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KINETIN-INDUCED MODIFICATION IN GROWTH CRITERIA, ION CONTENTS AND WATER RELATIONS OF SORGHUM PLANTS TREATED WITH CADMIUM CHLORIDE

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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 growth criteria, ions content and water relations of *Sorghum bicolor* plants. Also particular interest was focused on the effect of grain presoaking with kinetin in order to ameliorate the toxicity effects exerted by the different levels of $CdCl_2$. In general, the results showed, that the observed suppression in growth criteria (i.e. root length, root fresh and dry weights, plant height, shoot fresh and dry weights, cumulative leaf area as well as root/shoot ratio), ions content (i.e. K^+, Na^+, Ca^{2+} and Cd^{2+}), water relations (i.e. total leaf conductivity, transpiration rate and relative water content) in response to $CdCl_2$ was relieved either partially or completely when grains were presoaked in kinetin (50 ppm). The alleviating effect of kinetin for Cd^{2+} toxicity was more pronounced in response to 1 mM $CdCl_2$ treatment.

Key words: cadmium, growth, ions content, kinetin, sorghum, water relations

INTRODUCTION

Soil can be contaminated with cadmium by amendment with sewage sludge, urban and industrial pollution or by the application of farm chemicals, which contain cadmium as a contaminant. Cadmium accumulation in agricultural food products has been attributed mainly to phosphate fertilizers (Tiller 1988). Within plants, cadmium is selectively accumulated in certain tissues. Thus, the heavy metal content per unit dry matter decreases in the following order: leaves, storage roots, tubers, fleshy fruits and seeds (Marschner 1983). Cadmium has been reported to concentrate in the protein fractions of plant, which may have important implications for food production. Cadmium availability for plant uptake is controlled by many soil factors, such as soil pH, clay mineralogy and organic matter (Kabata-Pendias and Pendias 1984).

Juwarker and Shende (1986) found that, in a pot culture study with calcareous clay soil amended with varying rates of Cd, Pb and Cd+Pb, length of root,

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height of plant, length of ear head of barley were synergistically reduced due to the combined treatment. Cadmium reduced percentage germination, root and shoot length, fresh and dry weights in different plant species (Poschendieder *et al.* 1983, Naguib *et al.* 1986). While, Jyoii *et al.* (1994) reported that, the radicle length of *Vicia faba* plants decreased at all concentrations of Cd, whereas Co, Ni and Zn increased radicle length at low concentration, but were inhibitory at high concentration. The toxicity order was as follows Cd > Ni > Zn = Co. Abo-Kassem *et al.* (1997) found that, in wheat plants, root and shoot dry weights as well as relative water content were significantly reduced by 5 and 10 µM cadmium as cadmium sulphate. Recently, Vassilev *et al.* (1998) reported that, in Cd-treated barley plants with 54 µM Cd^{2+} for 12 days, the plant growth was inhibited.

Krupka and Moniak (1998) found that the level of heavy metal toxicity appeared to be related to the leaf age and stage of plant growth. Furthermore they deduced that the basic growth analysis (total leaf length and fresh weight content of leaf sections) being the most sensitive to cadmium toxicity. Addition of the chloride salts of Cd or Ni to the nutrient solution in which soybean cv. 'Crawford' plants grown, reduced the plant biomass (Malan and Farrant 1998). Furthermore, Vassilev *et al.* (1999) established that a soil Cd concentration of 45 mg/kg inhibited dry mass accumulation and suppressed development of barley plants.

Cadmium reduced root water uptake, stomatal opening, conductance, relative water content and transpiration in different Cd-treated plants (Poschendieder *et al.* 1983, Marchiol *et al.* 1996, Abo-Kassem *et al.* 1997, Vassilev *et al.* 1997, 1998, 1999).

