Effect of gamma irradiation or potassium on oxidative stress and antioxidant system of cadmium stressed *Brassica rapa* (L.) plant

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Abstract

The effect of cadmium chloride concentrations (25.50, 75, and 100 mg/kg soil), seeds pre-irradiated by low doses of gamma rays (15, 30, 45, and 60 Gy), potassium chloride (60 mg/kg soil) and the combination of cadmium + gamma and cadmium + potassium on *Brassica rapa* germination, oxidative stress and antioxidant system were investigated under laboratory and greenhouse conditions. Germination percentage decreased progressively by increasing cadmium chloride concentrations. Gamma irradiation and potassium treatment enhanced the germination and reduced cadmium toxicity when combined with it. All of the treatments relatively caused overproduction of H_2O_2. Peroxidase and catalase activities were increased by the cadmium concentrations while ascorbic acid was decreased.

Keywords: Brassica rapa; cadmium chloride; gamma doses; potassium; germination; H_2O_2; catalase; peroxides; ascorbic acid

Abd El-Hameed Abo Hamad, Sh., M. Abu Elyazeed Abd Elhaak and **K. Mahfouz Ghareeb Saad-Allah.** 2012. 'Effect of gamma irradiation or potassium on oxidative stress and antioxidant system of cadmium stressed *Brassica rapa* (L.) plant'. *Iranian Journal of Plant Physiology* 2 (3), 435 – 443.

Introduction

The accumulation of cadmium (Cd) in biotic systems as a consequence of human activities is becoming a major environmental problem. The application of sewage sludge, city waste, and Cd-containing fertilizers causes the increase of Cd content in soils (Williams and David, 1973). Plants are an important link in pathway by which excessive amounts of heavy metals are channeled into the food chain and biological cycles (Das et al., 1997).

Cadmium is rapidly taken up by plant roots and can be loaded into the xylem for its transport into leaves. Most plants are sensitive to low Cd concentrations which inhibit their growth as a consequence of alterations in the photosynthesis rate and the uptake and distribution of macronutrients and micronutrients (Sandalio et al., 2001; Benavides et al., 2005). 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., 2008 b, c) that may damage photosynthetic pigments and other bio-molecules such as lipids, proteins and nucleic acids. It causes leakage of electrolytes via membrane lipid peroxidation, a decrease in the ascorbic acid and glutathione contents and alteration in activities of antioxidant enzymes such as superoxide dismutase, catalase,

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Received: April, 2012
Accepted: May, 2012

ascorbate peroxidase, and glutathione reductase (Chaoui et al., 1997; Kuo and Kao, 2004; Anjum et al., 2008 a, b).

Potassium is an important and the most abundant macronutrient cation in plant tissues (Zhao et al., 2003; Jordan-Meille and Pellerin, 2008). 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). K^+ nutrition has been shown to decrease the uptake of Cd^{2+} as observed in wheat (Zhao et al., 2003).

Seed irradiation is one of the most effective methods to improve plant production, yield components and chemical composition (Selenia and Stepanenko, 1979). Many studies report that low doses of gamma rays stimulated seed germination, plant growth and oil production (Zheljazkov et al., 1996; Moussa, 2006; Melki and Sallami, 2008).

The aim of this study was to use gamma irradiation at low doses and potassium to ameliorate the adverse effect of Cd stress on germination percent and antioxidative potential of *Brassica rapa* (L.) plant.

Materials and Methods

Seeds of turnip *(Brassica rapa* L.) were obtained from the Crop Institute Agricultural Research Center, Giza, Egypt. Turnip seeds were divided into two sets, one of which was irradiated with four gamma ray doses (15, 30, 45 and 60 Gy) emitted from cobalt 60 source. Irradiation process was performed at the National Center for Research and Radiation Technology, Nasr City, Cairo, Egypt. Both irradiated and not-irradiated seeds of turnip were surface sterilized with 5% Clorox for 8 minutes and rinsed several times in distilled water. The irradiated seeds were irrigated with different concentrations of Cd Cl2 (0, 25, 50, 75 and 100 mg/kg soil). The seeds not irradiated received the same concentrations of $CdCl_2$ (0, 25, 50, 75 and 100 mg/kg soil) alone or combined with 60 mg KCl/kg soil. These seeds were allowed to germinate in 12.5 cm diameter and 3.5 cm height plastic dishes, containing 100 g clay-sandy soil (2:1 w/w), 10 seeds in each dish, and each treatment was replicated 4 times. The number of germinated seeds was recorded after 10 days and the germination percentage was calculated. Fresh samples from produced seedlings were used for the determination of H_2O_2, antioxidant enzymes and ascorbic acid.

