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II Potassium nutrition reduces cadmium accumulation and oxidative burst in mustard (*Brassica campestris* L.)

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Abstract

A greenhouse pot experiment was conducted to study the protective effect of potassium (K) nutrition against cadmium (Cd) toxicity in mustard (*Brassica campestris* L.). Cadmium treatment drastically reduced plant growth (plant dry mass, leaf area), photosynthetic traits (net photosynthetic rate, stomatal conductance and internal CO_2 concentration) and the contents of ascorbic acid (AsA), glutathione (GSH) and potassium (K) but significantly increased the contents of thiobarbituric acid reactive substances (TBARS), hydrogen peroxide (H_2O_2) and Cd ions in the leaves. K application was effective in decreasing the Cd-toxicity-induced oxidative burst as evident from the lowering of H_2O_2 and TBARS, increase of AsA and GSH contents as well as enhanced plant growth. These effects of K were associated with a sharp decline in Cd content of leaves. The results are indicative of the ameliorative role of K in mustard against the oxidative stress caused by Cd toxicity.

Key words: *Brassica campestris*; Cadmium toxicity; Oxidative damage; Potassium nutrition.

Abbreviations: APX, ascorbate peroxidase; AsA, ascorbic acid; CAT, catalase; Cd, cadmium; DAS, days after sowing; GR, glutathione reductase; gs, stomatal conductance; GSH, glutathione reduced; Ci, intercellular CO_2 concentration; PN, net photosynthetic rate; ROS, reactive oxygen species; SOD, superoxide dismutase; TBARS, thiobarbituric acid reactive substance.

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Introduction

Cadmium (Cd), a toxic element, is dispersed in the natural and agricultural environments mainly through human activities and has a long biological half-life (Wagner 1993). It is one of the non-essential heavy metals, toxic to flora and fauna, which is easily taken up by plant roots and translocated to the aerial plant parts (Zhao *et al*. 2003; Yang *et al*. 1998). Cadmium accumulation reduces photosynthesis, disturbs plant-water relations and the uptake and translocation of nutrients, and results in visible injury symptoms and/or plant death (Drazkiewicz *et al*. 2003; Hsu and Kao 2007; Anjum *et al*. 2008a). Cadmium is known to cause a burst of reactive oxygen species (ROS) in plant tissues, leading to the development of secondary oxidative stress (Qadir *et al*. 2004; Anjum *et al*. 2008b, c) that may damage photosynthetic pigments and other bio-molecules such as lipids, proteins and nucleic acids. It causes leakage of electrolytes via lipid peroxidation, a decrease in the AsA and GSH contents and alteration in activities of antioxidant enzymes such as superoxide dismutase (SOD, EC 1.15.1.1), catalase (CAT, EC 1.11.1.6), ascorbate peroxidase (APX, EC 1.11.1.11) and glutathione reductase (GR, EC 1.6.4.2) (Kuo and Kao 2004; Chaoui *et al*. 1997; Dixit *et al*. 2001; Chien *et al*. 2002; Mobin and Khan 2007; Anjum *et al*. 2008a, b).

Cadmium (Cd) toxicity symptoms i.e. necrosis appeared in older mustard leaves which led to defoliation (A & C). Potassium (K) application suppresses the symptoms of Cd toxicity in mustard and also inhibited early senescence and premature defoliation. Plants look healthy, green and turgid with K (B & D). In A & C, Cd_{100} + K_0 mg/kg soil; in B & D, Cd_{100} + K_{60} mg/kg soil.

