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AMELIORATING EFFECT OF KINETIN ON PIGMENTS, PHOTOSYNTHETIC CHARACTERISTICS, CARBOHYDRATE CONTENTS AND PRODUCTIVITY OF CADMIUM TREATED *SORGHUM BICOLOR* PLANTS

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The objective of this study was to investigate the effect of soil drench with three different concentrations of $CdCl_2$ on pigments content, photosynthetic activity, carbohydrate contents and productivity of *Sorghum bicolor* L. cv. 'Dorado' plants throughout various stages of plant growth and development. Also particular interest was focussed on the effect of grain presoaking with kinetin to ameliorate the toxicity effects exerted by the different levels of $CdCl_2$. In the majority of cases, grain pretreatment with kinetin increased photosynthetic pigments, photosynthetic activity, Hill reaction as well as carbohydrate contents in leaves of cadmium treated sorghum plants. In general, the observed decrease in yield and yield attributes of sorghum plants in response to Cd^{2+} treatments was accelerated particularly when grains were presoaked in kinetin. The ameliorating effect of kinetin was more pronounced at 1 mM $CdCl_2$. Grain priming with kinetin increased grain biomass (i.e. fresh and dry weights), carbohydrates, protein and ion contents in yielded grains of cadmium treated sorghum plants. Cadmium treatments altered the balance of growth bioregulators in developed grains of sorghum plants. Thus, $CdCl_2$ at all the used concentrations exerted a significant decrease in growth promotor levels with an increase in growth inhibitory substances equivalent to abscisic acid. On the other hand, grain priming with kinetin increased the growth promotory substances and reduced abscisic acid levels.

Key words: cadmium, carbohydrates, kinetin, photosynthetic activity, pigment, sorghum, yield

INTRODUCTION

Cadmium ions inhibited production of chlorophyll in dark-grown seedlings of barley by affecting the synthesis of 5-amino laevulinic acid and complexing of protochlorophyllide from 5-amino laevulinic acid (Stobart *et* al. 1985). Furthermore, Burstron *et al.* (1986) found that, chlorophyll (a+b) content is a better indicator of Cd toxicity than shoot dry weight or root length in wheat seedlings. Cadmium reduced pigmentation in radish, lettuce and wheat seedlings (Naquib *et al*. 1986). Rani *et al.* (1987) reported that, cadmium inhibited chlorophyll biosynthesis in leaf segments of *Vigna mungo* plants, but

illumination immediately after treatment decreased inhibition slightly. Photosynthetic rate was depressed in heavy metal treated soybean plants (Ghi-Ying *et al*. 1974**)**. The application of cadmium sulphate at concentration: 0, 1, 5 and 10 µM, reduced photosynthesis in wheat plants *(Triticum aestivum* cv. 'Sakha 69') (Abo-Kassem *et al*. 1997). Short-time exposure to Cd^{2+} inhibited photosynthetic $^{14}CO_2$-fixation of mesophyll chloroplast of lettuce plants (Weigel 1985). Long-term exposure to Cd^{2+} stress of barley plants (*Hordeum vulgare* cv. 'Obzor') inhibited the formation of photosynthetic apparatus and decreased its capacity for ^{14}C photoassimilation (Vassilev *et al*. 1997).

In *Phaseolus vulgaris* plants cv. 'Kentwood', grown hydroponically and exposed to excess concentrations of Cd, Co, Ni or Zn the excess Co, Ni and Zn caused abnormal starch accumulation in unifoliate leaves within 2–3 days, while cadmium being the most toxic, did not cause starch accumulation (Rauser 1978**)**. Moreover, Stadelmann *et al.* (1986**)** stated that, cadmium reduced the content of soluble carbohydrates and starch in Cd treated plants. The effect of heavy metals (Cd^{2+}, Cu^{2+} and Hg^{2+} at concentrations 10, 100 and 1000 µM) on 15-day-old seedlings of rice (*Oryza sativa* cv. 'ADT 42') grown in controlled environmental conditions, was shown to decrease the carbohydrate content (Hemalatha *et al*. 1997).

Cadmium affected plant yield and reduced pod fresh weight in Cd treated soybean plants (Ghi-Ying *et al*. 1974**)**. Moreover, cadmium at 6–12 ppm reduced grain yield of wheat by 1 per cent and straw yield by 12–17 per cent (Hofer and Schutz 1980). Furthermore, Poschendieder *et al.* (1983) proved that, seed number and size were reduced in Cd treated *Phaseolus vulgaris* plants with cadmium concentrations at 10, 80 or 160 ppm. At 48 ppm Cd, wheat grain yield was only 6 per cent of control, while potato tuber yield was reduced only by 16 per cent (Stadelmann *et al*. 1986). Cadmium reduced grain yield of barley in Cd treated plants (Juwarker and Shende 1986). Malan and Farrant (1998) found that, cadmium reduced plant biomass, seed production and mature seed mass due to decreased yields of lipids, proteins and carbohydrates in soybean plants which were treated with chloride salts of cadmium and nickel.

It has been suggested that plant hormones may be involved in delaying the senescence and chlorosis in plants treated with heavy metals (Godbold 1994). The role of cytokinins in plant responsed to adverse environmental conditions has been recently reviewed by Hare *et al*. (1997). In barley, Pb treatment caused the reduction in the content of chlorophyll, especially chlorophyll b content and the average number of grana, whereas in the presence of kinetin, the content of chlorophyll increased (Wozny *et al*. 1995). They suggested that kinetin diminished the inhibitory effect of Pb on the chlorophyll content. Kinetin reduced Na^+ and Ca^{2+} accumulation and also improved K^+ uptake under salinity. Kinetin enhanced shoot growth and grain yield, and also reduced

membrane injury by dehydration and improved the water status of plant under both aerobic and anaerobic conditions. Kinetin had a dominant effect on the stability of chlorophyll and soluble sugar contents (Gad-Allah 1999). Moreover, kinetin stimulated the synthesis of polysaccharides and the materials of the new cell walls in kinetin treated *Phaseolus vulgaris* plants (Robertson *et al*. 1999).

On the light of the above-mentioned reviews, it was of particular interest to investigate the effect of grain priming with kinetin on pigments, photosynthetic activity, Hill reaction, carbohydrates and yield as well as biochemical aspects of yielded grains of *Sorghum bicolor* plants stressed with different levels of cadmium chloride.

MATERIALS AND METHODS

Homogeneous grains of *Sorghum bicolor* (cv. 'Dorado') were surface sterilised by soaking in 1 mol m^{-3} $HgCl_2$ solution for 3 minutes, then washed thoroughly with distilled water, and then divided into two sets, which were soaked in distilled water or 50 ppm kinetin, respectively, for about three hours. After soaking, thoroughly washed grains were planted (10 grains per pot) on 30th March 1999 in earthenware pots (30 cm in diameters) filled with 3 kg soil (Sand, Clay 2/1 v/v). The pots were kept in greenhouse under a normal day/night and irrigated with normal tap water when required.

After two weeks, only five uniform seedlings were left in each pot. The plants of the first set were subdivided into four groups. The first group irrigated with normal tap water to serve as control, while the 2nd, 3rd and 4th groups irrigated continuously during the experimental period with 0.01, 0.1 or 1 mM of $CdCl_2$, respectively. The plants of the 2nd set were subdivided into four groups. The first one irrigated with normal tap water to serve as control of kinetin, while the 2nd, 3rd and 4th groups irrigated continuously during the experimental period with 0.01, 0.1 or 1 mM of $CdCl_2$, respectively. Twenty pots /treatment were used.

After thinning and before heading, the plants received 35 g (N) m^{-2} as ammonium nitrate and 35 g (P) m^{-2} as super phosphate.

Samples from the 3rd leaf of main shoot (numbered from the base) were taken after 43, 75, 97 and 114 days from sowing. The samples of each treatment were ten replicates for estimating yield components and three replicates for measuring photosynthetic pigments, photosynthetic activity, Hill reaction, carbohydrates and elements (K^+, Na^+, Ca^{2+} and Cd^{2+}).

Estimation of photosynthetic pigments. The plant photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) were determined at different

stages of plant development according to the spectrophotometric method as recommended by Metzner *et* al. (1965).

