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Effects of cadmium and kinetin on chlorophyll content, saccharides and dry matter accumulation in sunflower plants

M.A.A. GADALLAH

Botany Department, Faculty of Science, Assiut University, Assiut, Egypt

Abstract

Cadmium (Cd) and kinetin (Kin) significantly affected the growth and contents of chlorophyll (Chl) and of soluble and reserve (hydrolysable) saccharides in sunflower plants. Cd-treated plants had lower contents of Chl and soluble saccharides and produced less dry matter than control (Cd-untreated) plants. Chl *a* stability to heat (CSI) increased at all Cd concentrations. The same was true for Chl *b* stability (0 - 10 µM Cd). Spraying sunflower shoots with Kin solutions counteracted the deleterious effects of Cd. Kin application enhanced the Chl *a* and *b* contents, Chl *a*/*b* ratio, content of soluble saccharides and dry matter, and to less extent Chl stability. The relative role of Kin in affecting the parameters tested (as indicated by η^2 values) was predominant while that of Cd was subsidiary except for Chl *a* stability. The role of Cd × Kin interaction was dominant for hydrolysable saccharides. Hence spraying shoots of plants grown under increasing Cd concentration with Kin can partially alleviate inhibitory effects of cadmium.

Key words: chlorophyll stability index, *Helianthus annuus*, hydrolysable saccharides, soluble saccharides

Introduction

Cadmium is an ever increasing industrial pollutant particularly in areas associated with smelting of zinc and heavy road traffic (Varma and Katz 1978, Ernst 1980). Plants can accumulate Cd to varying extent (Pettersson 1977, Keul *et al.* 1980), and in some cases it reaches levels that may be toxic to animals (Lagerwerff 1972). Studies on the phytotoxic effects of Cd have principally focused on nutrition (Walker *et al.* 1977), on enzyme activities (Vallee and Ulmer 1972, Påhlsson 1989, Van Assche and Clijsters 1990), or on photosynthesis and related processes (Baszynski *et al.* 1980, Weigel 1985, Stiborová *et al.* 1987). Cadmium also reduces Chl content (Czuba and Ormrod 1973, Imai and Siegel 1973, Baszynski *et al.* 1980, Bishnoi *et al.* 1993, Ferretti *et al.* 1994) probably by affecting both the synthesis of

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5-aminolevulinic acid and of the protochlorophyllide reductase ternary complex with its substrates (Stobart *et al.* 1985). Cd-treated plants exhibited reduced growth rate of shoots and roots coupled with reduction in photosynthetic activity and reduced saccharide accumulation in root nodules (Huang *et al.* 1974).

Recently, some investigators use plant growth regulators (cytokinins, especially kinetin) to alleviate severe effects of stress, namely on Chl synthesis (Banerji and Laloraya 1967). The effect of Kin on the stimulation of Chl formation may be due to an influence on the synthesis of protochlorophyllide (Shlyk and Averina 1973). Kin treatment increases the Chl *a* and *b* contents in many crop plants grown under stress (Salama and Awadalla 1987, Gadallah 1994). In the present study we put forward the idea that Kin may in some way counteract the deleterious effect of cadmium on growth. In addition, some aspects of a possible dual effect of Cd and Kin on Chl content and Chl stability to heat and on content of saccharides were also investigated.

Materials and methods

Sunflower (*Helianthus annuus* L. cv. Geza 2) plants were grown under field conditions in plastic pots containing 1400 g air dry soil (sand-clay 2:1 v/v). The plants (5 per pot) were twice watered with 100 cm^3 portions of full strength 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 daily. After 5 weeks the distilled water used for irrigation was replaced by a solution containing 5, 10, 20 and 40 μM cadmium as $CdCl_2.2.5\ H_2O$. The control plants were watered with distilled water. The plants were irrigated with Cd solutions for a period of two weeks before starting treatments with Kin. Kin solutions (0, 5, 10 and 15 g m^{-3}) were applied three times at 5-d-intervals by spraying the shoots. Three pots were assigned at random to each treatment combination at every Cd concentration. A week after the last Kin treatment, plants were harvested and analyzed.

