Caspian J. Env. Sci. 2006, Vol. 4 No.1 pp. 1~8
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CJES
Caspian Journal of Environmental Sciences

[Review]

Influence of Cadmium Toxicity on Rice Genotypes as Affected by Zinc, Sulfur and Nitrogen Fertilizers

M. J. Hassan[1,2*], Z. Zhu[1], B. Ahmad[2] and Q. Mahmood[3]

1- Department of Horticulture, Zhejiang University, Hangzhou, 310029 China 2- Department of Agronomy, NWFP Agriculture University Peshawar Pakistan 3- Department of Environmental Engineering, Zhejiang University, Hangzhou, 310029 China Corresponding Author's E-mail: jaffar148@yahoo.com

Abstract

Cadmium (Cd) is among the most widespread and toxic heavy metal in several part of the world. Its toxicity in soil is becoming a severe threat to living organism worldwide. It is one of the main pollutants in paddy fields near industrial areas and highly toxic to plant growth and development of the plants. Cd can be easily taken up by plants and enter the food chain. Therefore, precautionary measurements should be done to reduce accumulation of Cd in crops to alleviate the risk of health hazards in response to Cd-polluted soils. Several strategies have been proposed for the successful management of the Cd-contaminated in crops. One approach, applicable on slightly contaminated soils, is selection of plant genotypes with high ability to repress root uptake and shoot transport of Cd which could be a reasonable approach to alleviate adverse effects of Cd toxicity in crops. Moreover, the toxic effect of Cd can be decreased by proper application of essential nutrients such as Zn, S and N fertilizers Cd contaminated soil.

Key words: Cadmium, Genotypes, Nitrogen, Rice, Sulfur, Toxicity, Zinc

Introduction

Cadmium (Cd) is one of the most toxic pollutants in the surface soil layer (Sanita di Toppi & Gabrielli, 1999). Its accumulation in crops and soils is increasing concern to crop production (Hall, 2002). A part of agricultural soils, all over the world are slightly to moderately contaminated by Cd due to industrial pollution, metal mining, manufacture and disposal as well as some agricultural practices such as extended use of superphosphate fertilizers, pesticides, sewage sludge and smelters dust spreading leads to dispersion of Cd (Angelova *et al.*, 1999). Cd is a relatively rare metal with no biological function, and is highly toxic to plants and animals (Alloway, 1995). Plants often accumulate a huge quantity of Cd without poisoning symptoms, which entering the food chain endangers human health as well (Fergusson, 1991).

Soils, waters, air, plants and animals are the routes by which human beings come into contact with Cd. Its high mobility rate in the soil-plant system can make it easy to enter into food chain and create risk to human health and environment (Grant *et al.*, 1998). It damages the kidney and elevates the risk to adopt osteoporosis, by inhibiting mineralization, vitamin D activation and calcium uptake (Jarup *et al.*, 1998). Cadmium toxicity caused death among Itai-Itai patients who were exposed to higher amount of Cd in rice and drinking water due to decrease in glomerular filtration rates.

Increase in international concern about the risks associated with long-term consumption of crops with Cd concentrations has led the international food standards organization, Codex Alimentarius Commission, to propose a 0.1 mg Cd kg^{-1} limit for cereals, pulses and legumes (Harris and Taylor, 2001). Plant-internal transport of Cd may be influenced by different factors, such as transpiration rate and plant internal chelators (Salt and Rauser, 1995). Previously it has been reported that the uptake and accumulation rate of Cd changes among plant species (Ozturk *et al.*, 2003) and genotypes of a given species (Dunbar *et al.*, 2003). Recently Hassan *et al.* (2005a) have observed differences between rice cultivars in their ability to absorb and accumulate Cd in roots and shoots. However, the mechanisms of its uptake and translocation in plants have not yet been sufficiently studied to date.

