EFFECT OF CADMIUM AND KINETIN ON TRANSPIRATION RATE, STOMATAL OPENING AND LEAF RELATIVE WATER CONTENT IN SAFFLOWER PLANTS

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SUMMARY: The effects of cadmium and kinetin on transpiration rate, stomatal opening and leaf relative water content were studied in safflower (Carthamus tinctorius) plants. Pollution of the root environment with concentration of cadmium ranging from 3 to 30 μM, supplied as $CdCI_2$, reduced leaf area, transpiration rate, stomatal opening and leaf relative water content. Transpiration rates were reduced to 59, 60, 55 and 48% of those in the control plants at 3, 10, 20 and 30 μM Cd, respectively. Reduction in transpiration due to higher cadmium contaminators is quite large at noon unlike that in the early morning or late afternoon. Kinetin greatly reduced or reversed the inhibitory effect of cadmium. Kinetin-treated plants generally showed a significantly higher transpiration rate, larger stomatal aperture and higher leaf turgidity than those untreated plants. Kinetin also increased leaf area significantly at higher Cd concentration. The highest kinetin concentration used was most effective in this respect. The results proved that cadmium mediated reduction in transpiration rate, stomatal opening and leaf turgidity are effectively reversed by kinetin.

Key words: Cadmium, Carthamus tinctorius, kinetin, Safflower, Transpiration, Turgidity.

INTRODUCTION

The study of the effects of some metals, especially of Al, Pb, Cu and Cd on the growth and development of crop plants represents not only a theoretically but also practically relevant problem with respect of increasing industrialization, and increasing accumulation of these xenobiotic elements in the soil and atmosphere. Cadmium is a major environmental contaminate. It is present in the air, in the water and in the soil especially in areas of heavy automobile traffic, near smelters, and in areas where oil is burned for heating purposes (14,24). Toxic effects of cadmium on some particular metabolic activities (23,37,41) and physiological processes (13,26) were demonstrated.

Higher plants grown on Cd containing substrates show disturbed water balance. Effects of Cd on stomatal function (5,22), water transport (2,25), cell wall elasticity (3), growth and photosynthesis (12, 29,35) have been reported. Poschenrieder *et al.* (32) reported that Cd-treated plants showed lower leaf relative water content and higher stomatal resistance than the controls. ABA, content was found to increase in response

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to Cd treatment leading to stomatal closure (32). In addition to ABA other compounds may be involved in stomatal closure. Blackmann and Davies (6) have suggested that a decrease of cytokinin export from roots may restrict stomatal opening.

Kinetin is one of the cytokinins receiving much attention in recent literature. A previous work (15,16, 34) revealed that kinetin has four confirmed effects:

1-Increasing water loss through its effect in increasing stomatal opening.

2-Affecting membrane stability and permeability and uptake of ions, which is clearly indicated by increasing content of some ions especially under osmotic stress.

3-Preserving chlorophyll integrity against degradation by either heat or stress.

4-Improving water retention properties, indicated by increased soluble protein content.

The effects mentioned above are extremely important in conjunction with adjustments of plant water relation to cadmium pollution. Accordingly, the investigation aimed to study the combined effects of cadmium and kinetin applied in different concentrations on transpiration rate, stomatal opening and leaf area and turgidity in *Carthamus* plants.

MATERIALS AND METHODS

Safflower (*Carthamus tinctorius L.*) plants were grown under field conditions in plastic pots containing 1400 g air dry soil (Sandy clay 2:1 V/V). Five plants were allowed to grow in each pot. The plants were twice watered at 10 day intervals with 100 ml portions of full strength Hoagland solution (18). The moisture content of the soil was kept at field capacity. This was achieved by watering the plants periodically by distilled water. When the plants were 5 weeks old distilled water was replaced by a solution containing 3, 10, 20 and 30 μM cadmium as $CdCl_2$. 2.5 H_2O. For each concentration, 15 plants were assigned at random. Control plants were watered with distilled water. The plants were allowed to adjust to the cadmium treatment for a period of two weeks before the treatment with kinetin. Kinetin solutions (0, 5, 10 and 15 ppm) were applied three times at 5 days intervals by spraying the shoots. For each kinetin concentration three pots were selected at random. A week after the last kinetin treatment, plants were used for analysis.