Plant nutrients play pivotal roles in protecting plant growth from various environmental stresses including the heavy metal stress (Cakmak and Romheld 1997; Cakmak 2005; Anjana *et al*., 2006; Anjum *et al*. 2008c, d). The mineral-nutrient status of plants has a regulatory role with reference to plant resistance to stress factors (Marschner 1995). Potassium (K) is an important macronutrient and the most abundant

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cation in plant tissues (Zhao *et al*. 2003; Jordan-Meille and Pellerin 2007). Increasing evidence suggests that raising K-nutrition status of plants can dramatically inhibit the generation of ROS by reducing the activity of NAD(P)H oxidases and maintaining photosynthetic electron transport (Cakmak 2005). In addition, enhanced K nutritional status induces a number of beneficial physiological effects. These include stimulation of root growth, increases of leaf area, chlorophyll content and the net assimilation rate (NAR). Plant water content is also closely regulated by the effects of K on closure and opening of stomata which maintains photosynthetic CO_2 fixation. Additionally K reduces undesirable excess uptake of ions such as Na and Fe as well as benefiting N metabolism as for example by stabilizing nitrate reductase (NR) (Khan 1991; Marschner 1995; Elstner and Osswald 1994; Mengel and Kirkby 2001; Umar 2006). K nutrition has been shown to decrease the uptake of Cd as observed in wheat (*Triticum aestivum* L.) (Zhao *et al*. 2004). The present study investigates whether K nutrition may protect plants from Cd-toxicity-induced oxidative damage by reducing the Cd availability to the plant thereby depressing the contents of H_2O_2 and TBARS in the mustard leaves.

Materials and methods (see details in the online version):

- Plant material and treatments
- Growth parameters and net photosynthetic traits
- Estimation of glutathione, ascorbate and K contents
- Estimation of thiobarbituric acid reactive substances and H_2O_2
- Estimation of cadmium content
- Statistical analysis

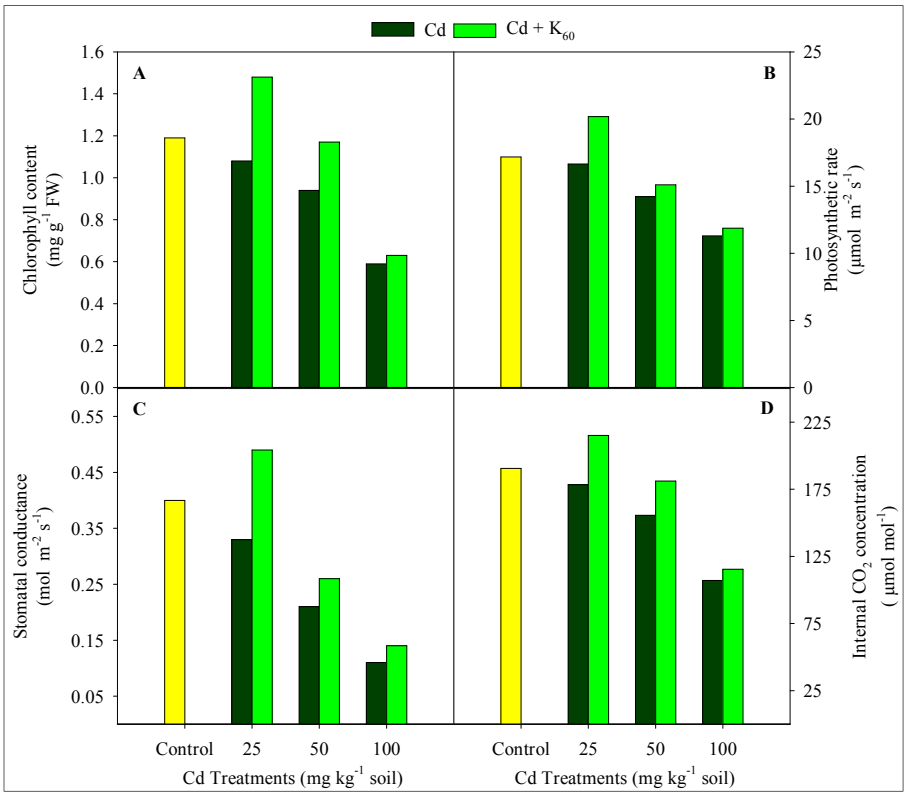

Fig. 1. Chlorophyll content, net photosynthetic rate, stomatal conductance and internal CO_2 concentration in *Brassica campestris* L. leaves as influenced by Cd stress and K nutrition at 30 d after sowing. Values are means ± SE (n = 3). Data followed by the same letter are not significantly different at P≤0.05 level as determined by the Duncan's multiple range test.