Cadmium inhibited potassium uptake in roots of oat (*Avena sativa* L.) plants to 80% of control value after 30 minutes in the presence of one millimolar $CdSO_4$ (Robert 1978). Cadmium inhibited absorption and accumulation of K^+, Ca^{2+} and Fe^{3+} in cucumbers as well as, at high concentrations, Cd caused efflux of K^+ but not of Mg^{2+} (Burzynski 1987). On the other hand, Abo-Kassem *et al.* (1997) demonstrated that cadmium inhibited the transport of Ca^{2+}, Na^+ and K^+, but increased the uptake of Zn, Fe and Cu in Cd-treated wheat plants with concentrations 1, 5 and 10 µM cadmium sulphate. Furthermore, Bipasha *et al.* (1997) found that greater uptake of cadmium in the roots of *Linum* spp. cv. 'Gorima' plants occurred than that in the shoot and the accumulation of cadmium occurred specifically in the cytoplasm of the regenerated roots. The same results were observed in Cd-treated wheat plants (Abo-Kassem *et al.* 1997) and in Cd-treated soybean cv. 'Crawford' plants (Malan and Farrant 1998). On the other hand, Vassilev *et al.* (1999) further found that the cadmium concentration in the roots was 10 times higher than in the above ground organs. In the over ground parts of plants, the highest concentration was found in the lower leaves of Cd-treated barley plants. Generally Thomas and Harrison (1991) reported, that Cd-uptake differs according to the varieties of lettuce used.

On the light of the above-mentioned reviews, it was of particular interest to investigate the effect of grain priming with kinetin on growth criteria, minerals and water relations of *Sorghum bicolor* plants stressed with different levels of cadmium chloride.

MATERIAL AND METHODS

Plant material and experimental design

Homogeneous grains of *Sorghum bicolor* (cv. 'Dorado') were surface sterilized by soaking in 0.001 M $HgCl_2$ 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 two sets were subdivided into four groups. The first group was irrigated with normal tap water to serve as control, while the second, third and fourth groups were irrigated with 0.01, 0.1 or 1 mM of $CdCl_2$, respectively.

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 growth criteria and three replicates for water relations and elements determinations, from one experiment (K^+, Na^+, Ca^{2+} and Cd^{2+}).

Estimation of leaf area

Leaf area of the 3rd leaf was estimated according to the following formula:

Leaf area = length × maximum width × 0.75 (Turner 1974).

Determination of K^+, Na^+, Ca^{2+} and Cd^{2+} ions

Sodium 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).

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

Measurement of total leaf conductance and transpiration rate

Total leaf conductance and transpiration rate of the third fully expanded leaf of sorghum plants were measured using a Li–1600 M Steady State Porometer. The atmospheric pressure (PRES SET) and aperture area of the apparatus were adjusted to 101.3 kpa and 1 cm^2, respectively (Ibrahim 1999).

The results were first subjected to analysis of variance (ANOVA). If ANOVA showed a significant ($P < 0.05$) effect, the least significant difference was used to compare treatments (Snedecor and Cochran 1976).

RESULTS AND DISCUSSION

The present results revealed that $CdCl_2$ particularly at 1 mM reduced both root and shoot growth of sorghum plants during the various growth periods (Figs 1–2). This negative effect of Cd^{2+} on root and shoot fresh weight may be probably due to that Cd^{2+} decreases the root water uptake and relative water content as suggested by other workers using different plant species (Poschendieder and Barcelo 1989, Marchiol *et al.* 1996, Abo-Kassem *et al.* 1997, Vassilev *et al.* 1997). Furthermore, the decrease in root and shoot dry weights as a result of Cd^{2+} treatment, particularly at 0.01 and 0.1 mM, could possibly due to the effect of Cd^{2+} in decreasing the ion contents (i.e. K^+, Na^+ and Ca^{2+}) of root and shoot as demonstrated in some salt stressed oil-producing plants (Heikal *et al.* 1980).

The present results are in conformity with the work of others (Greger *et al.* 1991, Landberg and Greger 1994, Abo-Kassem *et al.* 1997). Presumably the effect of Cd^{2+} in this case could be due to inhibition of RNA and protein syntheses and consequently cell division as suggested by Mukherjee and Sharma (1988).