Hill reaction assay. As described by Arnon (1949), 4 g of detached leaves were ground three times for 5 seconds at full speed in a chilled blender in 50 ml of 50 mM N-Tricine (N-Trishydroxyl methylglycine, pH = 7.8), 0.3 M sucrose and 2 mM $MgCl_2$. The resulting homogenate was filtered through four layers of nylon mesh. Chloroplast pellets were obtained by centrifugation at 2000 g for 10 minutes. The pellets were resuspended in 30 ml of 0.1 M NaCl and then centrifuged again at 5000 g for 5 minutes. The resulting pellets were resuspended in 1 mM N-Tricine (pH = 7.8), 10 mM NaCl and 10 mM $MgCl_2$ and then kept at 0–4 °C until used for the analysis.

Photosystem II activity, as indicated by the rate of 2,6-dichlorophenol-indophenol (2,6-DCPIP) photo-reduction (Trebst 1967) was monitored at 600 nm using spectronic 21 D spectrophotometer. The sample cuvette was illuminated by tungsten lamp (6000 lux) using head filter ($CuSO_4$ solution). The photosystem II reaction mixture contained 200 mM Na phosphate (pH = 6.7), 2 mM $MgCl_2$ and 0.5 mM DCPIP. The chlorophyll concentration range for this experiment was 10 µg/ml of the reaction mixture (4 ml).

Analysis of ^{14}C assimilation (^{14}C light fixation). As described by Gaber (1985) a definite fresh mass of 1st compound leaf discs was introduced into the fixation apparatus. An aqueous solution of ^{14}C-sodium carbonate of known activity (3.7 MBQ cm^{-3}) was pipetted into the apparatus followed by 0.2 ml of H_2SO_4 (10%). The evolved $^{14}CO_2$ passed through the pores in the upper part of the inner container to the main apparatus where it could be photosynthesised by the green leaf discs. The experiment was carried out in natural sunlight at 10 am, when light intensity was about 3500 lux. At the end of the fixation period (10 minutes), the leaf discs were quickly transferred to be killed and then extracted in a mortar by grinding. After being cooled, the insoluble fraction was separated from the soluble one by centrifugation at 2000 g for 20 minutes. The soluble and insoluble fractions were made up to a known volume (35 ml) and then 2 ml of samples were pipetted into the plastic tubes of scintillator counter. The radioactivity of the green leaf discs was measured using a Packard Scintillation Counter model 526. The count per minute (cpm) obtained were then calculated according to the efficiency of the apparatus used. The radioactivity measured is directly proportional to the amount of CO_2 fixed in soluble organic compounds, which was calculated as cpm/mg fresh mass of leaf.

Extraction and determination of total soluble sugars, sucrose and polysaccharides. Sugars were extracted by the method of Riazi *et al*. (1985). A known dry weight (0.03 g) was submerged in 10 ml 80% ethanol overnight with periodic shaking, then filtered through Whatman No. 1 filter paper, and the filtrate was made up to known volume with 80% ethanol. The total soluble sugars and su-

crose content of developing seeds were determined by anthrone method as described by Riazi *et al*. (1985). Glucose contents were estimated using the O-toluidine procedure of Feteris (1965**)** as modified by Riazi *et al.* (1985).

According to Naquib (1963) a known weight of the dried plant residue which remained after extraction of soluble sugars, was heated under reflux in 1.5 N H_2SO_4 for 4 hours at 100 °C. The solution was neutralised, cleared with basic lead acetate (137 g/l) and deleaded with Na_2HPO_4 (M/3). The solution was made up to known volume. Polysaccharides content were estimated according to the procedure adopted by Younis *et al.* (1969).

Estimation of protein. Protein content was determined using the method of Lowry *et al*. (1951).

Determination of K^+, Na^+, Ca^{2+} and Cd^{2+} ions. Na^+ and K^+ cations were estimated by the flame photometer. Standard Na^+ and K^+ solutions with known concentrations were used to draw a standard curve against its atomic absorption (Younis *et al*. 1994).

Ca^{2+} and Cd^{2+} cations were determined by the Atomic Absorption Spectrophotometry (BHF 80B biologie spectrophotometer). The samples were diluted with 0.8% $LiCl_3$ to suppress the interference of Na^+ and K^+.

Estimation of growth bioregulators. The method of extraction of hormones was that originally described by Shindy and Smith (1975). For determination of abscisic acid (ABA) in extracts, the wheat coleoptile bioassay developed and adopted by Wright (1956) was used. The amounts of either acidic or neutral auxins was estimated according to straight growth test of barley coleoptile adopted by Foda and Radwan (1962). Gibberellic acid in extracts was determined by the lettuce hypocotyl bioassay developed and adopted by Frankland and Wareing (1960). The content of cytokinins was estimated according to the method described by Esashi and Leopold (1969).

The results were first subjected to the analysis of variance (Anova). When Anova showed a significant ($P \leq 0.05$ at the least) effect, the least significant differences were used to compare treatments (Snedecor and Cochran 1976).

RESULTS AND DISCUSSION

Plant yield is a function of many factors among which the pigment content of the developing leaves is the most important (Ibrahim 1999). Thus, the presented data in Figure 1 show that $CdCl_2$ at all the used concentrations led to significant decreases ($P \leq 0.05$) in total chlorophylls (chl. a +b), carotenoids and total pigments during various stages of plant growth and development. Meanwhile, chl. a/b ratio appeared to be significantly decreased ($P \leq 0.05$) at all of the used concentrations particularly after 43 and 114 days from sowing. This

ratio decreased significantly ($P \leq 0.05$) at 0.01 mM $CdCl_2$ or non-significantly at 0.1 and 1 mM, respectively, on days 75 or 97 from sowing (Fig. 1). These results are in a good agreement with those obtained by others in different plant species, e.g. in wheat plants (Malik *et al.* 1992), in *Pisum sativum* plants (Baranwal 1995), in maize plants (El-Enany 1995), in *Phaseolus vulgaris* plants (Hemalatha *et al.* 1997), in *Pisum sativum* plants (Chugh and Sawhney 1999). The reduced chlorophyll content in Cd^{2+} treated plants may be due to the inhibition of biosynthesis and the activation of its enzymatic degradation (Somashekaraiah *et al*. 1992).

Grain priming with kinetin enhanced the production of total chlorophylls (chl. a+b), carotenoids and total pigments during the growth and development of sorghum plants subjected to cadmium stress (Fig. 1). Furthermore, kinetin may delay the senescence of sorghum leaves by retaining the chlorophyll content (Fig. 1). The stimulative effect exerted by kinetin on pigment biosynthesis might be presumably due to the fact that, kinetin can increase the rate of transpiration (unpublished data) and this might have increased the rate of translocation of minerals and cytokinins (CK) from root to the developing shoot. Thus, Richmond and Lang (1975) have shown that kinetin prevented chlorophyll loss in detached *Xanthium* leaves. Moreover, Uheda and Kuraishi (1978) found that kinetin increased both transpiration and chlorophyll synthesis. The present results are in a good harmony with those obtained by Godbold (1994), Moya *et al.* (1995), Wu *et al.* (1998), and Gad-Allah (1999). They stated that kinetin application increased water uptake, mineral, chlorophyll and soluble sugar contents in different plant species.

As can be seen from Figure 2 $CdCl_2$ at all concentrations applied resulted in a sharp decrease in the soluble, insoluble and consequently total photosynthates as well as Hill reaction in sorghum plants after 43 days from sowing. The magnitude of decrease appears to depend mainly on the concentration. The inhibition of photosynthetic $^{14}CO_2$ fixation in mesophyll cells of sorghum plants may be due to decreased activity of some photosynthetic enzymes such as ribulose–1,5-biphosphate carboxylase, glyceraldehyde–3-phosphate dehydrogenase and ribulose–5-phosphate kinase (Weigel 1985). Another explanation may come from the fact that cadmium inhibits the formation of photosynthetic apparatus and its capacity for ^{14}C photoassimilation (Fodor *et al.* 1996, Vassilev *et al*. 1996). These results are in agreement with those obtained by Abo-Kassem *et al.* (1997) and Vassilev *et al.* (1999) by using different plant species.

Grain priming with kinetin stimulates the accumulation of soluble, insoluble and total photosynthates as well as Hill reaction activity. This promotive effect induced by kinetin may probably be due to its stimulative effect on leaf expansion and photosynthetic pigments as well as transpiration rate of sor-

ghum plants subjected to cadmium stress. Furthermore, kinetin may exert its effect on photosynthetic machinery at the mesophyll and chloroplast level by increasing plastid biogenesis and consequently increases the number of proplastids or newly developed chloroplasts (Aldesuquy and Baka 1998).