Also the sterilized irradiated and not irradiated seeds were allowed to germinate under greenhouse conditions in plastic pots of 45 cm diameter and 40 cm depth, each pot was filled with 25 kg clay-sandy soil (2:1 w/w) and received the same Cd and K irrigation treatments. Each treatment was represented by 4 pots in which 20 seeds were sown. After 30 days of growth (early vegetative stage), fresh samples were taken for the determination of H_2O_2, antioxidant enzymes and ascorbic acid.

Hydrogen peroxide (H_2O_2) content

Content of H_2O_2 was determined in leaf tissue (100 mg) using the method given by Velikova et al. (2000). The amount of H_2O_2 was calculated using the extinction coefficient (0.28 µM−1 cm^{-1}) and expressed as n mol g^{-1} f. wt.

Assaying of peroxidase (POD) and catalase (CAT)

The activity of peroxidase [EC 1.11.1.7] and catalase [EC1.11.1.6] were assayed according to Kato and Shimizu (1987). Enzymes activity was expressed in units of µM of the substrate converted per min. per gram fresh weight.

Determination of ascorbic acid (AA)

Ascorbic acid was estimated in leaf tissue according to Oser (1979) and ascorbic acid content was measured by spectrophotometer (Model 4049 LKB Novasped). Ascorbic acid was calculated as mg/g d. wt using a prepared calibration curve by ascorbic acid.

Statistical analysis

The obtained results were statistically analyzed using the two ways analysis of variance (ANOVA) to determine the degree of significance (p) for the variations between the treatments and

F test was calculated for treatments and their interactions. All of the statistical methods were according to the method described by Bishop (1983).

Results

Seed germination percentage

Increasing the concentration of $CdCl_2$ significantly and progressively decreased the seed germination of *Brassica rapa* as compared to the control (Fig. I). The germination percentage decreased to less than 50% with the highest Cd stress treatment (100 mg/kg soil). Gamma irradiation doses and KCl as single treatments relatively enhanced the seed germination above that of the control. The most noticeable enhancement was by KCl treatment. The interaction of effects of $CdCl_2$ and gamma doses or KCl, to some extent alleviated the inhibitory effect of the different $CdCl_2$ treatments on seed germination. The marked effect was by 45 Gy irradiation under 25, 50, and 75 Cd treatments and by KCl under 100 Cd treatment.

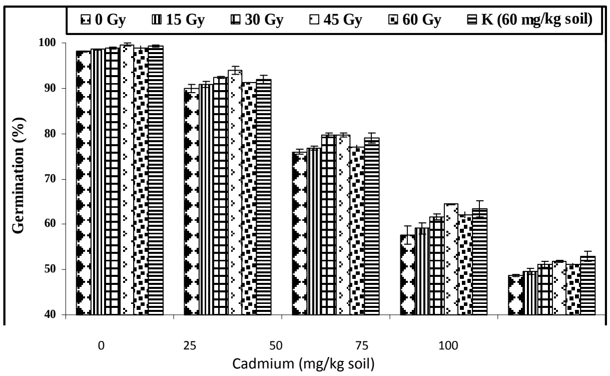

Fig. I. The effect of gamma irradiation or potassium application on the seed germination (%) of cadmium stressed *Brassica rapa* (L.) plant.

Oxidative stress (H_2O_2 production by the plant shoot)

Cadmium treatment had a considerable effect on H_2O_2 accumulation in the plant tissues at both seedling and early vegetative growth stages (Fig. II). Cadmium caused a concentration–dependant increase in the level of H_2O_2 in plant shoot system. Also using gamma irradiation