The maximum tolerable intake of Cd for

humans, recommended by FAO/WHO is 70 μg/day. Consumption of Cd contaminated rice with 3.7 mgCd kg^{-1} has reported a renal toxicity in Chinese population (Nordberg *et al.*, 1997). This level of Cd in rice is approximately 10-fold higher than safe limit (Nakashima *et al.* 1997). The Cd concentration in soil of Zhangtu (in Zhejiang province) is even more than 5-7 mg kg^{-1} and in Sheyang (in Zhejiang province) is 1-2 mg kg^{-1} much higher than normal level 0.4 mg kg^{-1} in soill (Kebata-Pendias and Pendias 2001). The Cd content in rice must not be more than 0.4 mg kg^{-1} in order to avoid occurrence of renal dysfunction due to Cd toxicity (Nakashima *et al.*, 1997). Therefore, precautionary measures are needed to decrease accumulation of Cd to reduce the risk of health hazards in response to Cd-polluted field.

Presently, the changes in antioxidant enzymes activities, photosynthetic rate and growth of rice cultivars as affected by Zn, S and N fertilizers on the alleviation of Cd toxicity has been reported by Hassan *et al.* (2005 abc). The differences in tolerance of these cultivars against Cd toxicity provide a base to study the mechanisms of Cd tolerance in crops. The over all aims of this paper are to focus the toxic effect of Cd in plants and to suggest strategies in order to decrease Cd accumulation in crops especially, rice one of the most stable food in China.

Efforts to alleviate Cd toxicity and accumulation in plants

The great effort has been given to develop the methods or techniques for alleviating the growth inhibition of crops by Cd toxicity and reducing its accumulation in plants. However, on the whole, the reports up to date provided the inconsistent results and the available strategies are not effective or practical in crop production. Following are some of the strategies which will we discuss in this review article.

Genotypic differences in response to Cd stress

Cd uptake can vary greatly among plant genotypes (Hassan *et al.*, 2005a; Bingham *et al.*, 1980) therefore it is possible for us to develop the species or cultivars with low Cd level in edible plant parts.This strategy has been successfully applied in sunflower and durum wheat (Penner *et al.*, 1995; Li *et al.*, 1995; Wang, 2002). The Cd-induced activities of antioxidant enzymes in barley could explain for the genotypic difference in their Cd tolerance (Wu *et al.* 2003). The difference in root uptake and shoot accumulation of Cd can be an important factor in explaining for genotypic variation in tolerance to Cd toxicity in plants.The retention of Cd in the root vacuoles cell might influence the symplastic radial Cd transport to the xylem and further transport to the shoots, resulting in genotypic difference in grain Cd accumulation in wheat (Stolt *et al.* 2003). Wang and Gong (1996) reported that compared with normal rice cultivars, the hybrid cultivars can absorb more Cd from the same contaminated soil and transport a larger proportion of Cd captured from roots to shoots and grains. The hybrid rice have the capacity to accumulate greater amount of Cd in root due to higher root activity, more root to shoot weight ratio and greater water consumption per gram of grain (Wu *et al.*, 1999). Recently we observed a significant difference between two rice cultivars for their sensitivity to Cd accumulation in dry matter, photosynthetic rate and antioxidant enzymes activities (Hassan *et al.*, 2005b). The amount of Cd absorbed by rice has significant positive correlation with biomass and grain yield (Wu *et al.*, 1999). The retention of Cd in the root cell vacuoles might influence the symplastic radial Cd transport to the xylem and further transport to the shoots, resulting in genotypic difference in grain Cd accumulation (Stolt *et al.*, 2003).