In comparing with control plants, soil drench with $CdCl_2$ at all concentrations caused marked decrease in glucose, sucrose, total soluble sugars (T.S.S.) and polysaccharides of sorghum leaves during various growth periods (Fig. 3). It is clear that the change in carbohydrate fractions during growth and development of sorghum leaves are consistent with the changes in photosynthetic pigments (Fig. 2). Thus, the observed decrease in photosynthetic pig-

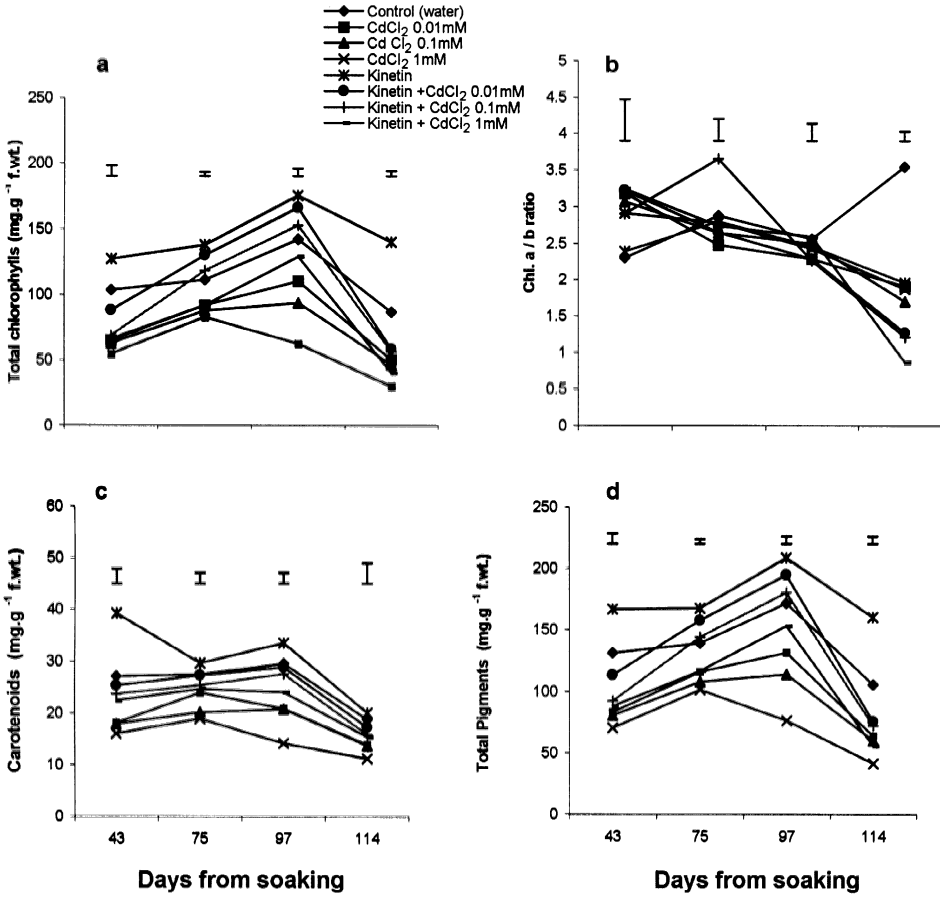

Fig. 1. Effect of grain presoaking in kinetin on pigment contents (a, b, c and d) of sorghum plants irrigated with various concentrations of $CdCl_2$. The vertical bars represent LSD values at $P = 0.05$

ments in Cd^{2+} treated plants was accompanied by a simultaneous decrease in carbohydrate contents in sorghum leaves. The massive reduction in all carbohydrate fractions in Cd^{2+} treated plants was probably due to the inhibition of chlorophyll biosynthesis leading to a decrease in carbohydrate contents as recommended by Somashekaraiah *et al.* (1992), Kupper *et al.* (1998), or to the retardation of photosynthetic activity containing PSI and PSII as well as its specific enzyme (Abo-Kassem *et al.* 1997, Vassilev *et al.* 1997, 1999). In this respect, Rauser (1978) reported that cadmium prevents starch accumulation in white bean plants.

In the majority of cases, grain priming with kinetin induced noticeable increases in glucose, sucrose, T.S.S. and polysaccharides contents in leaves as compared with the content detected in Cd^{2+} treated plants alone (Fig. 3). Thus the obtained results show that the interactive effect between kinetin and $CdCl_2$ on carbohydrate contents in sorghum leaves may be explained on the fact that kinetin increases the cumulative leaf area, production of photosynthetic pigments as well as its biogenesis and consequently stimulates the photosynthetic activity. The pronounced increase in soluble carbohydrates by kinetin treatments in Cd^{2+} treated plants may probably due to an increase in invertase activity that consequently led to a simultaneous increase in soluble carbohydrates (Howard and Witham 1983). In this connection, Erisman and Wegner (1967) found that there was an excessive accumulation of starch in case of *Lemna minor* plant as a result of kinetin treatment. In contrast to these reports, other workers reported that kinetin treatment resulted in starch breakdown and an increase in free sugars within the plant tissues (Berridge and Ralph 1971).

Generally, when the leaves of sorghum plants started to senesce, there was a gradual loss of chlorophylls and carbohydrate fractions. Soil drench with $CdCl_2$ appeared to induce clear senescence particularly if compared with control plants. On the other hand, the application of kinetin played an important role in delaying the senescence of sorghum leaves by retaining its chlorophyll and enhancing the formation of carbohydrate fractions. Therefore, the increase in CO_2 fixation induced by kinetin could be due to the increase in chlorophyll content as well as the increase in the enzymes responsible for CO_2 fixation. As a result of such changes caused by kinetin, there may be a rapid movement of the assimilates from the leaves (source) to the developing grains (sink) resulting in improved yield quality of the sorghum plants subjected to cadmium stress.

Cadmium chloride at all concentrations caused marked decrease in panicle length and weight, number of grains/panicle, 100-kernel weight, grain biomass (fresh and dry weights), grain yield, harvest index, and relative grain yield (Figs 4–5). These results are in harmony with those obtained by Poschen-

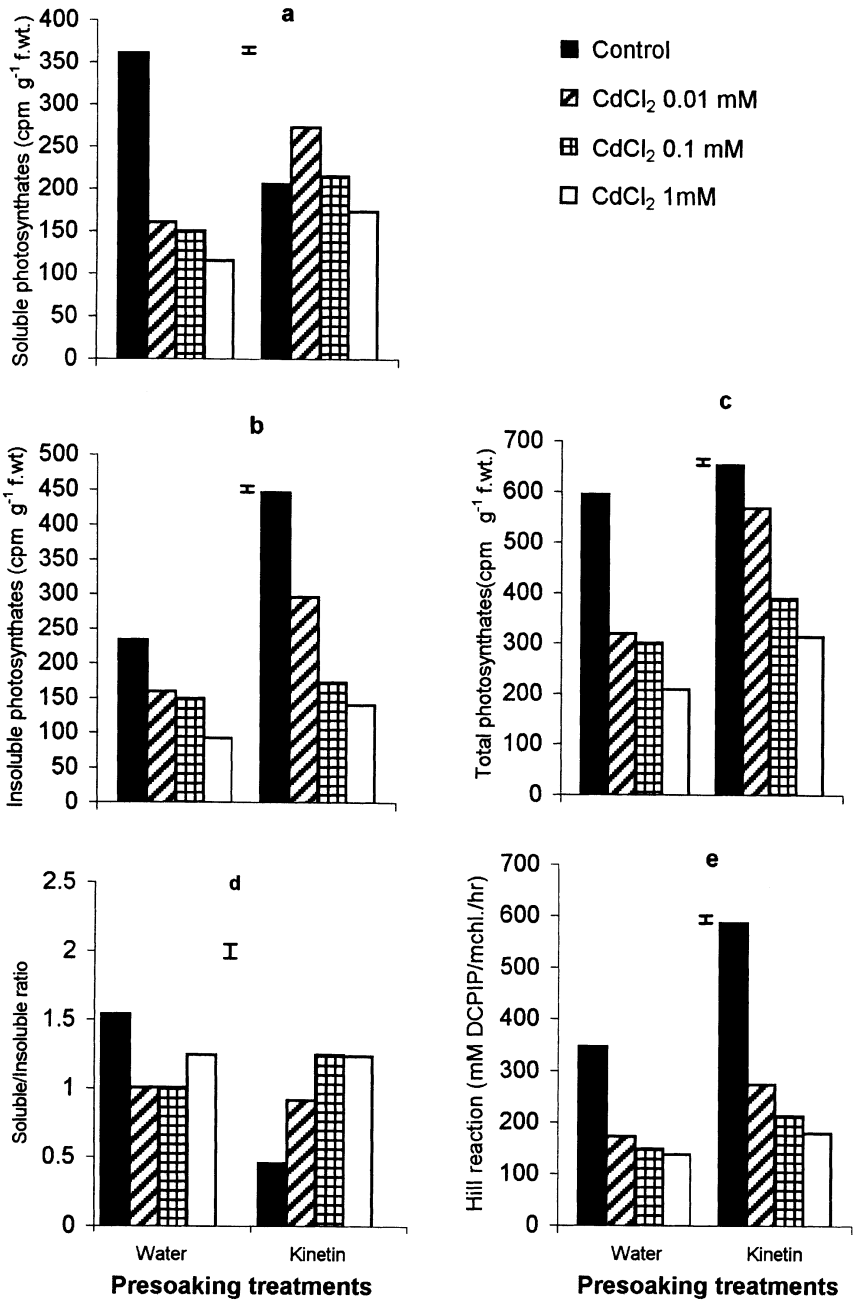

Fig. 2. Effect of grain presoaking in kinetin on photosynthetic activity (a, b, c and d) and Hill reaction (e) of sorghum plants irrigated with various concentrations of $CdCl_2$. The vertical bars represent LSD values at $P = 0.05$