Results

Plant growth parameters

All applications of Cd reduced plant dry mass and leaf area significantly (P≤ 0.05) (Table 1). Application of 100 mg Cd/kg soil caused maximum reduction, compared with the control. Plant dry mass was reduced by 15.90%, 30.78% and 46.67%, and leaf area by 16.84%, 42.16% and 42.44% due to application of 25, 50 and 100 mg Cd/kg soil, respectively, in comparison with control. K application (60 mg/kg soil) alleviated Cd toxicity and lowered the reductions caused by Cd. K alleviation effect was more pronounced with the lowest level of Cd (25 mg/kg soil) followed by 50 and 100 mg Cd/kg soil. With the application of 60 mg K/kg soil, plant dry mass increased by 38.41%, 31.11% and 16.35%, compared to that at 25, 50 and 100 mg Cd/kg soil, respectively. K application increased the leaf area maximally (28.23%) at 25 mg and minimally (3.76%) at 100 mg/Cd kg soil.

Photosynthetic traits

Relative to the control, the chlorophyll content, net photosynthetic rate, stomatal conductance and concentration of internal CO_2 decreased significantly at P≤ 0.05 with increase in Cd level in the

Table 1: Plant dry mass and leaf area of Brassica campestris L. as influenced by Cd stress and K nutrition at 30 d after sowing. Values are means ± SE (n = 3). Data followed by the same letter are not significantly different at P≤0.05 level as determined by the Duncan's multiple range test.

Treatment	Plant dry mass	Leaf area
mg/kg soil	*g/plant*	*cm²*
Control	1.95b ± 0.09	67.05a ± 3.35
Cd_{25}	1.64c ± 0.08	55.76b ± 2.75
Cd_{25} + K_{60}	2.27a ± 0.12	71.50a ± 3.57
Cd50	1.35d ± 0.06	38.78c ± 1.93
Cd_{50} + K_{60}	1.77c ± 0.08	43.16c ± 2.15
Cd_{100}	1.04e ± 0.05	22.32d ± 1.16
Cd_{100} + K_{60}	1.21d ± 0.06	23.16d ± 1.15
LSD at 5%	0.147	4.477

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soil (Fig. 1a-d). K supplementation to Cd-exposed plants improved the content of chlorophyll by 37.04%, 24.47% and 6.78% at 25, 50 and 100 mg Cd/kg soil, respectively (Fig. 1a). The net photosynthetic rate increased with K application by 21.20%, 6.19% and 5.04%, with 25, 50 and 100 mg Cd/kg soil, respectively (Fig. 1b), whereas stomatal conductance decreased by 17.50%, 47.50% and 72.50% with 25, 50 and 100 mg Cd/kg soil (without K), respectively. Data in Fig. 1c reveal that the stomatal conductance increased due to supply of K (60 mg/kg soil) to Cd-exposed mustard plants, the extent of increase varying with treatments. Stomatal conductance increased by 48.48%, 23.81% and 27.27% when 25, 50 and 100 mg Cd/kg soil was added (Fig. 1c). Concentration of internal CO_2 decreased maximally with 100 mg Cd/kg soil (43.79%) followed by 50 mg Cd/kg soil (18.31%) and 25 mg Cd/kg soil (6.37%). K supplementation to Cd-exposed plants improved the CO_2 level by 20.59%, 16.39% and 7.73% at 25, 50 and 100 mg Cd/kg soil, respectively (Fig. 1d).