Chl *a* and *b* contents were determined according to Todd and Basler (1965). Chl stability to heat (57 °C, 30 min) was assessed according to Murty and Majumder (1962). Chl stability index was modified as follows:

$$SCI = \frac{\text{Chl in heated sample}}{\text{Chl in fresh sample}} \times 100$$

Shoot soluble and reserve (hydrolysable) saccharides were determined according to Pucher *et al.* (1948) and Dubois *et al.* (1956), respectively. For dry matter determination the fresh shoots were dried in an aerated oven at 70 °C to constant mass.

Statistical evaluation of the effects of single factors and of their interaction on the parameters tested included: analysis of variance (F values), least significant differences (LSD) test, and coefficient of determination (η^2), respectively (Ostle 1963).

Results

In the absence of Kin (Table 1), Chl *a* and *b* contents decreased with increasing Cd concentration. The Chl *a* content amounted to 72, 64, 69, and 57 % of the control at 5, 10, 20 and 40 μM Cd, and that of Chl *b* was 88, 70, 64 and 57 %, respectively. The effect of Cd was greatly reduced in the presence of Kin. In contrast, spraying with Kin significantly increased Chl *a* content in both control and Cd treated plants. Chl *b* was significantly enhanced by the three Kin concentrations in the presence of Cd at concentration above 20 μM. At Cd concentration not exceeding 10 μM, Chl *b* was significantly higher in plants receiving 5 g m^{-3} Kin only. The lowest used Kin concentration was generally the most effective one.

Chl *a*/*b* ratio was slightly affected by Cd treatment. No significant changes in Chl *a*/*b* were found at Cd concentrations above 5 μM. The only significant decrease was observed at 5 μM Cd compared to the control (0 μM Cd). Kin treatment in general enhanced Chl *a*/*b* ratio in both control and Cd-treated plants except at 20 μM Cd receiving 5 g m^{-3} Kin. The enhancement by Kin was significant except in control (with 5 and 10 g m^{-3}) and 40 μM Cd (with 5 g m^{-3} Kin).

In the absence of Kin, treatment with all concentrations of Cd significantly increased Chl *a* stability. Chl *b* stability was significantly enhanced by 5 and 10 μM Cd but the opposite was true at 40 μM. Kin significantly increased Chl *a* stability in control and 40 μM Cd-treated plants. Chl *b* stability was significantly enhanced by Kin at 20 and 40 μM Cd. The reverse was true at 5 μM Cd by receiving 10 g m^{-3} Kin.

In plants not receiving Kin, the contents of soluble saccharides (SS; Table 2) were significantly lower in Cd-treated plants than in the control (5 μM Cd was an exception). Spraying with Kin significantly increased the SS content in both control and Cd-treated plants (statistically nonsignificant were only the differences at 5 and 10 μM Cd at the lower Kin application). Although the plants exposed to Cd had less SS in the absence of Kin, their hydrolysable saccharides (HS) were not significantly affected except at 40 μM Cd. HS fluctuated in an irregular manner in response to Kin treatment (Table 2).

In the absence of Kin, increasing Cd ion concentration over the range from 10 to 40 μM yielded a highly significant decrease in dry matter content of plants. Kin induced an increase in dry matter content, that was highly significant except with 5 and 10 g m^{-3} Kin in 0 and 5 μM Cd-treated plants. The highest used Kin concentration was the most effective one.

The effects of Cd, Kin as well as their interactions (Table 3) on the parameters tested were statistically significant. The coefficient of determination (η^2) indicated that the role of (Cd × Kin) interaction was predominant in affecting HS ($\eta^2 = 0.83$). The relative role of Kin was predominant for all parameters tested (except in CSI a and HS) while that of Cd was subsidiary. The role of Cd is predominant in affecting Chl *a* stability only.

Table 1. Chlorophyll (Chl) content, Chl *a*/*b* ratio, Chl stability to heat (CSI) and shoot dry mass content in sunflower at different cadmium (Cd) and kinetin (Kin) concentrations.