dieder *et al.* (1983) using *Phaseolus vulgaris* plants, Juwarker and Shende (1986) using barley plants, Malan and Farrant (1998) using soybean plants. Furthermore, the reduction in yield of soybean plants was attributed to the decrease in photosynthetic rate, carbohydrate accumulation, nitrogenase activity and consequently protein synthesis and accumulation in seed yield (Malan and Farrant 1998). In this respect, Ghi-Ying *et al.* (1974) reported that, the decrease in growth parameters of root and shoot nodule, ammonia, protein and carbohydrate contents of soybean plants caused the decline in yield production. Cadmium also reduced the yield production in clover plants by inhibiting the fixation efficiency of free nitrogen and this inhibitory effect of Cd^{2+} increased with the increase in cadmium concentrations (Strzelec and Oron 1987).

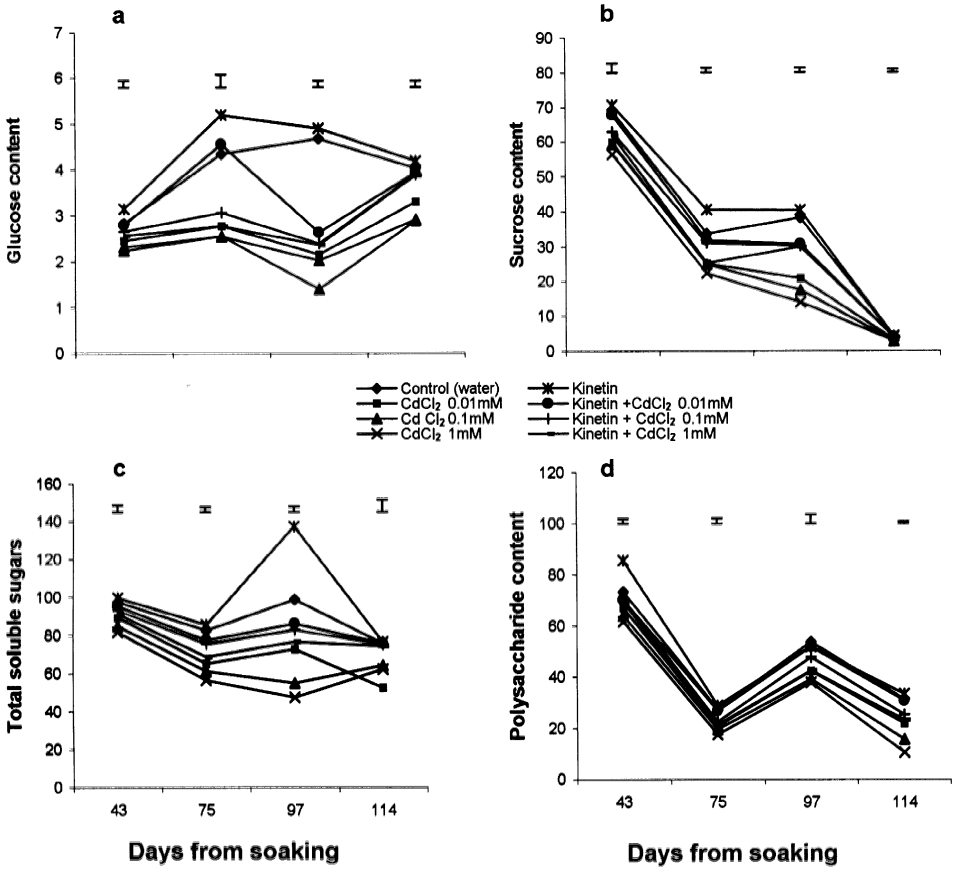

Fig. 3. Effect of grain presoaking in kinetin on carbohydrate contents (a, b, c and d) (mg g^{-1} d. wt.) of sorghum plants irrigated with various concentrations of $CdCl_2$. The vertical bars represent LSD values at $P = 0.05$

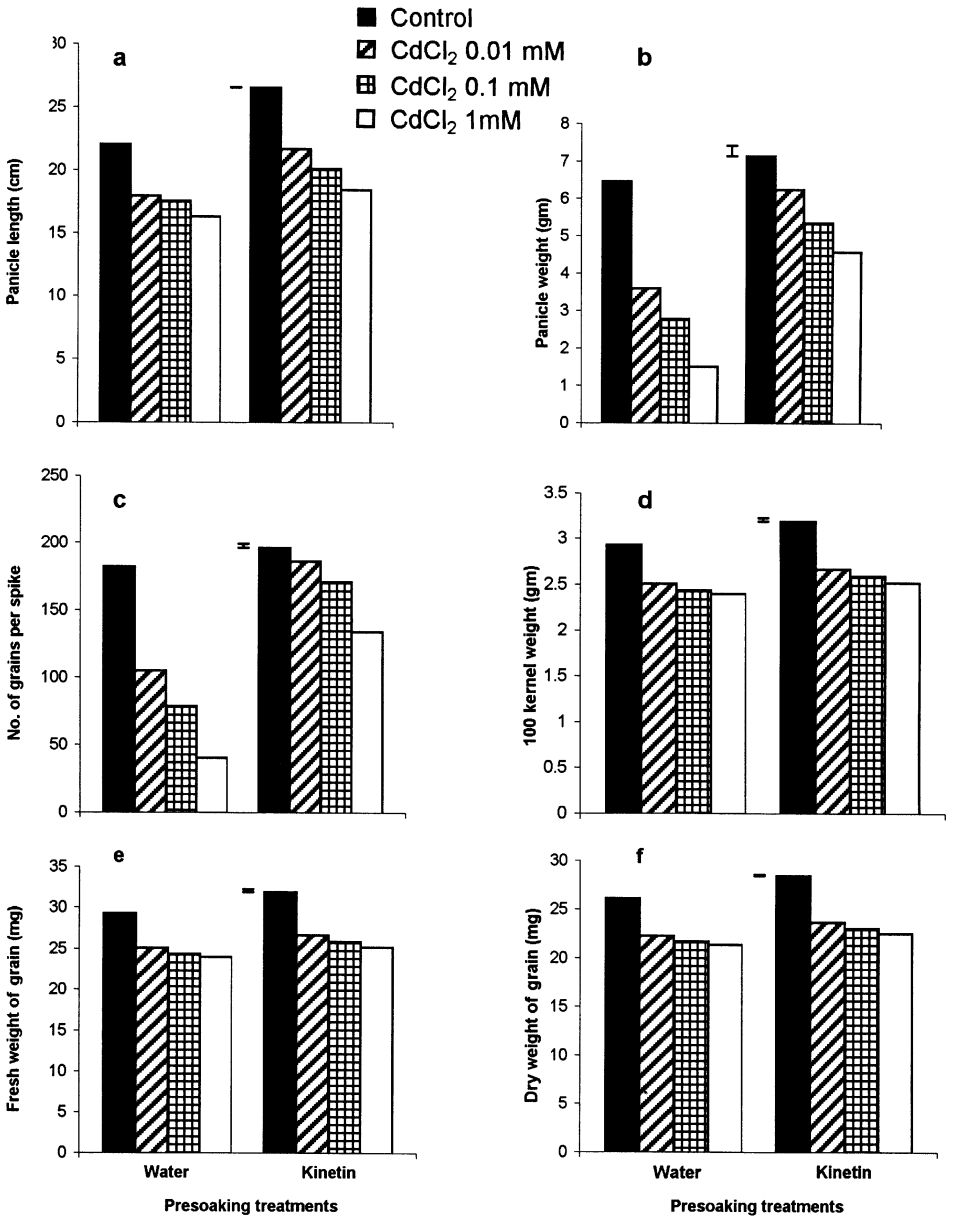

Fig. 4. Effect of grain presoaking in kinetin on yield and yield components (a, b, c, d, e and f) of sorghum plants irrigated with various concentrations of $CdCl_2$. The vertical bars represent LSD values at $P = 0.05$

In this investigation the application of kinetin reduced the toxicity of Cd^{2+} on yield and yield components of sorghum plants (Figs 4–5). The increase in yield production may be due to the increase in longevity of leaves which perhaps contributed to grain filling by enhancing the duration of photosynthate supply to grain (Ray and Choudhuri 1980).