The diurnal marsh of transpiration, stomatal aperture, leaf area, relative water content and concurrent effective climatic parameters were measured under field conditions. Transpiration rates were measured gravimetrically. Before starting transpiration measurement, the soil surface in the pots was covered with the projecting margins of the plastic bags lining the pots by wrapping them around the base of the stem. This act prevents water loss by direct evaporation from the soil and any measurable loss, hence, represents loss by transpiration. Each pot was weighed periodically during day time. This was carried out at 3 hours intervals, namely at: 7 a.m.; 10 a.m.; 1 p.m.; 4 p.m. and 7 p.m. Area of leaves for each pot was measured by disk method (38). Transpiration rate was expressed in (mg H_2O dm^{-2} min^{-1}). Stomatal aperture (μM^{-2}) on the upper and lower epidermis were determined in leaf strips by measuring the length and width of the aperture using a square ocular micrometer. The epidermis was removed from the leaf according to the methods described by Rodriquez and Davies (33). In measuring relative water content, the method of weatherley (39) and its modification by Weatherley and Barrs (40) was adopted, following the considerations given by El-Sharkawi and Salama (11).

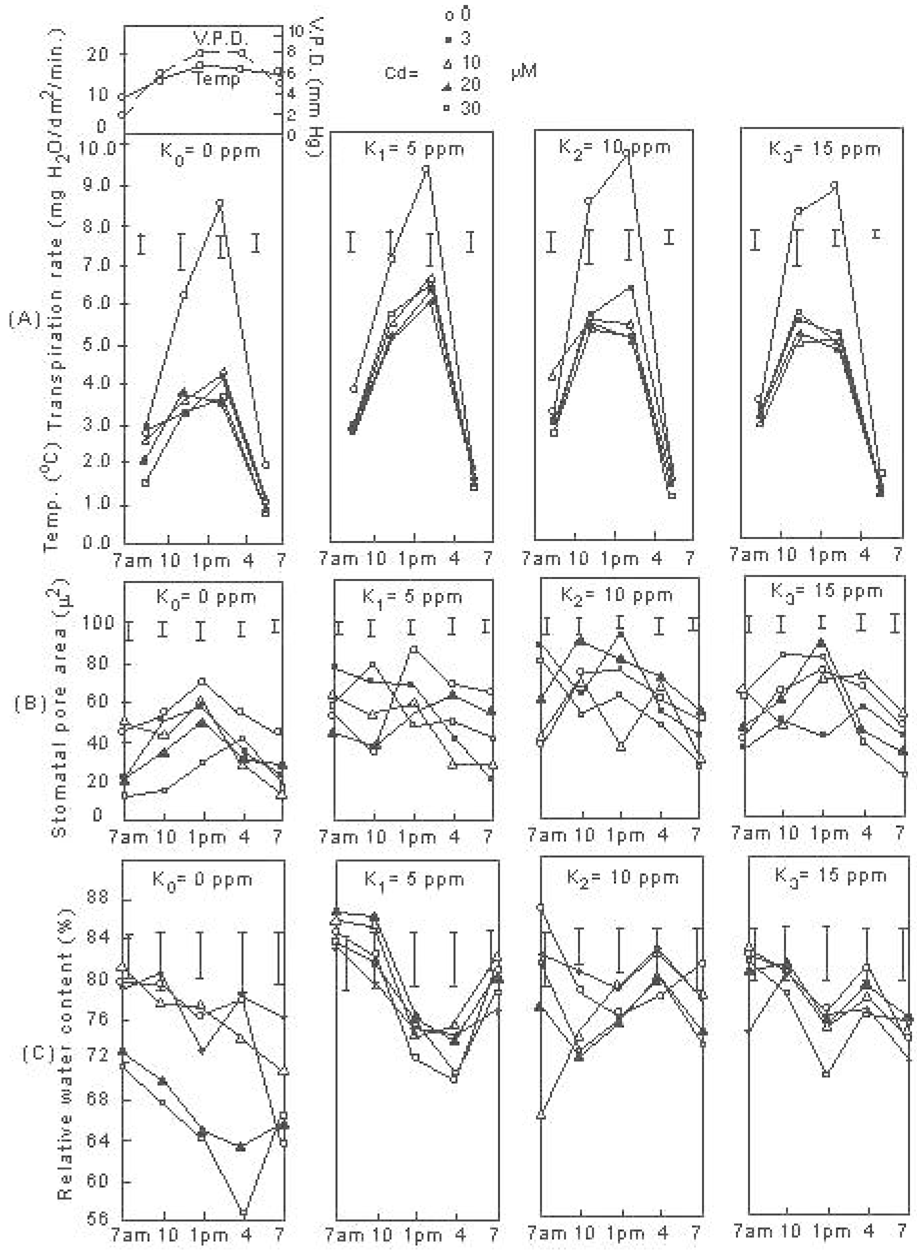
Wet and dry bulb temperature of air was measured by Aspiration Assman Psychrometer (36). Actual and saturation vapour pressures (mmHg) at the ambient temperatures were obtained from hygrometric tables and vapour pressure deficit (V.P.D.) was calculated.

