Asian Journal of Chemistry Vol. 20, No. 2 (2008), 1099-1108

Cadmium Stress Tolerance Through Potassium Nutrition in Soybean

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Cadmium is known to hamper plant growth through disruption in structure, nutrition, water relations and metabolism in addition to oxidative stress induction. While, potassium has crucial role in the energy status of plants, translocation/storage of assimilates and tissue water relations. Further, it activates over 60 enzyme systems, aids in photosynthesis, regulates stomatal opening and nutrients translocation and enhances synthesis of starch and proteins. These physiological and biochemical aspects of potassium could also be having a remedial role in counteracting the detrimental effects of cadmium. However, such relationship has rarely been studied in detail. Therefore, the study in view was envisaged for exploiting potassium to counter-balance the cadmium toxicity in soybean. Emphasis was laid on growth, physiology and biochemistry of two soybean genotypes differing in cadmium response. Following treatments were employed under factorial design in hydroponics: Check (no cadmium or potassium applied); cadmium only; potassium only; cadmium + potassium. Cadmium reduced growth, chlorophyll content and fluorescence, photosynthesis and stomatal conductance markedly in both genotypes. Enhanced malondialdehyde (MDA) content, super oxide dismutase (SOD) and peroxidase (POD) activities were also detected in plants exposed to cadmium than those with potassium alone or combined with cadmium. The activities of SOD and POD were found higher in cv. Liao-1 than in cv. Zhechun3. Apparently, potassium and cadmium behaved antagonistically; suggesting that potassium could be a candidate for cadmium detoxification in crops cultivated under polluted environments.

Key Words: Antioxidants, Biochemical aspects, Physiology, Growth, Pollution.

INTRODUCTION

Heavy metals are important environmental pollutants and their toxicity is a problem of increasing significance for ecological, evolutionary, nutritional and environmental reasons[1]. The current worldwide mine production of Cu, Cd, Pb and Hg is considerable[2]. Yalcin *et al.*[3] conducted

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a detailed study on heavy metals status in soils alongside the highways in Anatolia, Turkey and found that heavy metal accumulation is closely associated with traffic intensity. The sensitivity of plants to heavy metals depends on an interrelated network of physiological and molecular mechanisms[4]. Cadmium is a non-essential element and highly toxic metal pollutant of soils, that negatively affects plant growth and development. It inhibits plant growth by disturbing photosynthetic activity and uptake and translocation of mineral nutrients in plants[5,6].

The degree to which plants are able to take up cadmium depends on its concentration in the soil and its bioavailability, modulated by the presence of organic matter, pH, redox potential, temperature and concentrations of other elements[1]. The uptake of cadmium ions seems to be in competition for the same transmembrane carrier with nutrients[7,8], such as K, Ca, Mg, Fe, Mn, Cu, Zn, Ni. In general, cadmium has been shown to interfere with the uptake, transport and use of several elements (Ca, Mg, P and K) and water by plants[4,9,10].

Potassium is not an integral part of any major plant component, but it does play a key role in a vast array of physiological processes vital to plant growth, from protein synthesis to maintenance of plant water balance[11]. It also helps in the transport of water and nutrients throughout the plant in the xylem. When potassium supply is reduced, translocation of nitrates, phosphates, calcium, magnesium and amino acids is depressed. Potassium predominantly exists as a free or absorptive bound cation and can therefore be displaced very easily on the cellular level as well as in the whole plant[12].

Many factors affect cadmium transport and accumulation in plant-soil systems, such as soil pH, soil redox potential, cation exchange capacity, plant species and fertilizer application, *etc.*[13]. As an important macronutrient and one of the dominating factors of soil cation exchange capacity (CEC), potassium may possibly influence cadmium transportation and accumulation in soil-plant systems[14]. Few studies have yet been done on the relationship between potassium fertilizer and cadmium uptake by plants. However, it was pointed out by Grant *et al.*[15] that the effect of potassium fertilizers may be due to the accompanying anions of the salt. Zhao *et al.*[14] observed that an increase in the application of potassium fertilizers increased cadmium concentrations in both shoots and roots of two wheat cultivars irrespective of the forms of fertilizers, which suggested that potassium itself may increase plant uptake of cadmium. Contrastingly, Noraho and Gaur[16] reported that potassium caused non-competitive inhibition of intracellular cadmium uptake of *L. polyrhiza* plants and expected high levels of cations in the external environment to lower the cadmium accumulation efficiency. However, the mechanisms of K-Cd interactions in plant uptake of cadmium need to be elucidated further.