The importance of leaf area in controlling plant dry matter and growth rate has long been appreciated (Aldesuquy 2000). He concluded that the rate of leaf area expansion has a greater influence on dry matter production than do net assimilation rate. The delay in the total cumulative leaf area production in response to $CdCl_2$ treatments particulary at 1 mM (Fig. 2) could be due to the slow rate of movement of nutrients and hormones transported with transpiration stream from the root to shoot (Abo-Hamed *et al.* 1987). Moreover, Barcelo *et al.* (1988) related the negative effect of Cd^{2+} on specific leaf area with disorders on water supply in *Phaseolus vulgaris* plants. These authors suggested that reduced cell turgor potential and cell wall elasticity led to formation of small cells and intercellular space area in Cd^{2+} treated plants.

As regards the influence of grain presoaking in kinetin, the observed reduction in growth parameters of sorghum plants in response to Cd^{2+} treatments was either relieved or reduced as a result of kinetin treatment (Figs 1–2). For instance, kinetin increased root and shoot fresh and dry weights of sorghum plants treated with $CdCl_2$ as compared to the corresponding levels of $CdCl_2$ alone (Fig. 1). The repairing effect of kinetin on growth of Cd^{2+}-treated

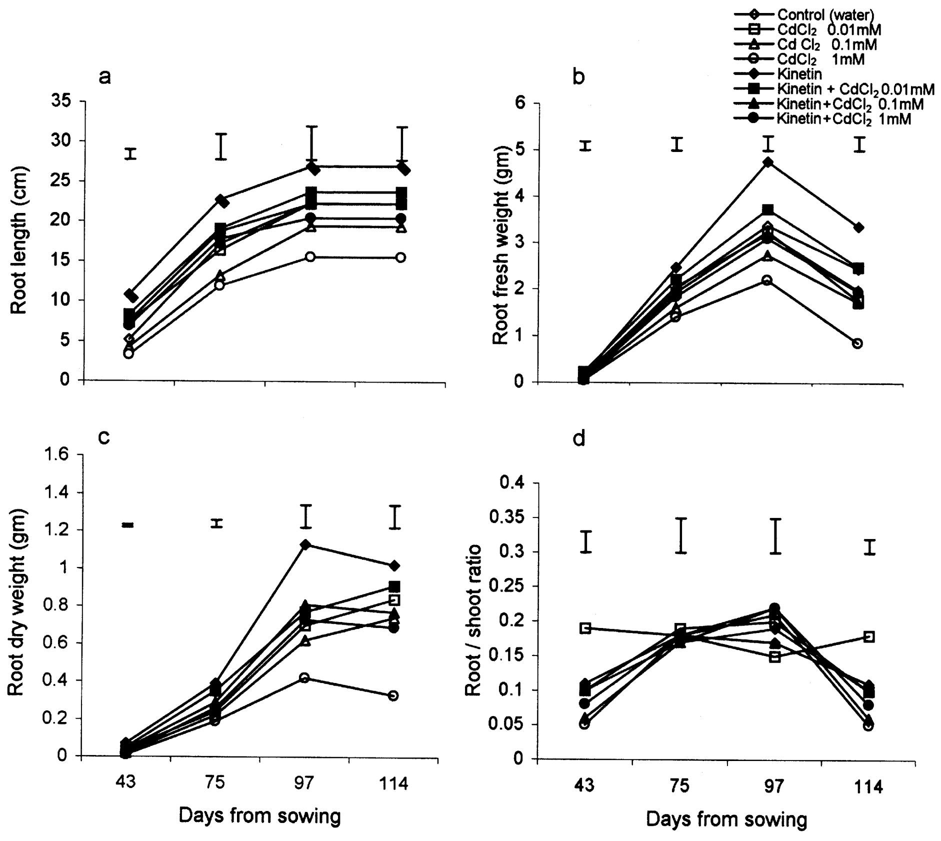

Fig. 1. Effect of grain presoaking in kinetin on root growth criteria (a, b, c and d) of sorghum plants irrigated with various concentrations of $CdCl_2$. The vertical bars represent LSD values at $P = 0.05$

plants may be due to increased water uptake by the root system of sorghum plants as reported in other studies (Heikal 1982, Younis *et al.* 1991).

The inhibition of growth of leaf area in response to cadmium applications was alleviated when the grains were treated with kinetin. This recovery may result from the fact that kinetin stimulates the rate of movement of nutrients and hormones from root which can accelerate the rate of leaf expansion in developing leaves (Richmond and Lang 1975).