In the present investigation grain priming with kinetin stimulated pigment production and photosynthetic activity which in turn increased carbo-

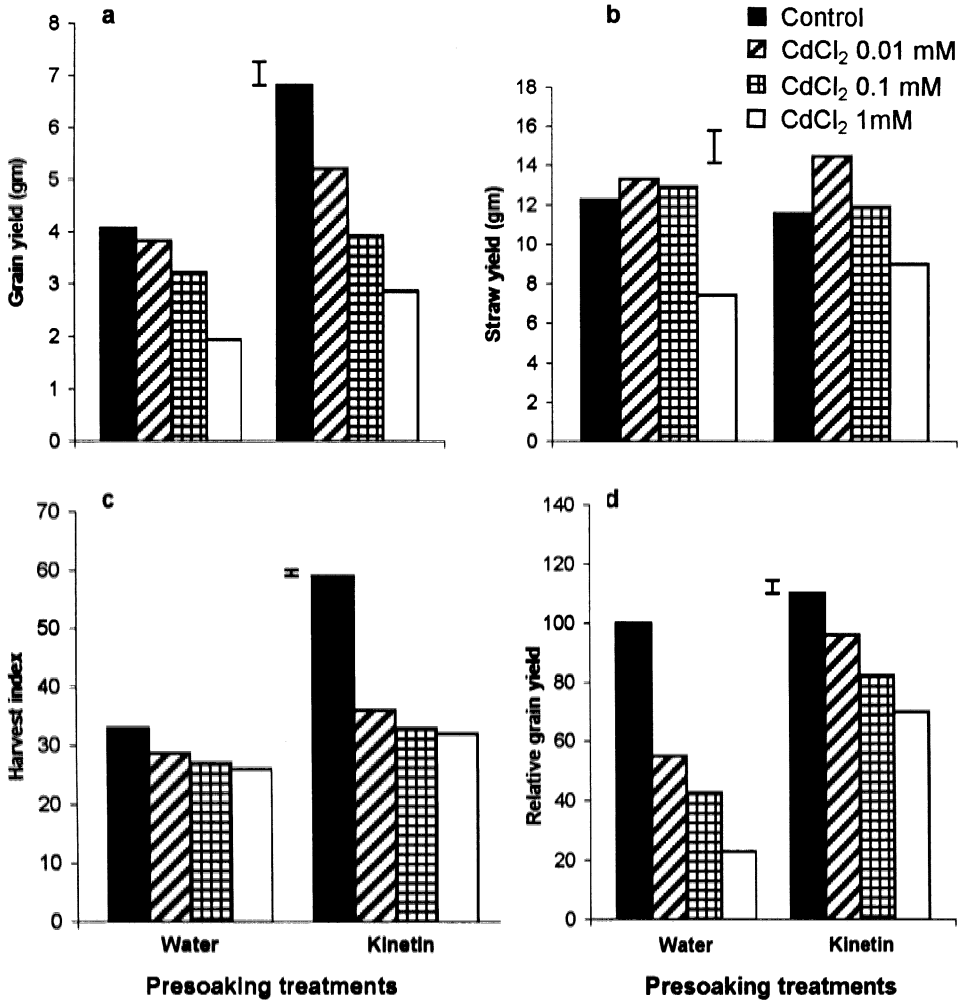

Fig. 5. Effect of grain presoaking in kinetin on yield and yield components (a, b, c and d) of sorghum plants irrigated with various concentrations of $CdCl_2$. The vertical bars represent LSD values at $P = 0.05$

hydrate accumulation in sorghum leaves and consequently increased the yield capacity of sorghum treated plants with $CdCl_2$. This stimulative effect exerted by kinetin increases the tolerance of sorghum plants against Cd^{2+} toxicity.

Cadmium chloride at all the used concentrations induced massive decrease in carbohydrate fractions (glucose, sucrose, total soluble sugars and polysaccharide), protein content as well as minerals (K^+ and Na^+, Ca^{2+}) in well developed grains of sorghum plants (Figs 6–7). On the other hand, the application of $CdCl_2$ enhanced the accumulation of Cd^{2+} in yielded grains (Fig. 7). The massive decrease in polysaccharide content of yielded grain, of sorghum plants in response to Cd^{2+} treatments may be due to that Cd^{2+} stimulated the degradation of polysaccharide (Rauser 1978) and at the same time increased the rate of dark respiration (Van Assche *et al.* 1988) during which total soluble sugars were consumed as respiratory substrate. Furthermore, Cd^{2+} application decreased pigment formation in sorghum leaves (Fig. 1), resulting in inhibition of the photosynthetic activity which in turn led to less accumulation of carbohydrate in developed leaves and consequently may decrease the rate of transport of carbohydrate form leaves to developed grains, where there is a good relationship between source (leaves) and sink (grain) in cereal plants.

It is clear from this investigation that Cd^{2+} treatment decreased the protein content in well-developed sorghum grains and this inhibitory effect may be explained mainly on the fact that, (1) cadmium increased the content of free amino acids due to the inhibition of protein synthesis in Cd^{2+} treated plants (Vassilev *et al.* 1997), (2) cadmium bound with three families of peptides forming high molecular weight Cd^{2+}-binding complexes such as (γ-glutamic acid-cysteine)$_n$-glycin [(g-Glu-Cys)$_n$-Gly] (g-Glu-Cys)$_n$-Glu, so the free peptides decreased and consequently protein synthesis inhibited (Wilfried and Philippe 1995).

The recorded decrease in K^+, Na^+ and Ca^{2+} contents of well-developed sorghum grains as a result of cadmium treatment to sorghum plants may be explained on the fact that, Cd^{2+} inhibits the accumulation of the previous elements in root and shoot and consequently inhibited the transport of K^+, Na^+ and Ca^{2+} from shoot to developed grains (Abo-Kassem *et al*. 1997). Irrigation of sorghum plants with $CdCl_2$ resulted in marked increase in Cd^{2+} content of yielded grains (Fig. 7). These results were in good agreement with those of Reuss *et al.* (1978) and Oliver *et al.* (1993).

The observed suppression in K^+, Na^+ and Ca^{2+} contents of yielded grains in response to Cd^{2+} treatment was relieved when grains were presoaked in kinetin (Fig. 7). The ameliorating effect of kinetin on mineral contents of yielded grains in response to Cd^{2+} treatment may probably be due to kinetin increase the uptake and transport of K^+, Na^+ and Ca^{2+} through the increased rate of transpiration (Fig. 7). These results were in accordance with those ob-

tained by Stegnar *et al.* (1978). Furthermore, kinetin appeared to have an inhibitory effect on Cd^{2+} content of sorghum grains and this may increase the tolerance of sorghum grains against Cd^{2+} toxicity. Till now there is no available report about the influence of kinetin on the uptake and transport of heavy metals in higher plants.