Parameter	Cd [μM]	Kin [g m^{-3}] 0	5	10	15	L.S.D. [%] 1	5
Chl *a* content [g kg^{-1}(f.m.)]	0	1.44	1.70	1.71	1.73	0.27	0.19
	5	1.03	1.44	1.35	1.44	0.20	0.14
	10	0.92	1.49	1.31	1.33	0.20	0.14
	20	0.99	1.50	1.37	1.53	0.32	0.22
	40	0.82	1.62	1.34	1.59	0.24	0.16
L.S.D.	1 %	0.24	0.26	0.23	0.23		
	5 %	0.16	0.18	0.16	0.16		
Chl *b* content [g kg^{-1}(f.m.)]	0	0.88	1.00	0.98	0.89	0.16	0.11
	5	0.77	0.85	0.79	0.77	0.08	0.06
	10	0.62	0.87	0.67	0.66	0.14	0.10
	20	0.56	0.87	0.68	0.76	0.19	0.13
	40	0.50	0.94	0.66	0.79	0.14	0.10
L.S.D.	1 %	0.13	0.16	0.14	0.11		
	5 %	0.09	0.11	0.10	0.08		
Chl *a*/*b*	0	1.64	1.70	1.75	1.94	0.30	0.20
	5	1.35	1.69	1.71	1.88	0.21	0.15
	10	1.48	1.71	1.96	1.99	0.29	0.20
	20	1.77	1.69	2.00	2.03	0.08	0.05
	40	1.65	1.73	2.05	2.05	0.22	0.15
L.S.D.	1 %	0.35	0.08	0.16	0.20		
	5 %	0.25	0.06	0.11	0.14		
CSI for Chl *a* [%]	0	57.01	75.65	77.35	76.95	8.21	5.65
	5	91.63	88.85	92.68	94.33	9.36	6.43
	10	91.99	91.28	92.78	94.81	9.73	6.58
	20	86.60	96.00	85.57	80.03	9.23	6.45
	40	77.28	85.33	86.47	86.65	6.38	4.49
L.S.D.	1 %	8.44	8.45	9.16	9.24		
	5 %	5.94	5.94	6.44	6.50		
CSI for Chl *b* [%]	0	72.17	66.79	77.03	76.42	9.82	6.75
	5	84.55	79.00	81.51	94.45	8.45	5.45
	10	82.42	80.19	90.39	97.35	10.13	6.96
	20	72.71	87.57	86.09	87.49	10.05	6.90
	40	56.33	80.11	92.96	96.12	6.67	4.59
L.S.D.	1 %	9.62	11.36	10.56	9.79		
	5 %	6.76	7.99	7.42	6.88		
Shoot dry mass [g]	0	4.92	5.20	5.45	5.98	0.84	0.58
	5	4.30	4.56	4.63	5.28	1.05	0.72
	10	3.70	5.05	4.97	5.42	0.98	0.67
	20	3.15	4.41	4.81	5.31	1.24	0.85
	40	2.50	4.16	3.81	4.51	1.16	0.80
L.S.D.	1 %	1.07	1.25	1.11	1.24		
	5 %	0.73	0.88	0.78	0.87		

Table 2. Soluble (SS) and hydrolysable (HS) saccharides in shoots of sunflower plants at different cadmium and kinetin concentrations.

Parameter	Cd [μM]	Kin [g m^{-3}] 0	5	10	15	L.S.D. [%] 1	5
SS [g kg^{-1}(d.m.)]	0	22.22	28.83	32.64	39.01	6.24	4.38
	5	21.94	20.98	38.80	33.87	8.93	6.17
	10	17.64	17.53	35.13	39.05	6.53	4.59
	20	16.84	31.46	45.17	45.20	5.89	4.05
	40	14.82	41.19	46.56	42.53	9.48	6.51
L.S.D.	1 %	6.33	5.93	7.41	8.84		
	5 %	4.46	4.08	5.09	6.06		
HS [g kg^{-1}(d.m.)]	0	8.90	2.52	8.61	16.01	3.38	2.32
	5	8.29	8.32	9.70	9.27	4.39	3.02
	10	7.13	30.84	5.99	9.55	5.18	3.56
	20	7.28	4.97	10.51	8.07	2.74	1.88
	40	6.17	6.04	11.91	6.27	3.56	2.45
L.S.D.	1 %	2.88	3.66	3.39	4.72		
	5 %	2.02	2.57	2.38	3.32		