TBARS and H_2O_2 contents

To evaluate the Cd-induced oxidative damage to membranes, contents of TBARS and H_2O_2 were determined. The presence of Cd in the soil caused a significant ($P \leq 0.05$) increase in TBARS content in mustard leaves (Fig. 2a-c). With application of Cd alone (without K), it was 613% (highest) at 100 mg, followed by 244% at 50 mg and 74.84% (lowest) at 25 mg Cd/kg soil. The application of K to Cd-exposed plants decreased the content of TBARS maximally (49.26%) at 25 mg, followed by 18.91% at 50 mg and 8.45% at 100 mg Cd/kg soil (Fig. 2a). The H_2O_2 content in mustard leaves with supply of Cd alone was also maximum (269%) at 100 mg, followed 110% at 50 mg and 38.04% at 25 mg Cd/kg soil. Application of K to Cd-exposed plants decreased the H_2O_2 content maximally (43.17%) at 25 mg, followed by 22.53% at 50 mg and 9.76% at 100 mg Cd/kg soil (Fig. 2b).

Cadmium content

Addition of Cd to the soil caused an increase in Cd content of mustard leaves, as expected (Fig. 2c). Plants grown in the soil without Cd also contained some Cd, although the contents were very low, resulting probably from soil contamination caused by agricultural chemicals. Significant differences in leaf Cd content ($P \leq 0.05$) were found in plants grown with 100, 50 and 25 mg Cd/kg soil (without K). With application of K (60 mg/kg soil) to the soil, Cd content of leaf decreased significantly, indicating an antagonistic effect of K nutrition on Cd uptake by plants. The leaf Cd content decreased by 25.53%, 12.21% and 5.25% at 25, 50 and 100 mg Cd/kg soil, respectively (Fig. 2c).

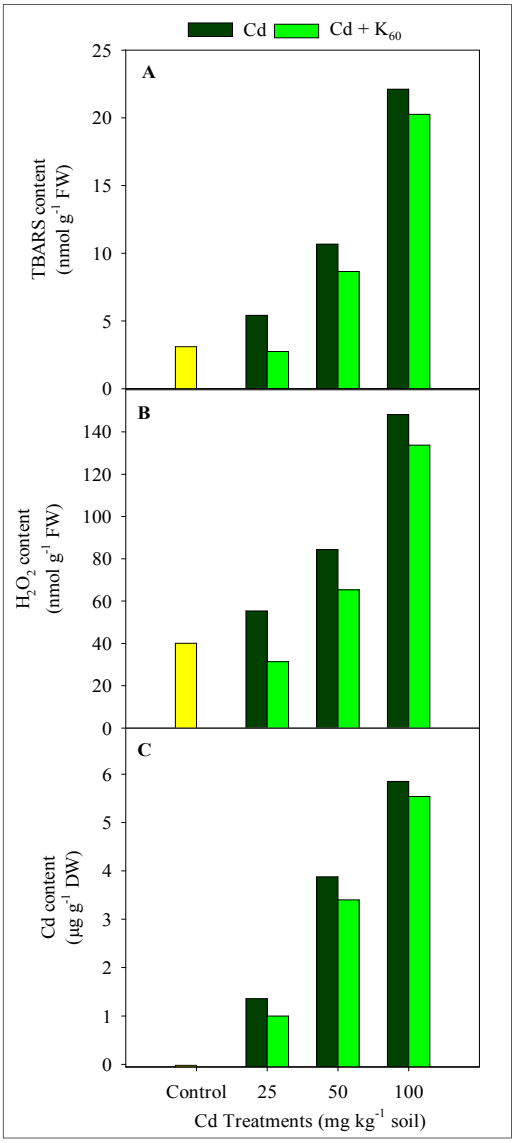

Fig. 2. TBARS, H_2O_2 and Cd contents in Brassica campestris L. leaves as influenced by Cd stress and K nutrition at 30 d after sowing. Values are means ± SE (n = 3). Data followed by the same letter are not significantly different at $P \leq 0.05$ level as determined by the Duncan's multiple range test.