In order to cope with highly toxic metals, or to maintain the level of essential metals within physiological ranges, plants have evolved complex mechanisms that serve to control the uptake, accumulation and detoxification of metals[1]. The exact ability of plants to accumulate cadmium varies significantly both between plants and among genotypes within a given species[4]. Differences of 25-50 % between cultivars have been reported. Variation in cadmium accumulation between different cultivars of spring bread wheat and durum wheat has been observed by Stolt and Hultin[17]. Legume crops are less tolerant to cadmium toxicity than cereals and grasses and frequently encounter strong inhibition of biomass production in the less than micromolar range of cadmium[18]. Considerable variability among 99 pea genotypes, in tolerance to cadmium and uptake of different heavy metals has been found[19].

The aim of the current study was to find the relationships between physiological and biochemical reactions to cadmium-stress in soybean genotypes at different potassium levels, in order to contribute to an understanding of the mechanisms of K-Cd interactions in plant growth and uptake of cadmium.

EXPERIMENTAL

Culture and treatments: This greenhouse experiment was conducted in hydroponic culture during 2004-05 at Zhejiang University, Huajiachi Campus, Hangzhou, China. Two soybean genotypes differing in aluminum tolerance were used, namely, Liao-1, relatively tolerant and Zhechun-3, relatively sensitive. Soybean seeds were surface sterilized in 0.2 % (w/v) $Na(OCl)_2$ for 20 min, rinsed 8 times with distilled water and germinated in moist quartz sand in a greenhouse. After the first complex leaf appeared (10 d after sowing), seedlings were selected for uniformity and transplanted on to a 4-L container, which was covered with a foamed plastic plate with evenly spaced holes and placed in a greenhouse. The composition of the basic nutrient solution was (mg L^{-1}): NH_4NO_3 330; $KH_2PO_4{\cdot}2H_2O$ 34, KNO_3 380 (in K treatments only); $MgSO_4$ 74, $CaCl_2$ 88, $FeSO_4{\cdot}7H_2O$ 13.9, $MnSO_4{\cdot}4H_2O$ 22.3, $ZnSO_4{\cdot}7H_2O$ 8.6, $CuSO_2{\cdot}5H_2O$ 0.025, H_3BO_3 6.2; $Na_2MoO_4{\cdot}2H_2O$ 0.25; KI 0.83; $CoCl_2{\cdot}6H_2O$ 0.025; $Na_2EDTA{\cdot}2H_2O$ 37.2; NH_4NO_3 380 (in no K treatments only). One week after transplanting to the basic culture solution. Cadmium as $CdCl_2{\cdot}5H_2O$ (@ 1.0 μmol L^{-1}) was added to the corresponding treatment containers and the solution pH was adjusted at 5.5 with HCl. The following four treatments of cadmium and potassium were employed: T1, Check (no Cd or K applied); T2, cadmium only; T3, K only; T4, Cd + K. The experiment was laid out according to two factors factorial completely randomized design (CRD) with three replications. The pH of solution in each container was adjusted every other day with HCl or NaOH as required. The nutrient solution in the growth containers was continuously aerated and renewed every 5 d.

Sampling and measurements: After 25 d treatments, the second fully expanded leaves were selected for measuring chlorophyll content/SPAD (Soil-Plant Analyses Development) value with a chlorophyll meter (Minolta SPAD-502, Japan). The rate of steady-state photosynthesis (Pn) and stomatal conductance (g_s) were measured by Infrared Analyzer (LI-6400 system, Li-COR Company, USA). The chlorophyll fluorescence yield (Y) was measured by means of PAM-fluorimetry (pulse amplitude modulation fluorimeter). To obtain Y, the following fluorescence parameters were recorded: F-Present fluorescence of plant sample before saturation pulse; F'm - maximal fluorescence of the illuminated plant sample; Y = (F'm - F)/F'm-actual yield of photochemical energy conversion[20].

After 28 d treatment, the upper second fully expanded leaves were sampled for the analysis of relevant enzymes. The activities of super oxide dismutase (SOD) and peroxidase (POD) and concentration of malondialdehyde (MDA) were simultaneously determined according to the methods given by Zhang[21]. The shoot and root length were also measured and plants were harvested and washed thoroughly with distilled water, separated into roots and shoots (stems and leaves), dried at 80 ºC and weighed.