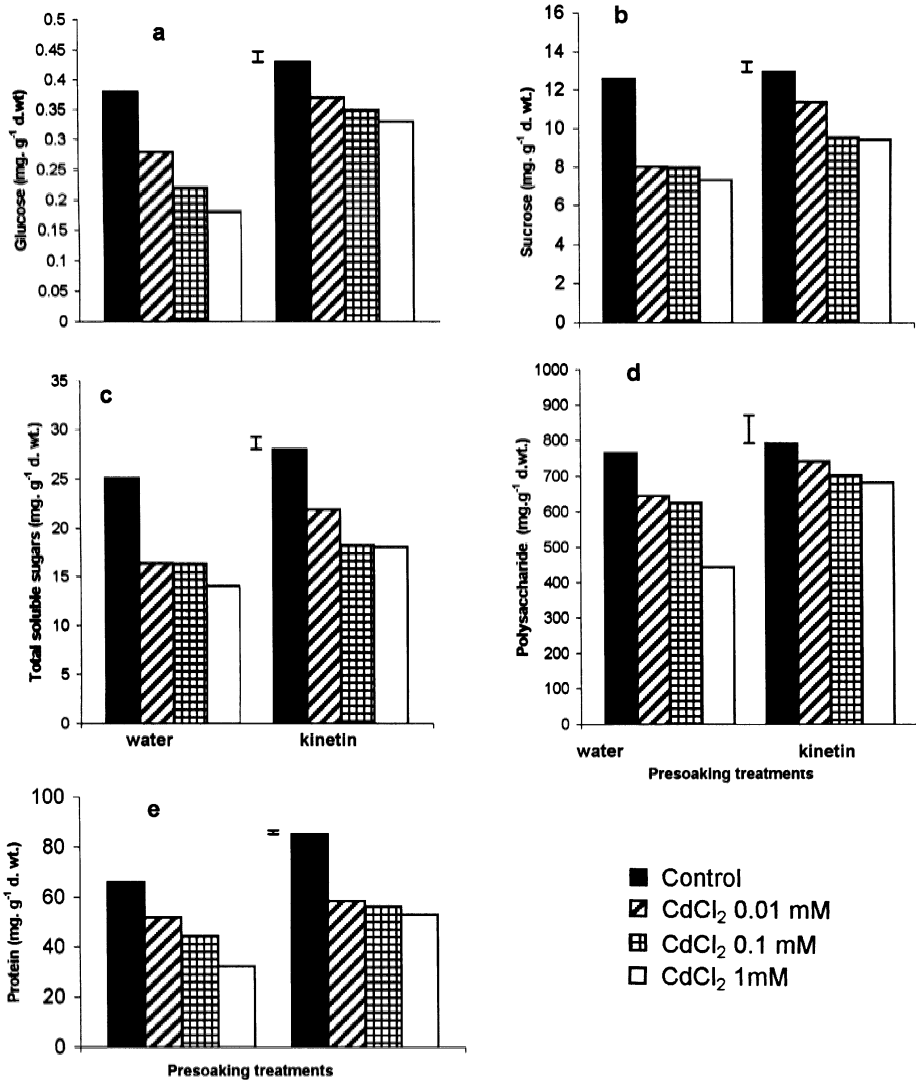

Fig. 6. Effect of grain presoaking in kinetin on carbohydrates content (a, b, c and d) and protein content (e) of yielded grains of sorghum plants irrigated with various concentrations of $CdCl_2$. The vertical bars represent LSD values at $P = 0.05$

Cadmium application obviously caused an increase in ABA levels in yielded grains with all doses used. This increase in ABA content detected in grains may probably be due to its biosynthesis within the grains or may be possibly translocated from the leaves. From another point of view, cadmium may act by interference with hormone metabolism by preventing the ABA catabolism in sorghum grains.

The decrease in free acidic auxins and neutral auxins in yielded sorghum grains as a result of cadmium treatment might be due to Cd^{2+} may stimulate the formation of IAA-oxidase and peroxidase leading to destruction of IAA in resting grains or due to decrease in IAA biosynthesis in developed grains. The noticeable decline in gibberellins of sorghum grains caused by Cd^{2+} application may result from conversion of free active gibberellins into bound inactive gibberellins. Another explanation may come from the fact that Cd^{2+} treatment may interfere with the metabolism of gibberellins; thus causing deactivation of gibberellins or inhibiting their biosynthesis.

As it is clear in $CdCl_2$ at all concentrations used inhibit the rate of transpiration (unpublished data) of sorghum plants. Thus its application may inhibit the translocation of cytokinins from developing root towards the yielded grains.

Grain pretreatment with kinetin acts by antagonizing the effect of Cd^{2+} on the internal bioregulators in sorghum grains. The exogenous application of

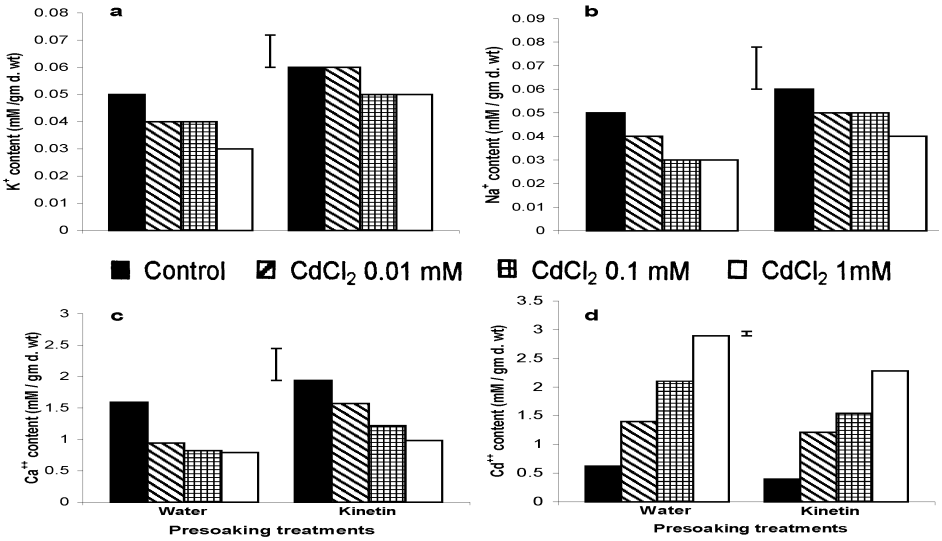

Fig. 7. Effect of grain presoaking in kinetin on ion contents (a, b, c and d) of yielded grains of sorghum plants irrigated with various concentrations of $CdCl_2$. The vertical bars represent LSD values at $P = 0.05$

kinetin may counteract the inhibitory effect of Cd^{2+} by reducing the ABA level and at the same time increases the production of growth stimulators within the developing grains. The increase in auxin level in sorghum grains by

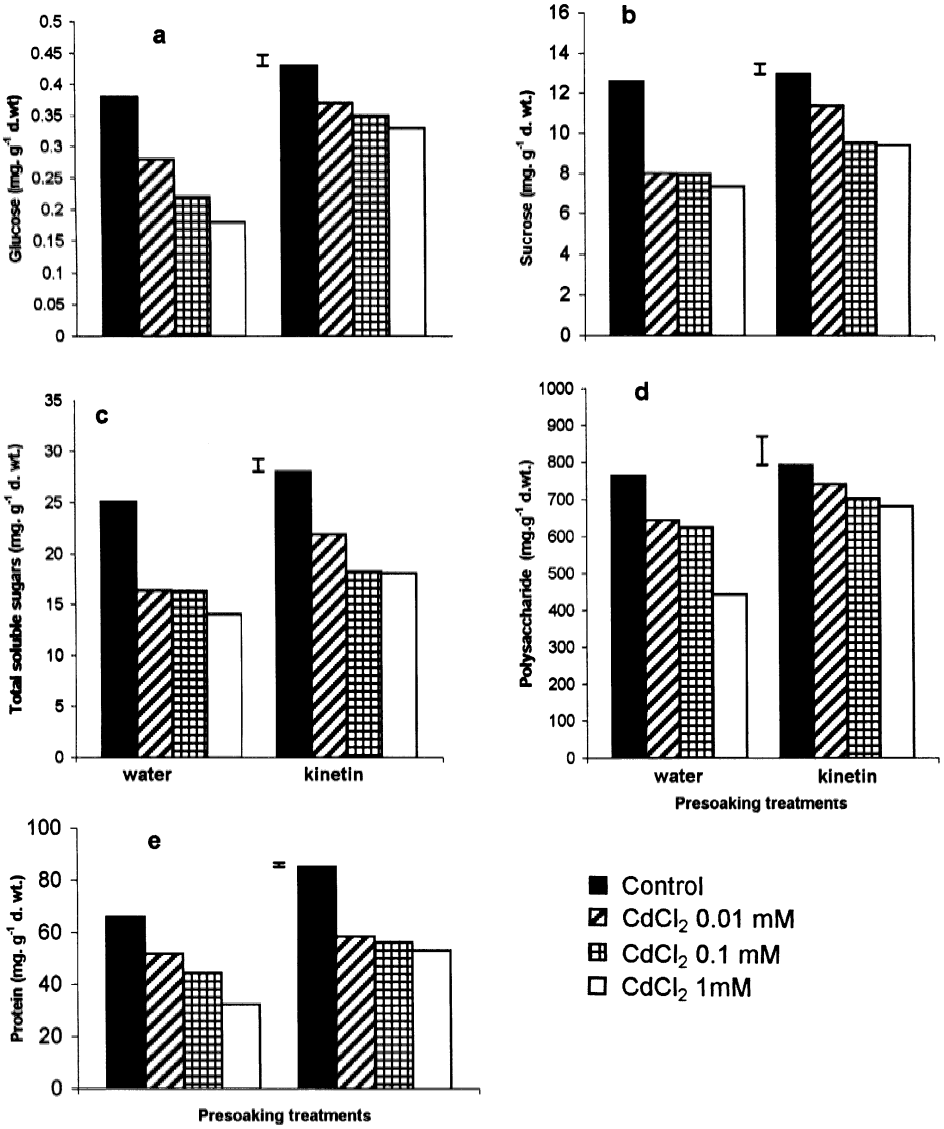

Fig. 8. Effect of grain presoaking in kinetin on growth bioregulators content (a, b, c, d and e) (μg g^{-1} f. wt.) of yielded grains of sorghum plants irrigated with various concentrations of $CdCl_2$. The vertical bars represent LSD values at $P = 0.05$