Table 2. Contents of AsA, GSH and K in *Brassica campestris* L. leaves as influenced by Cd stress and K nutrition at 30 d after sowing. Values are means ± SE (n = 3). Data followed by the same letter are not significantly different at $P \leq 0.05$ level as determined by the Duncan's multiple range test.

Treatment	AsA content	GSH content	K content
mg/kg soil	*nmol g/FW*	*nmol g/FW*	*% DW*
Control	190b ± 9.50	328a ± 16.40	3.62 ± 0.181
Cd_{25}	162c ± 8.10	259b ± 12.95	3.41 ± 0.171
$Cd_{25} + K_{60}$	216a ± 10.80	325a ± 16.25	3.59 ± 0.180
Cd_{50}	118e ± 5.90	193d ± 9.65	3.10 ± 0.155
$Cd_{50} + K_{60}$	147d ± 7.35	223c ± 11.15	3.22 ± 0.161
Cd_{100}	71f ±3.55	104e ± 5.20	2.83 ± 0.141
$Cd_{100} + K_{60}$	77f ± 3.85	113e ± 5.65	2.89 ± 0.145
LSD at 5%	13.44	21.38	0.291

Contents of ascorbate, glutathione and potassium

The content of ascorbate decreased by 14.74%, 37.89% and 63.63% at 25, 50 and 100 mg Cd/kg soil (without K), respectively. As shown in Table 2, the ascorbate content increased with addition of K (60 mg/kg soil) to the Cd-exposed plants, the increase being treatment dependent. The content of leaf ascorbate increased with K application by 33.33%, 24.58% and 8.45% on application of 25, 50 and 100 mg Cd/kg soil, respectively (Table 2). The influence of K and Cd treatments on glutathione content of leaves is shown in

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Fig. 1c. It decreased significantly ($P \leq 0.05$) at 100 mg Cd (68.29%), 50 mg Cd (41.16%) and 25 mg Cd/kg soil (21.04%). Application of K (60 mg/kg soil) reduced the decline in glutathione content substantially; the effect being more pronounced with the lowest Cd treatment (25 mg Cd/kg soil). Thus, maximum content of glutathione occurred with 25 mg Cd (25.48%), followed by the value of 15.54% with 50 mg Cd and 8.65% with 100 mg Cd/kg soil when supplemented with 60 mg K/kg soil (Table 2). The content of potassium decreased by 5.80%, 14.36% and 21.82% at 25, 50 and 100 mg Cd/kg soil (without K), respectively. As shown in Table 2, potassium content increased with addition of K (60 mg/kg soil) to the Cd-exposed plants, the increase being treatment dependent. The content of leaf K increased with K application by 5.28%, 3.87% and 2.12% on application of 25, 50 and 100 mg Cd/kg soil, respectively (Table 2).