Statistical analysis: Statistical analyses were carried out by one-way Anova using Student's t-test to compare the significance of difference between the treatments[22].

RESULTS AND DISCUSSION

Biometric traits: Soybean growth data in terms of length and dry biomass of roots and shoots are given in Table-1. Cadmium reduced these growth parameters of soybean plants as compared to control (no cadmium or potassium). Potassium treatment caused an increase over control; also it improved the growth significantly when applied to cadmium treated plants as against cadmium applied alone. The results showed that the negative influence of cadmium toxicity on soybean growth could be remedified through potassium nutrition. The significance of potassium in the development of resistance against environmental stresses has well been understood, so it also appeared to fight against cadmium stress in the current study. As regards the genotypic difference for cadmium tolerance, Zhechun-3 performed better than Liao-1 for all the plant growth characteristics. The interactions between treatments and genotypes were statistically non significant for shoot length; while there were significant differences for root length and dry biomass of roots and shoots. The results clearly show that potassium had a positive role in cadmium tolerance and there was also a genotypic difference in soybean cultivars for resistance to cadmium stress on plant growth.

TABLE-1
EFFECT OF CADMIUM AND POTASSIUM ON GROWTH TRAITS OF SOYBEAN PLANTS

Treatments (T)	Length (cm)		Biomass (g plant^{-1})	
	Root	Shoot	Root	Shoot
T1 No Cd or K	15.55 b	30.90 b	2.31 b	3.55 b
T2 Cd only	12.55 c	25.95 c	2.02 c	3.13 c
T3 K only	17.50 a	43.40 a	2.80 a	4.13 a
T4 Cd + K	15.60 b	40.65 a	2.55 a	3.92 a
Genotypes (G)				
G1 Liao-1	16.70 A	29.90 B	2.36 A	3.56 B
G2 Zhechun-3	13.40 B	43.55 A	2.51 A	3.85 A
T × G	*	ns	*	**

*The different letters after data within a column represent significant difference at 95 % probability.

Physiological parameters: Cadmium also exerted a negative impact on chlorophyll contents (expressed as SPAD value), net photosynthesis (A), stomatal conductance (g_s) and chlorophyll fluorescence yield (Table-2); with cadmium having the lowest values among all treatments in this study. Potassium applied either alone or with the combination of cadmium gave higher values for these plant characteristics as compared in cadmium alone treatment or in check (no cadmium or potassium). This was obviously due to the recognized role of potassium in the photosynthesis. As the cadmium toxicity damages chlorophyll and reduces photosynthesis in plants, so these effects can be countered by the application of higher dose

TABLE-2
EFFECT OF CADMIUM AND POTASSIUM ON PHOTOSYNTHETIC CHARACTERISTICS IN SOYBEAN

Treatments (T)	Chlorophyll content (SPAD value)	A (μmol CO_2 m^{-2} s^{-1})	g_s (mmol m^{-2} s^{-1})	Chlorophyll fluorescence (Y)
T1 No Cd or K	24.60 b	3.35 b	0.145 a	0.786 b
T2 Cd only	19.60 c	2.35 c	0.085 b	0.765 c
T3 K only	31.70 a	5.35 a	0.160 a	0.803 a
T4 Cd + K	21.65 c	3.50 b	0.095 b	0.790 b
Genotypes (G)				
G1 Liao-1	22.83 B	3.63 A	0.088 B	0.781 B
G2 Zhechun-3	25.95 A	3.65 A	0.155 A	0.791 A
T × G	ns	**	**	**

*The different letters after data within a column represent significant difference at 95 % probability.

of potassium to plants. Between the genotypes, there was non significant difference for net photosynthesis; however, they differed significantly for SPAD value, gs and influorescence. Zhechun-3 showed higher values for these photosynthesis parameters. The interaction between treatments and genotypes was highly significant except that for SPAD value, showing the positive influence of both potassium and genotypes on cadmium toxicity tolerance in soybean. Thus the improved potassium nutrition in the resistant cultivars would be helpful in the successful growth of crops in the soils exposed to cadmium toxicity problem.