Concerning the mineral composition of the tested plant tissues, in this study cadmium chloride treatment induced considerable changes in the concentration of cations in root and shoot throughout growth and development of

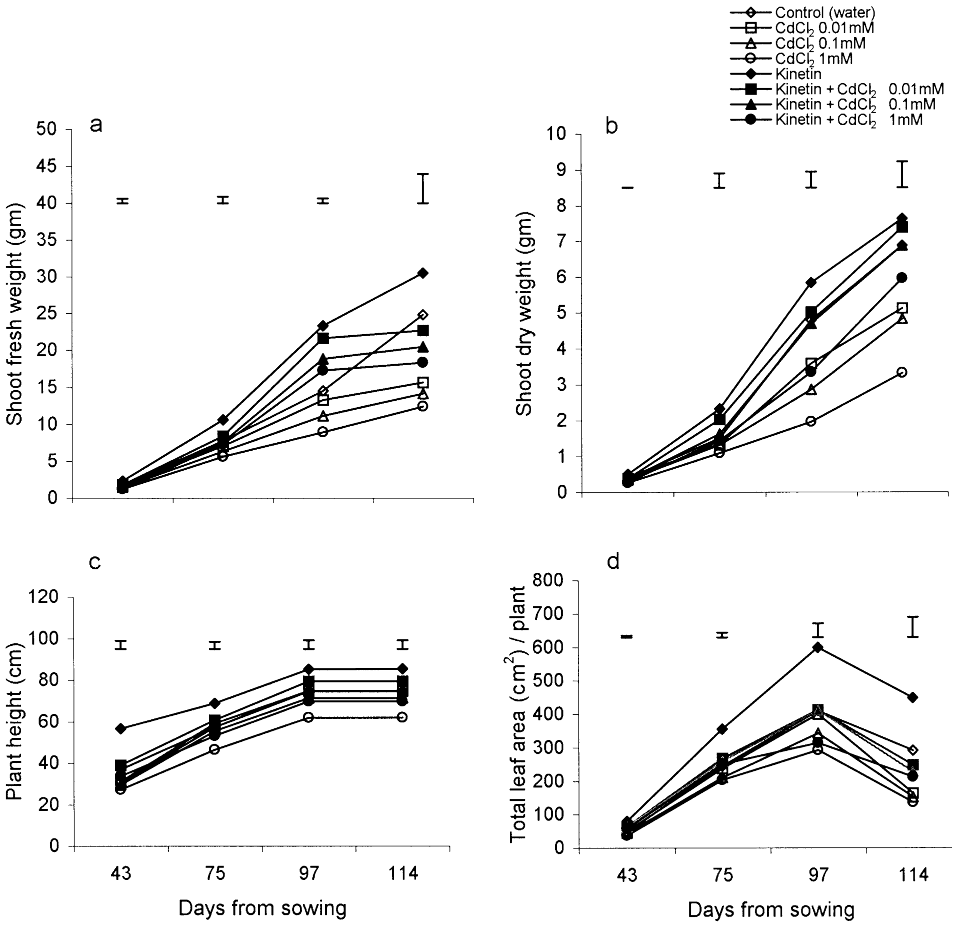

Fig. 2. Effect of grain presoaking in kinetin on shoot growth criteria(a, b, c and d) of sorghum plants irrigated with various concentrations of $CdCl_2$. The vertical bars represent LSD values at $P = 0.05$

Sorghum bicolor plants. The magnitude and the pattern of these changes differed according to the used level of $CdCl_2$ (Figs 3–4). It is clear that cadmium application at all the studied concentrations (0.01, 0.1 or 1 mM) caused a reduction in the ions content (i.e. K^+, Na^+ and Ca^{2+}) in both roots and shoots of sorghum plants at all growth periods (Fig. 4). These results were in accordance with those of Robert (1978) using *Avena sativa* plants; Burzynski (1987) using *Phaseolus vulgaris*, cucumber and wheat plants; Thomas and Harrison (1991) using *Lactuca sativa* plants and Abo-Kassem *et al.* (1997) using wheat plants.