Discussion

Cadmium greatly lowered the Chl content than the control. The decrease in leaf Chl content with increasing Cd-concentration was in accordance with results of Czuba and Ormrod (1973), Imai and Siegel (1973), Baszynski *et al.* (1980) and Stobart *et al.* (1985). 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 effect of Cd on the free and photoconvertible protochlorophyllide reductase is probably due to its interference with the sulfhydryl site on the reductase protein (Ernst 1980).

Kin-treated plants generally had higher Chl content than untreated plants (Salama and Awadalla 1987, Gadallah 1994). The enhancement of Chl content by Kin may be due to the effects of Kin on both Chl synthesis (Beevers 1968) and degradation (Sabater and Rodriquez 1978). The effects of Kin on the stimulation of Chl formation may be due to an influence on synthesis of protochlorophyllide (Shlyk and Averina 1973) and formation of 5-aminolevulinic acid (Stobart *et al.* 1972).

The Chl *a*/*b* ratio only slightly changed in response to Cd. This means that the effect of Cd on the destruction and/or inhibition of Chl formation was identical for both Chl. The Chl *a*/*b* ratio was enhanced by Kin. This indicates that the production or stimulation of Chl *a* synthesis by Kin was favoured over that of Chl *b*. Generally, increasing Cd concentration and Kin treatment increased the Chl stability to heat. Cd-treated plants had less SS than the control. The reduction in SS may be attributed to the inhibition of photosynthetic activity (Huang *et al.* 1974). Kin-treated plants showed higher soluble saccharides than the untreated plants. Increased SS showed higher soluble saccharides than the untreated plants. Increased SS concentration may result from increased starch hydrolysis, their increased synthesis or decreased conversion to other products (Meidner 1967).

Table 3. F and η^2 values for the effect of cadmium (Cd), kinetin (kin) and their interactions (Cd × Kin) on chlorophyll (Chl) *a* and *b* content, Chl *a*/*b* ratio, Chl stability index (CSI), dry mass (d.m.), soluble (SS) and hydrolysable (HS) saccharides content of sunflower.

Source of variance	Chl *a* F	η^2	Chl *b* F	η^2	Chl *a*/*b* F	η^2	CSIa F	η^2
Cd	35.00**	0.28	15.00**	0.43	14.29**	0.18	39.94**	0.67
Kin	97.50**	0.59	18.75**	0.40	70.00**	0.68	7.93**	0.10
Cd × Kin	5.00**	0.13	2.50	0.17	2.86	0.14	4.45**	0.23
	CSIb F	η^2	d.m. F	η^2	SS F	η^2	HS F	η^2
Cd	16.63**	0.31	4.64**	0.20	8.30**	0.10	19.47**	0.14
Kin	37.41**	0.53	20.73**	0.66	76.45**	0.72	6.25**	0.03
Cd × Kin	8.10	0.46	1.18	0.14	4.78**	0.18	39.57**	0.83

* - significant at 5 % confidence level, ** - significant at 1 % confidence level

Cadmium-treated plants exhibited a reduced growth rate compared with untreated plants. The growth reduction (measured as dry matter content) associated with Cd treatment was probably caused by inhibition of protein synthesis (Foy *et al.* 1978), CO_2 fixation (Stiborová *et al.* 1987) and different reaction steps of the Calvin cycle (Weigel 1985). Kin application stimulated the growth rate (biomass production) even under inhibitory concentration of Cd. The Kin effect may be due to higher Chl content which leads to higher photosynthesis and accumulation of dry matter.

The results of this study indicate that Kin can alleviate the inhibitory effect of Cd on Chl content and growth. Such interaction may play a role in the adaptive response to Cd pollution.

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