Biochemical components: The final product of membrane lipids peroxidation is malondialdehyde (MDA), which accumulates when plants are subjected to oxidative stress. Therefore, the concentration of MDA is commonly considered as a general indicator of lipid peroxidation as well as the stress level[23]. Data indicated that MDA contents in the leaves of plants had the highest values under cadmium treatment and they were reduced with potassium application (Table-3). Potassium exerted a reductive effect on MDA content and activities of SOD and POD enzymes, both under its alone or combined treatment with cadmium. These parameters also had higher values in check (no cadmium or potassium); that was due to the reason that in this treatment potassium was not applied to the plants. Another clear observation was that Liao-1 genotype had significantly higher contents of these antioxidants than in Zhechun-3. So, the genotypes appear to have a significant role in cadmium stress tolerance and under this study Zhechun-3 seems to be more tolerant to cadmium toxicity as it had less activity of antioxidant enzymes. The interactions between treatments and genotypes were highly significant at $p < 0.01$ for all the component parameters. The data gives an indication that with the combination of enhanced potassium nutrition and cultivation of tolerant soybean genotypes can overcome the problem of cadmium toxicity to this crop.

TABLE-3

EFFECT OF CADMIUM AND POTASSIUM ON MDA CONTENT AND ENZYMES ACTIVITY IN SOYBEAN

Treatments (T)	MDA (μg g^{-1} fresh weight)	SOD (U g^{-1} fresh weight h^{-1})	POD (U g^{-1} fresh weight h^{-1})
T1 No Cd or K	40.45 a	93.65 b	36.90 a
T2 Cd only	48.90 a	98.80 a	32.90 a
T3 K only	28.90 b	74.55 d	15.85 b
T4 Cd + K	35.65 b	82.70 c	18.65 b
Genotypes (G)			
G1 Liao-1	39.20 A	93.40 A	34.65 A
G2 Zhechun-3	37.75 A	81.35 B	17.50 B
T × G	**	**	**

*The different letters after data within a column represent significant difference at 95 % probability.

The effects of cadmium on different metabolic processes are controlled by several plant factors such as species and/or variety[4], water and nutritional status[24]. Potassium has a crucial role in the energy status of the plant, translocation and storage of assimilates and maintenance of tissue water relations[11]. Both these elements (Cd and K) have contrasting effects on the plant growth and physiology, however, scientific literature lacks information regarding the effect of potassium on the dynamics of cadmium in plants. Current research work provides a look into, whether potassium could nullify the harmful effects of cadmium on plants. The results on soybean growth characteristics revealed that without potassium application, cadmium reduced the root length, plant height and fresh biomass of both the soybean cultivars significantly. Dong *et al.*[25] also reported the negative impact of different levels of cadmium on the growth of tomato plants grown under hydroponic conditions. Abo-Kassem *et al.*[26] established 20 % inhibition of RGR-relative growth rate (due to decreased NAR-net assimilation rate) in wheat plants subjected to a 15 d treatment with cadmium in concentration 10 μM. Potassium in conjunction with cadmium reduced the deleterious effects of cadmium on plant growth parameters. In *vice versa*, Li[27] reported that exposure of algae to heavy metals often resulted in the loss of cellular potassium. Zhao *et al.*[14] observed that cadmium concentrations in shoots and whole plants of spring wheat increased significantly ($p < 0.01$) with increasing potassium addition. However, Noraho and Gaur[16] found that cations, including Ca, Mg, K, Na, Cu, Fe, Ni and Zn, inhibited (up to 40 %) extracellular binding and intracellular uptake of cadmium by *Lemna polyrhiza* in solution culture. Monovalent cations (Na and K) caused non-competitive inhibition of intracellular cadmium uptake. They expected that high levels of cations and metals in the external environment should lower the cadmium accumulation efficiency. Genotypes of soybean also differed in their response to cadmium stress, Zhechun-3 showed better growth. A number of studies have indicated such differences in different crop species, Belimov *et al.*[19] reported a considerable variability among 99 pea genotypes in tolerance to cadmium and uptake of different heavy metals.

The physiological traits of soybean plants *viz.*, chlorophyll content (SPAD value), net photosynthetic rate, stomatal conductance and influorescence yield were affected negatively by cadmium. However, they were improved with K treatment. The countering effect of potassium on cadmium could be due to inhibition of cadmium uptake by potassium. According to Noraho and Gaur[16] potassium caused non-competitive inhibition of intracellular cadmium uptake by *L. polyrhiza*. Most researchers connect the reduction of chlorophyll in cadmium-treated plants with inhibition of its biosynthesis[28]. Based on the expressed symptoms and the established lower concentrations of Mg and Fe in the leaves of cadmium-treated sugar beet plants, Greger and Ogren[29] suggested that lower chlorophyll concentrations in these plants were a result of the deficiency of these nutrients. Chlorophyll concentrations in cadmium-treated plants could also be lowered by the activation of its enzyme degradation. Somashekaraiah[30] established that after a 6 d treatment with 100 µM cadmium in *Phaseolus vulgaris* plants, the lipoxygenase activity increased, while chlorophyll concentrations and activity of the antioxidative enzymes such as SOD and catalase decreased significantly.