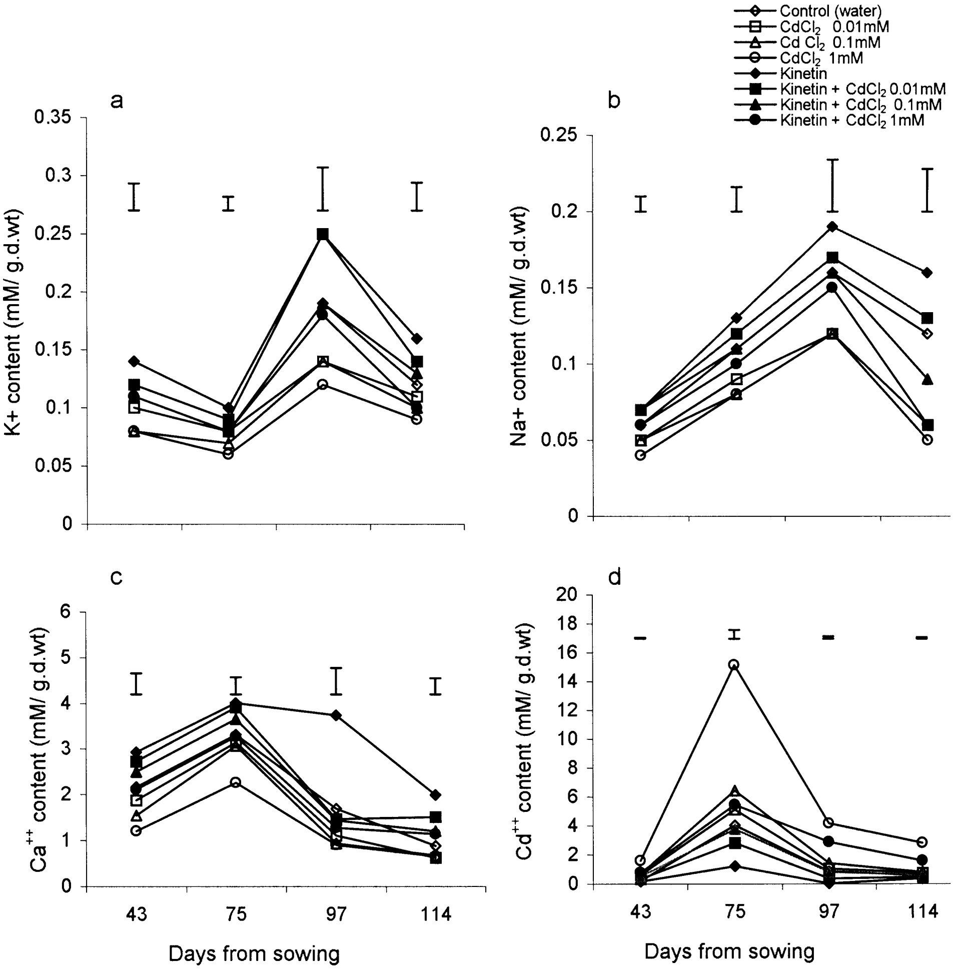

Fig. 3. Effect of grain presoaking in kinetin on ion contents (a, b, c and d) of roots of sorghum plants irrigated with various concentrations of $CdCl_2$. The vertical bars represent LSD values at $P = 0.05$

The accumulation of cadmium in Cd^{2+}-treated plants was generally depending on the increase of cadmium concentration and the age of the plant. In roots, the cadmium accumulation was greater than that in shoot (Figs 3–4). These results are comparable to those data obtained by Abo-Kassem *et al.*