kinetin treatment may be explained on the basis that kinetin may either stimulate the synthesis of auxin or act as an inhibitor of IAA-oxidase (Einest 1977, Saleh and Hemberg 1980).

Grain priming with kinetin resulted in an increase in gibberellins within the grains of sorghum plants treated with $CdCl_2$ (Fig. 8). Such increase might have resulted from induction of influx of certain metabolites particularly sugars into the grains, thus leading to an increase in the osmotic uptake of water and consequently the sharp rise in fresh weight of the grains (Fig. 4) which follows by an increase in extractable gibberellins activity of the endosperm (Fig. 8). This postulation is in accord with the opinion of Eeuwens and Schwabe (1975) in *Pisum sativum* plants.

The peak of endogenous cytokinin activity in cereal grains during anthesis (Wheeler 1972) which is preceded by high activity of this hormone in the xylem sap coincides with the phase of rapid cell division in the endosperm (Evers 1971). These interrelations are often taken as indication for the eminent importance of the cytokinin synthesis in roots with regard to the development of sink capacity in the grain (Herzog 1982). The noticeable increase in cytokinin content of yield grains of sorghum plants treated with Cd^{2+} after treatment with kinetin may come from the fact that after grain maturation, the cell division of the endosperm ceased and consequently there is a high amount of cytokinins in yielded grains, hence the produced cytokinin within the developed grains may be utilised during the developmental process (Van Staden 1983).

It is clear form this study that kinetin application play an important role in ameliorating the toxic effects of Cd^{2+} on growth, development and metabolism of sorghum plants by enhancing the production of photosynthetic pigments that led to massive increase in photosynthetic activity and Hill reaction. Furthermore, kinetin increases the yield capacity of sorghum plants by inducing remarkable increases in yield components (i.e. panicle length, panicle weight, 100-kernel weight, grain biomass, grain yield, harvest index and relative grain yield) as well as increases in protein and carbohydrates contents in yielded grains. In future, this study will be extended to include further investigations on the effect of kinetin on some different metabolic pathways, different enzymes, endogenous hormonal levels, ultrastructure of chloroplast in sorghum plants treated with cadmium.

REFERENCES

Abo-Kassem, E. M., El-Din, A. S., Mohamed, Y. A. H. and Foda, E. A. (1997): Effect of different cadmium concentrations on growth, photosynthesis and ion relation of wheat plants. – *Egypt. J. Physiol. Sci.* **21**: 41–51.

Aldesuquy, H. S. and Baka, Z. A. M. (1998): *Interactive effect of seawater and plant hormones on the pigment content and chloroplast ultrastructure of wheat flag leaf.* – The 6th conference of Egypt. Bot. Soc. **1**: 51–64.

Arnon, D. I. (1949): Copper enzymes in isolated chloroplasts. Polyphenol oxidase in Beta vulgaris. – *Plant Physiol.* **24**: 1–15.

Baranwal, A. K. (1995): Effect of cadmium on the leaf content of certain pigments in pea plants. – *New Agriculturist* **6**: 201–204.

Berridge, M. V. and Ralph, R. K. (1971): Kinetin and carbohydrate metabolism in Chinese cabbage. – *Plant Physiol.* **47**: 562–566.

Burstron, K. W., King, J. B. and Morgan, E. (1986): Chlorophyll as an indicator of the upper critical tissue concentration of cadmium in plants. – *Water, Air and Soil Pollution* **27**: 147–154.

Chugh, L. K. and Sawhney, S. K. (1999): Photosynthetic activities of Pisum sativum seedling grown in presence of cadmium. – *Plant Physiol. and Biochem.* **37**: 297–303.

Eeuwens, C. J. and Schwabe, W. W. (1975): Seed pod wall development in Pisum sativum L. in relation to extracted and applied hormones. – *J. Experim. Bot.* **26**: 1–14.

Einest, J. W. (1977): Two effects of cytokinins on the auxin requirement of tobacco callus cultures. – *Plant Physiol.* **59**: 45–49.

El-Enany, A. (1995): Alleviation of cadmium toxicity on maize seedlings by calcium. – *Biol. Plant.* **37**: 93–99.

Erisman, K. H. and Wegner, F. (1967): Effect of a growth inhibiting kinetin concentration on chlorophyll content, photosynthesis rate and starch production of Lemna minor. – *Flora Abt. A. Physiol. Biochem.* (Jena) **158**: 433–442.

Esashi, Y. and Leopold, A. C. (1969): Cotyledon expansion as a bioassay for cytokinin. – *Plant Physiol.* **44**: 618–622.

Evers, A. D. (1971): Entwicklung des Endosperms im Wiezenkorn. – *Ber. getreidechem Tagg.* (Detmold) **6**: 89–93.

Feteris, A. W. (1965): A serum glucose method without protein precipitation. – *Amer. J. Medical Technol.* **31**: 17–21.

Foda, H. A. and Radwan, S. S. A. (1962): Straight growth test for hormones and inhibitors using coleoptiles of some Egyptian plants. – *Ain Shams Sci. Bull.* **8**: 381–399.

Fodor, F., Sárvári, É., Láng, F., Szigeti, Z. and Cseh, E. (1996): Effects of Pb and Cd on cucumber depending on the Fe-complex in the culture solution. – *J. Plant Physiol.* **148**: 434–439.

Frankland, B. and Wareing, P. F. (1960): Effect of gibberellic acid on hypocotyl growth of lettuce seedlings. – *Nature* **185**: 255–256.

Gaber, A. M. (1985): *Effect of water stress on respiration and photosynthesis.* – PhD thesis, Mansoura University, Egypt.

Gad-Allah, M. A. (1999): Effects of kinetin on growth, grain yield and some mineral elements in wheat plants growing under excess salinity and oxygen deficiency. – *Plant Growth Regulation* **27**: 63–74.

Ghi-Ying, H., Fakhri, A. and Larry, N. (1974): The inhibition of soybean metabolism by cadmium and lead. – *Plant Physiol.* **54**: 122–124.

Godbold, D. I. (1994): Aluminium and heavy metal stress: from the rhizosphere to the whole plant. – *J. Agron. and Crop Sci.* 232–264.

Hare, P. D., Cress, W. A. and Van Staden, J. (1997): The involvement of cytokinins in plant responses to environmental stress. – *Plant Growth Regulation* **23**: 79–103.

Hemalatha, S., Anburaj, A. and Francis, K. (1997): Effect of heavy metals on certain biochemical constituents and nitrate reductase activity in Oryza sativa L. seedlings. – *J. of Environ. Biol.* **18**: 313–319.

Herzog, H. (1982): Influence of ear size, leaf area and cytokinin applications on kernel development in wheat. – *Z. Acker- und Pflanzenbau* **151**: 205–209.

Hofer, H. and Schutz, E. (1980): Contribution to the determination of threshold toxicity levels for heavy metals in plant production. – *Mitt. f. schweizerische Land Wirtschaft* **28**: 66–77.

Howard, H. F. and Witham, F. M. (1983): Invertase activity and the kinetin-stimulated enlargement of detached radish cotyledons. – *Plant Physiol.* **73**: 304–308.

Ibrahim, A. H. (1999): *Control of growth of sorghum plants grown under stress conditions.* – PhD thesis, Fac. Sci., Mansoura University, Egypt.