Statistical inferences necessary to evaluate the effects and relative roles (shares) of single factors and their interaction are included: analysis of variance (F values) and coefficient of determination, (η^2) respectively (30).

RESULT

Transpiration rate and concurrent effective climatic parameters are shown in Figure 1-a. Transpiration generally followed both temperature and V.P.D. of air in their fluctuation. A maximum in transpiration was attained shortly after midday (2. p.m.) where a peak was observed. Transpiration rates were significantly lower in Cd treated plants at all times of measurement except in the morning (7-10 a.m). Decline in transpiration due to increase in cadmium ion concentration is quite large at midday unlike that in the early morning or late afternoon. The three kinetin concentrations used yielded highly significant increases in transpiration rate most of times of measurements compared to untreated (ko) plants.

Figure 1: Diurnal fluctuation in transpiration rate (A), stomatal pore area (B) and relative water content (C) in safflower at different cadmium (Cd) and kinetin (K) concentrations with the corresponding effective climatic parameters. (Vertical bars represent I east significant differences at 5% level).



Comparison of the transpiration mean daily values among the three kinetin concentrations (Table 1) indicated that the concentration K_2 (10 ppm) was the most effective concentration in increasing transpiration at low Cd concentrations (0 to 10 μM), while K_1 (5 ppm) was more effective at high Cd concentrations (20 and 30 μM).

Stomatal Pore Area

Figure 1-b shows the diurnal fluctuations in stomatal pore area. In the absence of kinetin, there was a progressive increase in stomatal aperture during morning, the rate of which was dependent on cadmium concentration, to a peak at 1 p.m. A decline in stomatal

Table 1: Mean daily transpiration (mg. H_2O. dm^{-2}, Min^{-1}), stomatal pore area (μm^2), leaf relative water content (R.W.C. %) and leaf area (dm^2) in safflower plants, under different cadmium (Cd) and kinetin (Kin.) concentrations.

	Cd	Kinetin (ppm)				L.S.D	
	(μM)	0	5	10	15	1%	5%
Transpiration rate (mg H^2 O. dm^{-2}. Min^{-1}							
	0	4.77	5.27	5.77	5.46	0.89	0.61
	3	2.82	3.93	4.22	3.79	0.97	0.67
	10	2.88	4.16	4.21	3.60	1.10	0.75
	20	2.61	3.88	3.77	3.66	1.07	0.73
	30	2.30	4.08	3.57	3.78	0.95	0.65
L.S.D.	1%	0.73	1.06	1.26	0.57		
	5%	0.51	0.74	0.88	0.40		
Stomatal pore area (μm^2)							
	0	53.52	61.93	59.29	57.01	5.95	4.09
	3	37.76	55.12	68.15	44.11	7.32	5.03
	10	40.11	46.81	49.17	60.69	9.62	6.61
	20	33.79	50.92	70.65	53.79	6.00	4.12
	30	24.22	55.32	53.77	56.20	7.76	5.33
L.S.D.	1%	7.10	4.27	6.84	7.85		
	5%	4.99	4.41	4.81	5.52		
Relative water content (%)							
	0	75.54	78.08	80.21	78.69	3.18	2.19
	3	77.54	77.99	80.53	75.85	4.57	3.14
	10	76.46	80.76	76.31	77.85	4.81	3.30
	20	67.47	80.82	75.94	78.26	3.48	2.39
	30	65.31	78.17	78.65	76.36	5.91	4.06
L.S.D.	1%	4.05	3.09	4.99	4.64		
	5%	2.85	2.17	3.51	3.26		
Leaf area (dm^2)							
	0	4.29	4.16	4.21	4.34	0.94	0.50
	3	4.29	3.94	3.96	4.37	2.63	1.41
	10	4.10	4.26	4.10	4.67	1.25	0.67
	20	3.60	4.07	3.93	4.73	2.45	1.32
	30	3.00	4.19	4.04	4.42	1.41	0.75
L.S.D.	1%	0.96	1.31	1.76	1.71		
	5%	0.56	0.76	1.02	0.99		

Table 2: F and η^2 values for the effect of Cd and kinetin and their interaction on transpiration rate, stomatal opening, relative water content and leaf area.