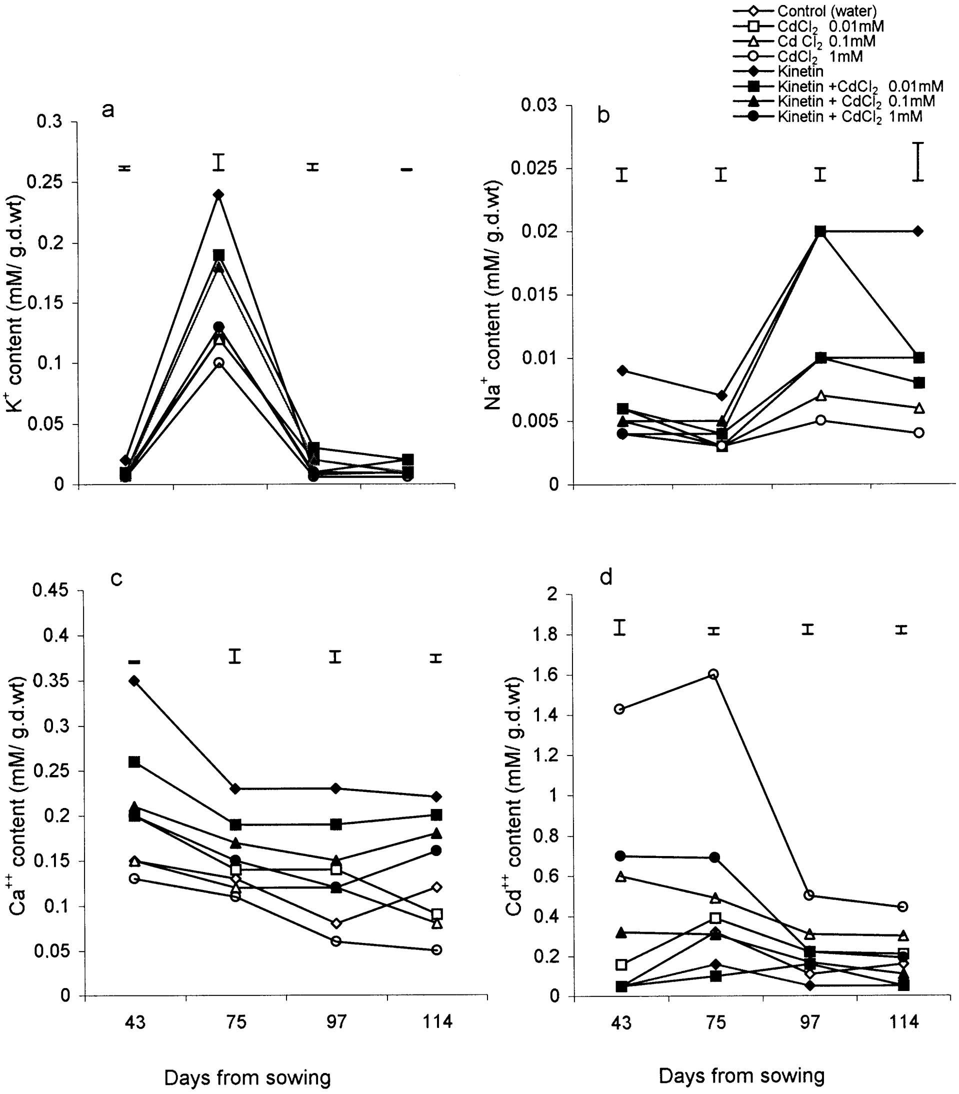

Fig. 4. Effect of grain presoaking in kinetin on ion contents (a, b, c and d) of shoots of sorghum plants irrigated with various concentrations of $CdCl_2$. The vertical bars represent LSD values at $P = 0.05$

(1997), Bipasha *et al.* (1997), Malan and Farrant (1998), Vassilev *et al.* (1999). Translocation of Cd^{2+} from root to shoot was demonstrated in maize plants (Yang *et al.* 1995), who reported that Cd^{2+} translocated from roots to shoots of maize plants and the higher Cd^{2+} concentrations in the xylem sap in maize was higher than that in other species.

The low levels of ions in Cd^{2+}-treated plants may be due to that cadmium lowered ATP content, inhibited ATP-ase activity of the plasma membrane and inhibited K^+ carriers which are bound to plasma membrane (Robert 1978), as well as a consequent reduction of water uptake and translocation might have occurred (David *et al.* 1995). Furthermore, the decrease in Ca^{2+} content in roots by Cd^{2+} treatment may probably be due to the competition between Cd^{2+} and Ca^{2+} on the active sites of specific carriers and/or the antagonism between them in the soil solution.

Cadmium accumulated in root of treated sorghum plants rather than in shoots (Fig. 3) may be result from reduction in transport from root to shoot due to formation of a high molecular weight Cd^{2+}-binding complex (phytochelatins) in roots as suggested by Wilfried and Philippe (1995).