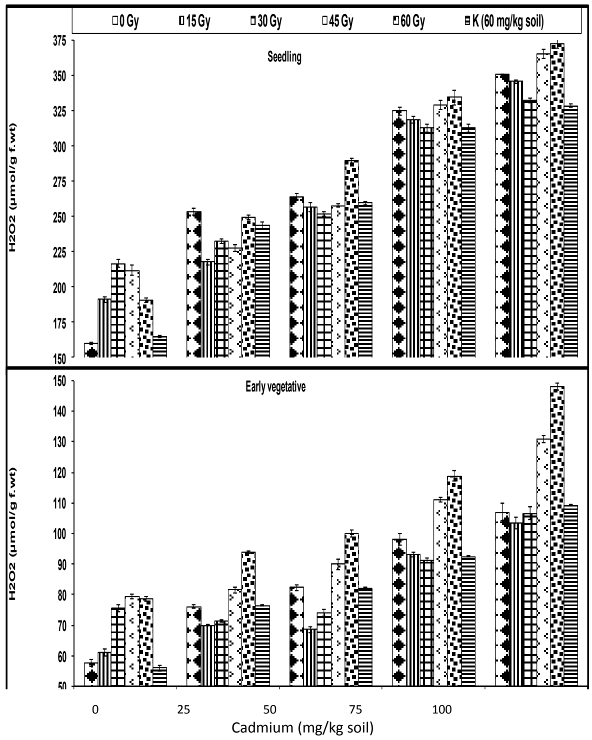

Fig. II. The effect of gamma irradiation or potassium application on the leaves H2O2 content (µmol/g f. wt) of cadmium stressed Brassica rapa (L.) plant at two growth stages.

showed a considerable accumulation of H_2O_2. The applied KCl concentration caused a minute increase in H_2O_2 at the seedling stage but it decreased H_2O_2 at the early vegetative stage. Interacting of gamma doses with $CdCl_2$ in most cases, specifically with the low irradiation doses, effectively lowered the accumulation of H_2O_2 compared with $CdCl_2$ single treatment during both seedling and vegetative stages. Interacting of KCl with $CdCl_2$ in most cases decreased the accumulation of H_2O_2 in the plant shoot compared with $CdCl_2$ single treatments.

Peroxidase (POD) and Catalase (CAT) activities

The activity of two antioxidant enzymes, peroxidase and catalase, in the plant shoot at the seeding and early vegetative stages was affected by the study treatments (Figs. III and IV). Cadmium chloride application had resulted in increasing the activity of POD and CAT linearly with the concentration. Gamma irradiation doses

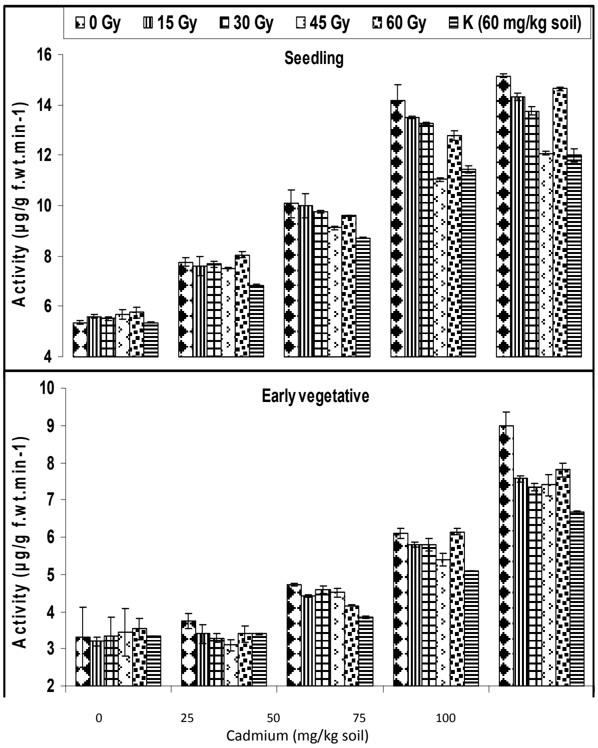

Fig. III. The effect of gamma irradiation or potassium application on the leaves peroxidase enzyme activity (µg/g f.wt.min-1) of cadmium stressed Brassica rapa (L.) plant at two growth stages.

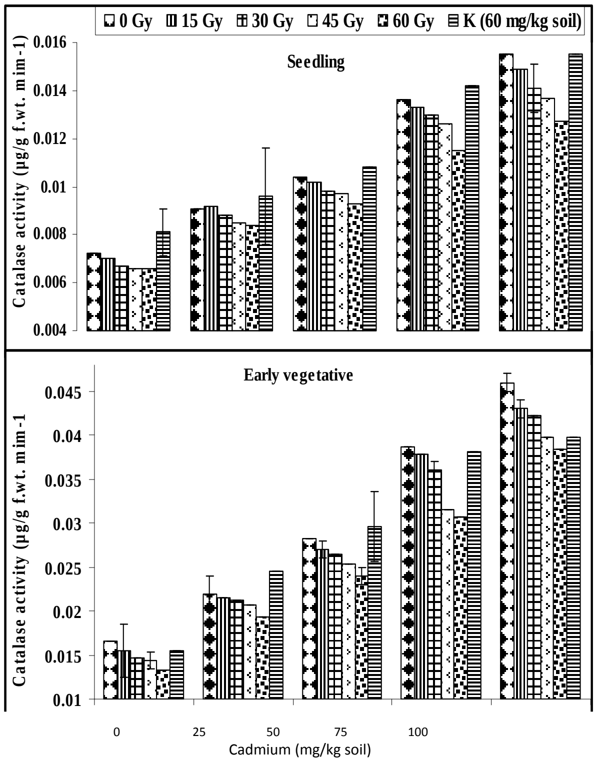

Fig. IV. The effect of gamma irradiation or potassium application on the leaves catalase enzyme activity (µg/g f.wt.min-1) of cadmium stressed Brassica rapa (L.) plant at two growth stages.