Source of variance	Transpiration rate		Stomatal opening		Relative water content		Leaf area	
	F	η^2	F	η^2	F	η^2	F	η^2
Cd.	62.58**	0.64	6.22**	0.13	12.35**	0.14	3.00*	0.14
Kin.	40.58**	0.31	43.62**	0.67	55.48**	0.46	3.96*	0.43
Cd x Kin.	1.42	0.05	3.38**	0.20	12.19**	0.40	3.21**	0.43

pore area was observed at early afternoon. Plants at 10 μM cadmium showed a tendency for decreasing stomatal opening in the morning (7-10 a.m.). Spraying with kinetin caused some changes in the pattern of fluctuation at some Cd concentrations. For example, plants received 5 ppm kinetin (except 30 μM Cd) exhibited a tendency for decreasing stomatal aperture in the morning (7-10 a.m.).

In the absence of kinetin, all concentrations of Cd induced a highly significant decrease in stomatal pore area all over day time compared to cadmium-untreated plants (10 μM Cd was an exception at 7 a.m.). Kinetin in general induced significant increases in stomatal opening during the day time with some exception. For example, kinetin concentration K_2 (10 ppm) decreased the stomatal pore area at 7 a.m. and 1 p.m. at 10 μM Cd concentration.

Data of Table 1 shows that there is a significant decrease in average daily stomatal opening with increased Cd concentration. Kinetin treatments significantly increased stomatal aperture in both control and Cd-treated plants. K_1 is the effective concentration in increasing stomatal opening in Cd-untreated plants, K_2 at 3 and 20 μM and K_3 at 10 and 30 μM Cd.

Relative Water Content

In the absence of kinetin, (Figure 1-c) plants at low Cd concentration (not exceeding 3 μM) showed increasing turgidity in the morning (7-10 a.m.), followed by decrease to a minimum at 1 p.m. Recovery of turgidity occurred in the early afternoon. At Cd concentrations 10 μM, turgidity progressively decreased early in the morning toward a minimum at evening 7 p.m. Plants at high Cd concentrations (20 to 30 μM) have an initial decreasing turgidity in the morning to a minimum. Application of kinetin appreciably affected the pattern and magnitude of diurnal fluctuation in the relative water content (Figure 1-c).

Turgidity of plants treated with low Cd concentration not exceeding 10 μM showed non significant changes in response to Cd all over day time except at 7 p.m. With increasing cd concentration, the turgidity significantly decreased than those not treated with cadmium all over day time. Generally, diurnal R.W.C. was higher in plants of kinetin treatment most of times of measurements.

The data of mean daily turgidity (Table 1) indicated that high concentration of cadmium ion (20 to 30 μM) yielded significant decrease in the leaf relative water content than Cd-untreated plants. Kinetin-treated plants at 0, 20 and 30 μM Cd showed significantly higher turgidity than their analogous (ko) plants. The effective concentration, seems to be K_2.

Statistical analysis (Table 2) show that the effect of cadmium (Cd), kinetin (Kin.) and their interaction (Cd x Kin.) on the three parameters tested are highly significant except the Cd x Kin. interaction effect on transpiration. The magnitude of the relative effect of each factor and their interaction (as indicated by η^2 values) is quite versatile. The relative role of Cd is predominant in affecting transpiration rate only ($\eta^2 = 0.64$) but that of kinetin is subdominant ($\eta^2 = 0.31$). The role of kinetin is predominant for stomatal opening and leaf relative water content ($\eta^2 = 0.67$ and 0.46 respectively) but that of (Cd x Kin.) interaction is subdominant ($\eta^2 = 0.20$ and 0.40 respectively) and the role of Cd is a minor one ($\eta^2 = 0.13$ and 0.14 respectively).

