203	0.077	0.266		
Amino acids (mg/g dm)	0	21.423^{bA}	18.628^{cB}	16.597^{cC}	1.175	2.157
	10	23.884^{bB}	26.117^{bB}	29.247^{bA}	3.097	5.685
	20	30.042^{aC}	34.914^{aB}	38.042^{aA}	2.123	3.897
LSD	5%	2.628	2.222	1.908		
	1%	4.824	4.078	3.503		

Means which are not significantly different (P= 0.05) are followed by the same letter

Table 4. Na^+/K^+ and Ca^{2+}/Mg^{2+} ratios of shoots and roots of *Pisum sativum* plant supplemented with different levels of cadmium or cadmium plus kinetin (10 and 20μM)

Parameter	Kin (μM)	Cd (μM)			LSD (%)	
		0	25	50	5	1
Shoots Na^+/K^+	0	1.283^{aC}	1.713^{aB}	2.560^{aA}	0.404	0.742
	10	0.654^{bC}	0.725^{bB}	1.108^{bA}	0.069	0.127
	20	0.526^{bB}	0.564^{cB}	0.762^{cA}	0.103	0.189
LSD	5%	0.381	0.067	0.170		
	1%	0.700	0.123	0.313		
Ca^{2+}/Mg^{2+}	0	3.133^{aC}	4.301^{aB}	5.839^{aA}	0.681	1.250
	10	3.030^{aC}	4.044^{aB}	4.712^{bA}	0.446	0.818
	20	2.730^{bC}	3.498^{bB}	4.366^{bA}	0.275	0.506
LSD	5%	0.307	0.555	0.566		
	1%	0.564	1.020	1.039		
Roots Na^+/K^+	0	1.663^{aC}	2.621^{aB}	4.538^{aA}	0.214	0.393
	10	1.329^{bC}	1.373^{bB}	1.499^{bA}	0.0.33	0.061
	20	1.212^{cB}	0.973^{cC}	1.449^{cA}	0.038	0.071
LSD	5%	0.054	0.184	0.107		
	1%	0.100	0.338	0.196		
Ca^{2+}/Mg^{2+}	0	3.529^{aC}	5.518^{aB}	8.766^{aA}	0.895	1.643
	10	3.465^{aB}	3.022^{cC}	4.486^{cA}	0.278	0.510
	20	3.095^{bC}	-4.4307^{bB}	6.355^{bA}	0.390	0.717
LSD	5%	0.184	0.832	0.362		
	1%	0.280	1.528	0.665		

Means which are not significantly different (P = 0.05) are followed by the same letter

plants may be an indication of the role of these cations in regulation of the physiological activities of these plants (Drazic and Mihailovic 2005). Foliar application of kinetin had an inhibitory effect on the Na^+/K^+ and Ca^{2+}/Mg^{2+} ratios.

To conclude, the exogenous kinetin application may ameliorate the negative effect of Cd on pea plants via accelerating photosynthesis and modification of some metabolites (e.g. proline, free amino acids and soluble sugars).

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