Juwarker, A. S. and Shende, G. B. (1986): Interaction of cadmium and lead: Effect on growth, yield and content of cadmium and lead in barley (Hordeum vulgare). – *Indian J. Environ. Health* **28**: 235–3.

Kupper, H., Kupper, F. and Spiller, M. (1998): In situ detection of heavy metals substituted chlorophylls in water plants. – *Photosynthesis Res.* **58**: 123–133.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951): Protein measurement with the Folin phenol reagent. – *J. Boil. Chem.* **193**: 265–275.

Malan, H. L. and Farrant, J. M. (1998): Effect of metal pollutants cadmium and nickel on soybean seed development. – *Seed Sci. Res.* **8**: 445–453.

Malik, D., Sheoran, I. S. and Singh, R. (1992): Carbon metabolism in leaves of cadmium treated wheat seedlings. – *Plant Physiol. and Biochem.* **30**: 223–229.

Metzner, H., Rau, H. and Senger, H. (1965): Untersuchungen zur synchrohisier einzelner pigmentmangel Mutanten von Chlorella. – *Planta* (Berlin) **65**: 186–194.

Moya, J. L., Ros, R. and Picazo, I. (1995): Heavy metal phytohormone interactions in rice plants: Effects on growth, net photosynthesis and carbohydrate distribution. – *J. Plant Growth Regul.* **14**: 61–67.

Naquib, M. I. (1963): Colourimetric estimation of plant polysaccharides. – *Zucker* **16**: 15–23.

Naquib, M. I., Hamed, A. A. and Al-Waheel, S. A. (1986): Effect of cadmium on growth criteria of some crop plants. – *Egypt. J. Bot.* **25**: 1–12.

Oliver, D. P., Moss, H. J. and Tiller, K. G. (1993): Cadmium in wheat grain and milling products from some Australian flour mills. – *Aust. J. Agric. Res.* **44**: 1–11.

Poschendieder, C., Cabot, C. and Barcfio, J. (1983): Influence of high concentrations of cadmium on the growth, development and photosynthetic pigment of Phaseolus vulgaris. – *Anales de Edafologia y Agrobiologia* **42**: 315–327.

Rani, S. M., Muthuchelian, K. and Paliwal, K. (1987): Differential toxicity of Cu^{2+} and Cd^{2+} on chlorophyll biosynthesis and nitrate reductase activity in Phaseolus mungo L. – *Annals of Plant Physiol.* **1**: 126–135.

Rauser, W. E. (1978): Early effects of phytotoxic burdens of cadmium, cobalt, nickel and zinc in white beans. – *Can. J. Bot.* **56**: 1744–1749.

Ray, S. and Choudhuri, M. A. (1980): Regulation of flag leaf senescence in rice by nutrients and its impact on yield. – *RISO* **29**: 9–14.

Reuss, J. O., Dooley, H. L. and Griffis, W. (1978): Uptake of cadmium from phosphate fertilizers by pea, radish and lettuce. – *J. Environ. Qual.* **32**: 128–133.

Riazi, A., Matsuda, K. and Arslan, A. (1985): Water stress induced changes in concentrations of proline and other solutes in growing regions of young barley leaves. – *J. Expt. Bot.* **36**: 1716–1725.

Richmond, A. E. and Lang, A. (1975): Effect of kinetin on protein content and survival of detached Xanthium leaves. – *Science* **125**: 650–651.

Robertson, D., Wojtaszek, P. and Bolwell, G. P. (1999): Stimulation of cell wall biosynthesis and structural changes in response to cytokinin and elicitor treatment of suspension cultured Phaseolus vulgaris cells. – *Plant Physiol. and Biochem.* (Paris), **37**: 611–621.

Saleh, A. N. and Hemberg, T. (1980): The influence of kinetin on the endogenous content of indolacetic acid in swelling seeds of Phaseolus, Zea and Pinus and young plants of Phaseolus. – *Physiol. Plant.* **50**: 99–105.

Shindy, W. W. and Smith, O. E. (1975): Identification of plant hormones from cotton ovules. – *Plant Physiol.* **55**: 550–554.

Snedecor, G. W. and Cochran, W. G. (1976): *Statistical methods.* 6th ed. – Oxford IBH Publishing Co., New Delhi.

Somashekaraiah, B., Padmaja, K. and Preasad, A. (1992): Phytotoxicity of cadmium ions on germinating seedlings of mung bean (Phaseolus vulgaris): Involvement of lipid peroxides in chlorophyll degradation. – *Physiol. Plant.* **85**: 85–89.

Stadelmann, F. X., Frossard, R. and Moeri, P. B. (1986): Influence of cadmium on yield, physiological properties and quality of Italian rye grass, radish and spinach. – *Schriftenreihe. Verb and Deutscher Landwirtschaftlicher Kongress berichte* **15**: 575–585.

Stegnar, P., Gogala, N. and Pohleven, F. (1978): The uptake of cadmium, zinc, phosphorus and plant hormone kinetin by ectomycorrhizal fungi. – *Acta Bot. Croat.* **37**: 67–73.

Stobart, A. K., Griffiths, W. T., Bukari, I. A. and Shearwood, R. P. (1985): The effect of Cd^{2+} on the biosynthesis of chlorophyll in leaves of barley. – *Physiol. Plant.* **63**: 293–298.

Strzelec, A. and Oron, J. (1987): Effect of cadmium on the development of Rhizobium strains and the activity of their symbiosis with leguminous plants. – *Roczniki gleboznawczze* **38**: 101–109.

Trebst, A. (1967): *Measurement of Hill reaction and photoreduction.* – In: Colowick, S. P. and Kaplan, N. O. (eds): Methods in Enzymology. **24**: 146–165.

Uheda, E. and Kuraishi, S. (1978): The relationship between transpiration and chlorophyll synthesis in etiolated squash cotyledons. – *Plant and Cell Physiol.* **19**: 825–831.

Van Assche, F., Cardinaels, C. and Clijsters, H. (1988): Induction of enzyme capacity in plants as a result of heavy metal toxicity; dose – reponse relations in Phaseolus vulgaris L. treated with zinc and cadmium. – *Environ. Pollut.* **52**: 103–115.

Van Staden, J. (1983): Cytokinins, seed development and germination. – *South African J. Bot.* **2**: 257–261.

Vassilev, A., Kerin, V. and Atanassov, P. (1996): Effect of cadmium pollution of soil upon productivity and seedling qualities of two winter barley (H. vulgare L.) cultivars. – *Bulg. J. Agric. Sci.* **2**: 333–340.

Vassilev, A., Yordanov, I. and Tsonv, T. (1997): Effects of Cd^{2+} on the physiological state and photosynthetic activity of young barley plants. – *Photosynthetica* **34**: 293–302.

Vassilev, A., Tsonev, T. and Yordanov, I. (1999): Physiological response of barley plants (Hordeum vulgare) to cadmium contamination in soil during ontogenesis. – *Environ. Pollut.* **103**: 287–293.

Weigel, H. J. (1985): The effect of Cd^{2+} on photosynthetic reactions of mesophyll protoplats. – *Physiol. Plant.* **63**: 192–200.

Wheeler, A. W. (1972): Changes in growth substance contents during growth of wheat grain. – *Ann. Appl. Biol.* **72**: 327–332.

Wilfried, E. R. and Philippe, M. (1995): Retention of cadmium in roots of maize seedlings. – *Plant Physiol.* **109**: 195–202.

Wozny, A., Scneider, J. and Gwozdz, E. A. (1995): The effects of lead and kinetin on greening barley leaves. – *Biol. Plant.* **37**: 541–552.

Wright, S. T. (1956): Studies in fruit development in relation to plant hormones. III – Auxins in relation to fruit morphogenesis and fruit drop in the black currant (Ribes nigrum). – *J. Hort. Sci.* **31**: 196–202.

Wu, Z. L. and Hu, G. and Wang, K. (1998): Study of protective effects of kinetin on paddy rice seedlings affected by chilling injury. – *J. Southwest Agric. Univ.* **20**: 351–354.

Younis, A. E., Younis, M. E. and Gabr, M. A. (1969): Studies on the effect of certain enzymic poisons on the metabolism of storage organs. II. Differential effects of iodoacetate on the respiratory metabolism and permeability barriers in radish root slices. – *Plant and Cell Physiol.* **10**: 95–101.

Younis, M. E., Abbas, M. A. and Shukry, W. M. (1994): Salinity and hormones interaction in affecting growth, transpiration and ionic relations of Phaseolus vulgaris. – *Biol. Plant.* **36**: 83–89.