Leaf Area

In the absence of kinetin (Table 1), increasing Cd concentration was associated with reduction in plant leaf area, where Cd_3 and Cd_4 induced significant decrease compared to control. Kinetin application significantly increased leaf area (especially in Cd_4 treated plants) compared to kinetin untreated analogous. The highest kinetin concentration used was most effective in this respect. Kinetin effect was most pronounced at 20 μM Cd than the lower Cd concentrations.

Applied kinetin and its interaction with Cd (Cd x Kin.) had equally dominant role in affecting leaf interaction area ($\eta^2 = 0.43$ for each) and the role of cadmium was subdominant ($\eta^2 = 0.14$).

DISCUSSION

Transpiration rate of safflower plants was largely reduced with increasing Cd concentration Table 1. The reduction in transpiration rate by cadmium agrees with the data reported by Bazzaz *et al.* (5). Carlson and Bazzaz (7), Carlson *et al.* (8) and Lamoreaux and Chaney (26). Such reduction in transpiration in response to Cd may be due to (a) increasing resistance to water flow in the stem (2), (b) inhibition of stomatal opening and increasing stomatal resistance (4). The data from the present study on stomatal openings supports the view of Bazzaz *et al.* (4) where Cd-treated plants have smaller stomatal aperture (Figure 1-b). Kinetin-treated plants generally have higher transpiration rate than their analogous not received kinetin (ko). This is in agreement with findings of Gadallah, (15,16). Such effect of kinetin is due to stimulation of stomatal opening Table 1. Kinetin concentration giving the maximal effect varied with Cd concentration. The variation in response to kinetin concentration may be due to the interaction of the hormone with some cellular components, in exerting their effects (21).

Cadmium had a marked influence on stomatal openings. The data of Figure 1-b indicate that safflower plants treated with Cd have smaller stomatal aperture than Cd untreated control. The inhibition of stomatal opening could be induced by increase of leaf ABA level in cadmium treated plants leading to stomatal closure (32). Both K^+ (17) and Ca^{+2} (19) play an important role in the regulation of stomatal opening. An interference of Cd with K^+ fluxes has been found in root cells (27). Therefore, the inhibition of stomatal opening may suggest that Cd has a direct effects on the ion and water movement in the guard cells.

Kinetin treatment, contrary to cadmium, increased stomatal pore area in both control and Cd-treated plants. This is attributed to the combined effects of kinetin: [1] Reduction of CO_2 concentration inside leaves and higher photosynthesis in the guard cells than in the mesophyll (28) thus favoring stomatal opening, [2] kinetin causes stomatal to open osmopassively and turgorpassively (31). In some cases, the response of stomatal opening to kinetin at different Cd concentrations was larger than that by the control. This may be due to the reduction by kinetin of ABA activities in Cd-treated plants.

Cadmium-treated plants at concentration higher than 10 μM had lower leaf relative water content than control plants, is an extension of previous findings of Poschenrieder *et al.* (32) and Barcelo *et al.* (1,2). Such decrease in leaf turgidity due to either increasing resistance to water flow in the stem (2) and/or alteration of cell wall properties induced by Cd (32). Kinetin sprayed plants had higher turgidity than unsprayed plants. This is mainly due to effects of kinetin on membrane permeability to water and solutes (42). There appears to be an antagonism between Cd and kinetin on the parameters tested.

Leaf growth decreased in response to Cd treatment especially in the absence of kinetin (Table 1). Growth retardation by Cd is a well-known phenomenon (12, 20, 29, 35, 43). This reduction in leaf area could be assumed due to [1] decreased activities of many enzymes involved in the fixation of CO_2 (9), [2] inhibition of photosynthetic capacity due to reduction in R.W.C. (10) and [3] changes in the thylakoid organization, reduction of chlorophyll contents and inhibition of photosynthetic activity (12). Sometimes, kinetin application had ameliorative effects and counteracted the deleterious effects of Cd e.g. K_3 induced significant increase in leaf area of Cd treated plants at higher cadmium concentrations (Cd_3-Cd_4). This interactive additive effects is clearly obvious from the η^2 table in which kinetin interaction with Cd (Cd x Kin.) had a dominant role in affecting leaf area ($\eta^2 = 0.43$).

The results of this study proved that, the inhibitory effects of cadmium was counteracted or reversed by application of kinetin. This fact positively leads to the suggestion that under conditions of hazarduous occurrence of cadmium pollution, spraying crop plants with kinetin is surely beneficial.

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