Discussion

Cadmium treatment causes oxidative stress in plants through increase in the production of H_2O_2 (Kuo and Kao 2004; Schutzendubel *et al*. 2001; Olmos *et al*. 2003) and induction of lipid peroxidation (Chien et al. 2002; Gallego *et al*. 1996a, b; Kuo and Kao 2004; Anjum *et al*. 2008a; Singh *et al*. 2008). Our results have shown not only that Cd increased the content of H_2O_2 and TBARS (Fig. 2a-b.) but also that it lowered the GSH and AsA contents (Table 2). Pigment loss (Fig. 1) and lipid peroxidation (Fig. 2a-b) were also prominent in Cd-treated mustard leaves. All these observations suggest that the Cd-induced toxicity in mustard leaves is mediated through oxidative stress. Glutathione (GSH) functions as a direct antioxidant of ROS and is involved in the generation of AsA, which is utilized as a substrate for APX (Noctor and Foyer 1998). Our results indicate that the decrease in GSH content is one of the earliest steps in the Cd-induced oxidative stress in mustard leaves; it was maximum at the highest Cd-level (Table 2). It may be supposed that the decrease in GSH may favour the accumulation of ROS in the form of H_2O_2 and TBARS in Cd-treated mustard leaves. Previous studies by Qadir *et al*. (2004) and Anjum *et al*. (2008a) and a review by Schutzendubel and Polle (2002) suggest that the depletion of GSH is apparently a critical step in Cd toxicity. Cd induced a significant accumulation of H_2O_2 in mustard leaves (Fig. 2). H_2O_2 accumulation has also been observed in Cd-treated pine and pea roots, pea leaves, and tobacco cells (Olmos *et al*. 2003; Romero-Puertas *et al*. 2003; 2004; Schutzendubel *et al*. 2001; Hsu and Kao 2007). There are reports showing that NADPH oxidase is possibly involved in Cd-induced H_2O_2 production in pea leaves and tobacco cells (Olmos *et al*. 2003; Romero-Puertas *et al*. 2004).

That the Cd-induced oxidative damage in mustard leaves is reduced by K nutrition can be inferred from observations that K supplementation prevented Cd-induced depressions in PN, gs, Ci and in the contents of chlorophyll (Fig. 1a-d), AsA and GSH (Table 2) and increases in the contents of H_2O_2 and TBARS (Fig. 2a-b). An adequate K supply plays a central role in the maintenance of PN and the related processes (Bendnarz and Oosterhuis 1999; Umar 2006). As evidenced in the present study, Cd-induced decrease in leaf K content may also contribute Cd-induced changes in mustard plants (Table 2) which accord with the findings of Anjum *et al*. (2008b). The decrease in PN and gs appears to be related to K-deficiency, which is in agreement with the findings of Cakmak and Engels (1999) and Zhao *et al*. (2001). ROS are highly toxic causing degradation of Chl. It is generally accepted that K supply strongly controls the process of photosynthetic CO_2 fixation as well as utilization of photoassimilates (Cakmak 1994). The role of GSH and AsA in scavenging processes against heavy metals and other stress conditions has been extensively investigated (Gallego *et al*. 1996a, b; Noctor and Foyer 1998). AsA contributes directly to ROS scavenging and by means of ascorbate peroxidase (APX). The decrease in AsA and GSH contents in mustard leaves treated with Cd suggests that AsA and GSH contents may be regulated by the synthesis and oxidation. GSH is the precursor of phytochelatins, cysteine-rich peptides synthesized via phytochelatin synthase (Cobbett and Goldsbrough 2002). GSH is severely depleted in response to Cd due to its increased consumption in phytochelatin production (Schutzendubel and Polle 2002).

The present results indicate that K nutrition decreased the Cd-induced decline in the AsA and GSH contents (Table 2). The capacity of K to scavenge H_2O_2 in mustard leaves was at a maximum with 60 mg K/kg soil plus Cd (Fig. 2b). In considering a possible mechanism for the depression of Cd-induced oxidative damage by K nutrition, it may be supposed that K might inhibit Cd uptake from the medium i.e. an antagonistic effect between Cd and K uptake (Zhao *et al*. 2004). This is supported by our findings which have shown that the Cd content in mustard leaves treated with K_{60} + Cd levels was lower than in those treated with Cd alone (Fig. 2c). Our findings suggest that increase in the K nutrient status of plants may prevent, though slightly (27%), the uptake of Cd. K_{60} + Cd levels treatment inhibited, almost completely, the Cd-induced generation of H_2O_2 and lipid peroxidation (TBARS) in mustard leaves (Fig. 2a-b). It may thus be concluded that K nutrition by depressing Cd uptake is able to protect plants from Cd-induced oxidative burst thereby avoiding H_2O_2 and TBARS generation.

References (see details in the online version).

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