Net photosynthesis in present research was significantly reduced due to cadmium treatments @ 1.0 µmol L^{-1}. Costa and Spitz[31] reported declined net photosynthesis in lupin plants above cadmium concentration of 0.1 µmol L^{-1} in the solution culture. Similar results were also observed by Barceló and Poschenrieder[24] for soybean/beans. Vassilev and Yordanov[28] established that growth inhibition is mainly due to disorders both in dark respiration and photosynthesis and the factors limiting photosynthesis have stomatal and non-stomatal nature. Marchiol *et al.*[32] reported the equal stomatal limitation of photosynthesis in control and cadmium-treated soybean plants. Most of the investigations on photosynthesis response to *in vivo* cadmium stress are focused on non-stomatal limitations. Decreased photosynthetic rate is a consequence of the negative cadmium effects on a number of different sites of this process and mainly on the biochemical reactions of Calvin's cycle[28].

Similarly, stomatal conductance (g_s) in soybean leaves was reduced by cadmium stress as compared to control and potassium applied alone or in addition to cadmium had significantly higher g_s values. Ashraf and Bashir[33] found that salt stress also caused a marked reduction in net CO_2 assimilation rate (Pn), transpiration rate (E) and stomatal conductance (g_s) in the species of *P. vulgaris* than in *S. aculeata*. Costa and Spitz[31] reported that above 1.0 µmol L^{-1} of cadmium in the solution culture, biomass, photosynthesis rate, stomatal conductance and water potential in lupin plants were reduced significantly over control, as also shown previously for soybean, bean and lettuce[24,34].

The chlorophyll fluorescence is a sensitive indicator for the status of photochemical reactions originates from PS II chlorophyll[35] and considered being more sensitive to stress[36]. The overall quantum yield (Y) of photochemical energy conversion, assessed as: $Y = (F'm-F)/F'm$; was introduced by Genty *et al.*[20]. This parameter is quite reliable. At higher light intensities (1000-1600 µmol m^{-2} s^{-1} PAR) the decrease of Y in cadmium-treated leaves is more expressed, as at these conditions cadmium provokes losses in the photochemical efficiency of PS II[37]. They observed a significant reduction of Y in cadmium-treated plants compared to control. The current study results also revealed significantly lower Y values under cadmium treatments; while K application improved it and the resistant genotype Zhechun-3 gave higher values. Wu *et al.*[38] have also shown genotypic differences in effect of cadmium on photosynthesis and chlorophyll fluorescence of barley plants.

Cadmium enhances the level of lipid peroxidation and alteration in antioxidant systems[30]. In the current study, MDA content and antioxidant enzymes (SOD and POD) activities were enhanced due to the application of cadmium in both the soybean genotypes. However, the less tolerant cv. Liao-1 showed higher figures compared to more tolerant cv. Zhechun-3 for these parameters. Unusual metal and cysteine-rich proteins, generally named metallothioneins, have been recognised as some of the major metal-binding proteins in various kinds of plants and microorganisms[39]. Now days, it has been suggested that cadmium-binding complexes similar to the metallothionein exist in several higher plants including soyabean[40]. Superoxide dismutase in leaves, roots and stolons were increased in the presence of Cd^{2+} when compared to control plants of phragmites[41]. Peroxidases induction is a general response of higher plants to uptake of toxic amounts of metals. It has been observed[42] in roots and leaves of various species after application of toxic doses of Zn, Cd, Cu, Ni and Pb. It appears that toxic metals change POD activity both quantitatively and qualitatively. It also appears that the increase in POD activity is a defensive response to most if not all metals, which may cause damage or disturb normal function of the plants. The results are in line with the findings reported by Hassan *et al.*[43], who studied the influence of cadmium toxicity on growth and antioxidant enzyme activity in rice cultivars.

ACKNOWLEDGEMENT

The authors wish to express sincere thanks to Chinese Ministry of Education for its financial support (the Project-sponsored by SRF for ROCS, SEM).

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(*Received*: 22 January 2007; *Accepted*: 1 October 2007) AJC-5944