slightly raised the activity of POD, but decreased the activity of CAT. KCl had less or no effect on POD activity, but it stimulated CAT at the seedling stage and diminished it at the early vegetative stage. Combination of $CdCl_2$ treatments with gamma doses or with KCl treatment had lowered the activity of both enzymes in most cases compared with $CdCl_2$ single treatments, but their activity remained higher than that of the controls.

Non enzymatic antioxidants (ascorbic acid content)

Fig. V shows that ascorbic acid (AA) content in *B. rapa* leaves was greater at seedling than at early vegetative stage. At both stages AA decreased significantly by the study treatments, i.e., $CdCl_2$ concentrations, gamma doses, and KCL treatment compared with the control. The fractional combination between $CdCl_2$ and gamma doses increased AA content relative to the content of plants receiving $CdCl_2$ alone. Combination of KCl with 25 and 50 mg $CdCl_2$ lowered AA content at both seedling and early vegetative stages, but with 75 and 100 mg $CdCl_2$ increased AA in comparison with $CdCl_2$ treatments.

Discussion

Increasing the concentration of $CdCl_2$ during the germination stage had suppressed the seed germination of *B. rapa* as found also by Asgharipour et al. (2011) and Heidari and Sarani (2011). Reduction in seed germination can be attributed to alterations of properties of cell membrane as Barcelo and Kahle (1992) indicated that Cd affected water relations not only by decreasing water absorption and transport, but also by lowering water stress tolerance. Study results reflected that all the used gamma irradiation doses enhanced the seed germination percentage, to some extent, higher than that of the control value. These results are well-matched with those obtained by many authors, e.g., Sheppard and Evenden (1986), Amjad and Anjum (2002), and Melki and Marouani (2010).

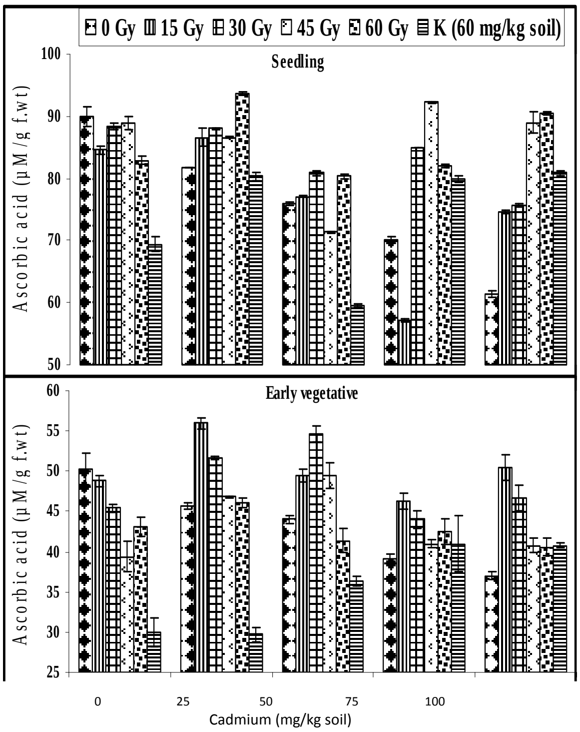

Fig. V. The effect of gamma irradiation or potassium application on the leaves ascorbic acid content (μmol/g f. wt) of cadmium stressed *Brassica rapa* (L.) plant at two growth stages.

Enhancement of germination percentage as a result of gamma irradiation was due to increasing the absorption of water and mineral salts that are needed for plant survival as was reported by Brown et al., (1987). Potassium as a single treatment had resulted in stimulation of the seed germination and when it was combined with cadmium treatments it had resulted in improving the germination percentage over that of cadmium single treatments. Potassium is an integral part of the membrane functions (Hopkins, 1995) and its ion is a metal activator for pyruvate kinase and other essential enzymes, regulating respiration and carbohydrate metabolism. Moreover, potassium plays a role in *de-novo* synthesis of specific enzyme proteins (Bewley and Black, 1985). Potassium is known to be the activator of many enzymes involved in photosynthesis, starch and protein synthesis and respiration (Bhandal and Malik, 1988) and activates α- amylase in the seeds (Shad et al., 2004).