The different groups of hormones can influence ion uptake by affecting membrane properties and transport processes (van Steveninck 1976). Thus, the present results clearly show that grain pretreatment with kinetin generally increased K^+, Na^+ and Ca^{2+} contents in roots and shoots of untreated and Cd^{2+}-treated plants if compared with the corresponding levels in case of $CdCl_2$ alone (Figs 3–4). This positive effect of kinetin may presumably be due to the increase in ATP content, activation of ATPase of plasma membrane and consequently the stimulation of K^+ carriers bound to plasma membrane (Dai *et al.* 1998). Kinetin also increased the rate of water uptake by root as well as the transpiration and consequently increases the uptake and translocation of K^+, Na^+ and Ca^{2+} contents which were driven by transpiration (David *et al.* 1995, Robertson *et al.* 1999, Zhang and Schmidt 1999). Furthermore, kinetin altered membrane composition (Merillon *et al.* 1993), its selectivity (Dhakal and Erdei 1986) and increased membrane fluidity (Vodnik *et al.* 1999).

It is clear that kinetin plays an important role in increasing the tolerance of sorghum plants to cadmium stress by decreasing the accumulation of free Cd^{2+} content in root and consequently in shoot (Figs 3–4). This repairing effect induced by exogenous application of kinetin could be due to: (1) kinetin may increase the production of Cd^{2+}-binding protein (phytochelatins) particularly in root (Yang *et al.* 1995), (2) kinetin may increase the detoxification of Cd^{2+} by increasing the accumulation of Cd^{2+} in trichomes on leaf surface as that reported in *Brassica juncea* L. (David *et al.* 1995), (3) Kinetin may increase the vacuolar storage of Cd^{2+} as reported by Chardonnes *et al.* (1998) in *Silene vulgaris* plants.

The importance of the internal water balance in plant–water relations is generally accepted because of the close relationship between the balance and turgidity, to the rates of physiological processes that control the quality and quantity of growth (Aldesuquy and Ibrahim 2000). It is well-known that in many plant species, cadmium disturbs the plant–water relations (Barcelo *et al.* 1986, Costa *et al.* 1994). In the present work, the data in Figure 5 revealed that application of different concentrations of $CdCl_2$ to sorghum plants (except 0.01 mM) decreased most of the water relations parameters, e.g. total leaf conductivity, transpiration rate and relative water content (RWC). On the other hand, 0.01 mM has exerted little effect on such parameters.

Kinetin might have presumably increased the relative water content (Fig. 5) and mineral contents (Figs 3–4) of sorghum plants as well as its trans-