Cadmium exposure had a considerable effect on H_2O_2 production in *B. rapa* leaves. The increase in H_2O_2 content after Cd exposure was reported by (Stroinski and Zielezinska, 1997). Loss in antioxidative defenses was sufficient to explain the observed H_2O_2 accumulation (Polle, 2001; Schutzendubel and Polle, 2002). Produced H_2O_2 is involved in a variety of reactions against abiotic stresses and signaling cascades for all aspects of plant growth and activities (Mazid et al., 2011). Gamma irradiation for *B. rapa* seeds resulted in accumulation of H_2O_2 in the plant leaves. In this concern, Xienia et al. (2000) reported that gamma irradiation induced oxidative stress with overproduction of reactive oxygen species (ROS) such as superoxide radicals, hydroxyl radicals and hydrogen peroxides (H_2O_2).

The increased H_2O_2 in the irradiated plants might be due to the inhibition of antioxidant enzyme activities for H_2O_2 detoxification or to the enhanced H_2O_2 production through the enzyme – mediated reactions (Dwyer et al., 1996; Hurkman and Tanaka, 1996; Wi et al., 2006). Potassium application seemingly didn't affect H_2O_2 accumulation in *B. rapa* leaves. The same result was obtained in rice seedlings by Mehraban et al. (2008). Similarly, potassium deprivation had no significant effect on H_2O_2 accumulation in *Zea mays* (Tewari et al., 2004), *Morus* alba (Tewari et al., 2007) and barley (Hafsi et al., 2010).

Cadmium chloride treatments effectively increased the activity of the peroxidase enzyme (POD) in *B. rapa* leaves. In this respect, Dinakar et al. (2009), Martinez et al. (2010), and Semane et al. (2010) reported increase in POD activities in *Spartina densiflora, Arachis hypogaea* and arabidopsis even in low concentrations. The increased activities of POD by cadmium stress suggests that the plant depends on this antioxidative enzyme for elimination of H_2O_2 under Cd stress (Mandakini et al., 2005). Van Assche et al. (1988) attributed enzyme induction to the increase of *de-novo* protein synthesis, or to the activation of enzymes already present. Antioxidants and POD are involved in the compensatory mechanisms of inhibition of free radicals formed upon irradiation (Rogozhin et al., 2000). This may explain the slight increase in peroxidase activity as a result of gamma irradiation. Potassium chloride treatment had no effect on POD activity. This may suggest that potassium treatment did not affect H_2O_2 exertion, so POD activity was not affected.

Cadmium chloride treatments significantly increased catalase (CAT) enzyme activity in *B. rapa*. CAT activity increased as heavy metals concentration increased and decreased at higher concentration for long – term exposure (Arleta et al., 2001; Salama et al., 2009; Liu et al., 2011). The increase in CAT activity after Cd treatments may be due to the scavenging role of CAT to H_2O_2, which could be quenched by the induction of specific enzymes like CAT (Elstner et al., 1988). The reduction of CAT activity in highest concentration of Cd may be due to the long-term stress exposure (Chaoui et al., 1997). Gamma irradiation significantly retarded the activity of CAT in *B. rapa* shoots at all the investigated gamma doses. Inhibition of CAT activity was also reported under irradiation stress (Liang et al., 2000; Al-Rumaih and Al-Rumaih, 2008).

Cadmium chloride significantly decreased the ascorbic acid (AA) content. The decrease in AA concentration under Cd stress has been observed in the leaves of *Pisum sativum* (Romero-Puertas et al., 2007) and *Brassica campestris* (Anjum et al., 2008). The decrease in AA content in response to Cd suggests that AA content may be regulated by the synthesis and oxidation (Umar et al., 2008). Trautner and Somogyi (1964) indicated that there is a close relationship between the biosynthesis of ascorbic acid and carbohydrate metabolism. The decrease in ascorbic acid content by gamma irradiation may be due to increased metabolism of ascorbic acid and biosynthesis of carbohydrates or its oxidation to dehydroascorbic acid (Trautner and Somogyo, 1964). Similarly, potassium treatment resulted in decreasing AA content in *B. rapa*. Reder et al. (1943) found that potassium fertilization caused a decrease in ascorbic acid content of field- grown turnip greens.

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