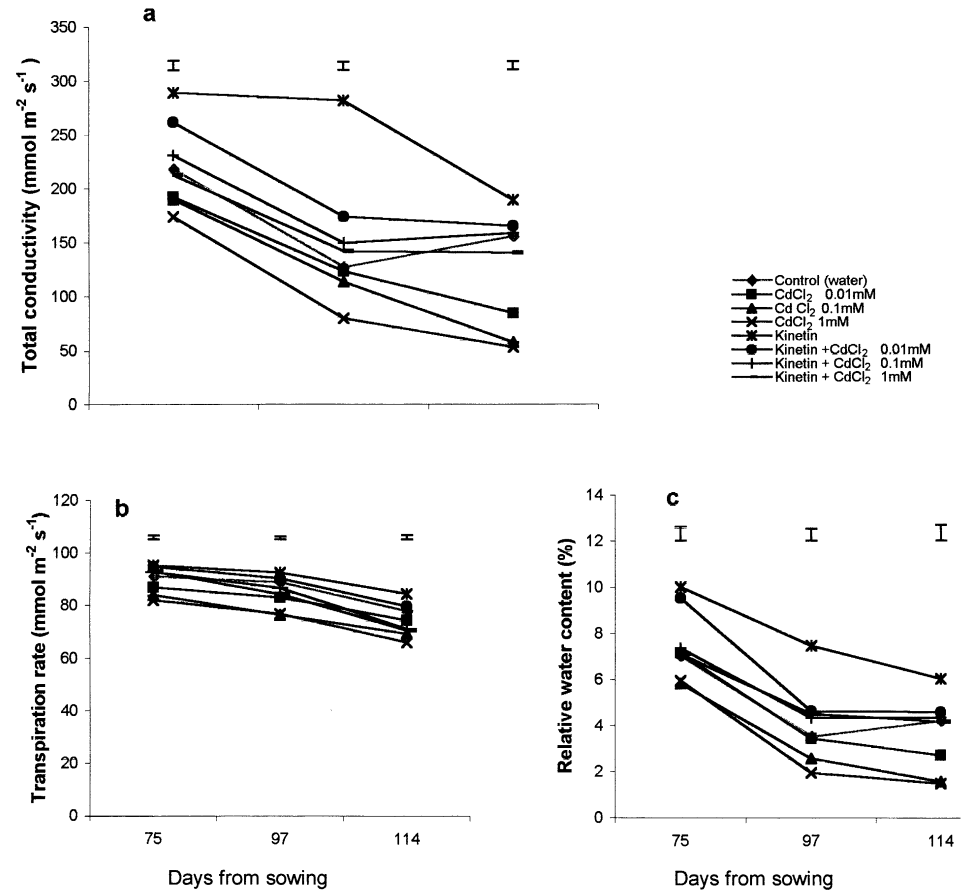

Fig. 5. Effect of grain presoaking in kinetin on water relations (a, b and c) of sorghum plants irrigated with various concentrations of $CdCl_2$. The vertical bars represent LSD values at $P = 0.05$

location via transpiration stream. These results are in agreement with those obtained by Heikal *et al.* (1982) using flax plants; Younis *et al.* (1991) using *Pisum sativum* plant who stated that treatment with kinetin induced increase in water uptake and inorganic matter in plants subjected to salinity stress.

The negative effect exerted by cadmium on total leaf conductance, transpiration rate and RWC may be mediated through the production of abscisic acid (ABA) resulting in stomatal closure and increasing stomatal diffusive resistance and consequently decreasing the rate of transpiration in Cd^{2+}-treated sorghum plants (Weigel 1985, David *et al.* 1995). The effect of cadmium on the transpiration rate may result also from decreased root/shoot ratio (Fig. 1) leading to hampered water supply to shoot and to negative cadmium effect on the stomatal functioning (Richmond and Lang 1975). According to Barcelo *et al.* (1990), cadmium may induce closure of stomata. The cadmium effects on water regime of sorghum plants are in agreement with the results obtained by other workers (Barcelo *et al.* 1986, Poschendieder and Barcelo 1989, Costa *et al.* 1994, Leita *et al.* 1995, Marchiol *et al.* 1996, Vassilev *et al.* 1997, 1998, Abo-Kassem *et al.* 1997) for other plant species under cadmium-stress conditions. Furthermore, Lamoreaux and Chaney (1978), Barcelo *et al.* (1986) and Marchiol *et al.* (1996) reported that hydraulic conductivity into xylem vessels decreased from two to four times depending on the applied cadmium stress and characteristic species. On the other hand, Barcelo *et al.* (1988) considered that the reasons for reduced water movement were the decreased vessels radius and number due to cadmium induced inhibition of division, elongation and differentiation of cambium cells.

Total leaf conductivity, transpiration rate and relative water content of sorghum plants treated with $CdCl_2$ were increased by grain pretreatment with kinetin (Fig. 5). The beneficial effect of kinetin was mainly due to the increased water uptake that led to powerful water supply to shoot (David *et al.* 1995, Robertson *et al.* 1999, Zhang and Schmidt 1999). This was confirmed by relatively high changes in RWC (Fig. 5). Furthermore, the application of kinetin may induce stomatal opening by diminishing the level of ABA and finally leading to an increase in the transpiration rate of sorghum plants treated with cadmium. In this connection, both kinetin and gibberellic acid were reported to suppress the accumulation of ABA-like inhibitors in leaves of nasturtium plant (Chin and Beevers 1970).

It is clear from this study that grain priming with kinetin displayed a positive role in improving growth and turgidity of *Sorghum bicolor* plants treated with $CdCl_2$ by inducing modification in growth criteria, minerals contents, transpiration rate, total leaf conductance as well as the relative water content.

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