The transport of Cd in plants closely related with the plant metabolism (Kabata-Pendias and Pendias, 2001). The mechanisms affecting the root uptake and shoot transport of Cd can also affect the expression of Cd toxicity in plants (Kochian *et al.*, 2002; Dunbar *et al.*, 2003). Great difference exists among the rice cultivars in the translocation of Cd absorbed by roots into shoots (Liu *et al.*, 2003; Hassan *et al.*, 2005a). In submerged paddy soils different rice cultivars possess different redox abilities in roots, thus creating different rhizospheric environments and accessing different amount of plant-available Cd and mineral nutrients (Liu *et al.*, 2001). Hu and Kao, (2003) observed that in second leaves of rice plants the decrease in chlorophyll content of cv. Tainung 67 (TNG 67) was less than cv. Taichung Native-

1 after Cd treatment, while the decrease in photosynthetic rate and chlorophyll content due to Cd toxicity is genotypic dependent (Hassan *et al.*, 2005b). The different species of cereal crops possess different abilities in the exuding of phytochelatins (PCs), but still further researches are needed in rice on the difference of PSs exudation among cultivars and its relationship with the uptake and transport of Cd (Liu *et al.*, 2003). However, the mechanisms of Cd uptake and translocation by rice plants have not yet been sufficiently studied to date.

Effect of Zn on Cd toxicity

Cadmium and Zinc (Zn) have many physical and chemical similarities. The fact that Cd is a toxic heavy metal and Zn an essential element makes this association interesting as it raises the possibilities that the toxic effect of Cd may be preventable by Zn. Zinc is involved in membrane integrity, enzyme activation, gene expression and regulation, carbohydrate metabolism, aerobic root respiration, protein synthesis, structural integrity of ribosomes, detoxification of superoxide radicals, phytohormone activity and disease resistant (Kim *et al.*, 2002).

The co-existence of essential elements in the ecosystem leads to interactions that may be additive, antagonistic or synergistic to Cd toxicity (Siedlecka, 1995). Evidence for a common pathway of remobilization for Cd and Zn in plant shoots, as shown by competitive inhibition of Cd movement by Zn, is contradictory. Many researchers showed that addition of Zn to soil reduced crop Cd concentrations (Choudhary *et al.*, 1995). But others reported that Cd uptake was increased by addition of Zn to soils (Moraghan, 1993). Chaoui *et al.* (1997) observed no antagonistic Cd-Zn interaction at uptake level, while Maclean (1976) and Smilde *et al.* (1992) reported that Cd and Zn had a synergistic effect on the accumulation of Zn. Smith and Brennan (1983) reported a synergistic interaction between Cd and Zn, while Cataldo *et al.* (1983) observed antagonistic interaction between Cd and Zn. According to Choudhary *et al.* (1995) application of Zn inhibits Cd uptake and accumulation in plants, while some studies failed to show any relationship (Oliver *et al.*, 1997). White and Chaney (1980) reported that Cd uptake in xylem and phloem was reduced by Zn application. In Contrast Hassan *et al.* (2005b) found that increasing the Zn level from 0.2 to 1 µmol in medium had reduced Cd uptake and accumulation in roots, while increased has been noted in shoots Cd content of rice cultivars.

Heavy metals interfere with the normal metabolic functions even at micro-concentration and affect different cellular components (Prasad and Hagemeyer, 1999). Zinc is an important component of many vital enzymes, a structural stabilizer for proteins, membrane and DNA-binding protein (Vallee and Auld, 1990), alcohol dehydrogenase, RNA polymerase, respiration enzyme activators, and the biosynthesis of plant growth hormones (Wahbeh, 1984), while Cd is an extremely toxic element without any metabolic significance (Aravind and Prasad 2003). Hassan et al (2005b) reported that addition of Zn to the medium solution has drastically alleviated Cd toxicity by decreasing melondialdehyde (MDA) content and antioxidant enzymes activities in two different rice cultivars grown in nutritent culture soulation. Zinc is known to have a stabilizing and protective effect on the biomembranes against oxidative and peroxidative damage, loss of plasma membrane integrity and also alteration of the permeability of the membrane (Bettger and. O'Dell, 1981). The balance between free radical generation and free radical defense determines the survival of the system. Therefore, Zn may have a role in modulation of free radicals and their related processes through antioxidant properties (Zago and Oteiza, 2001).

Role of S in alleviation of Cd toxicity

Sulfur is available to plants primarily in the form of anionic sulfate (SO_4^-) present in soil. It is actively transported into roots and then distributed, mostly un-metabolized, throughout the plants. In response to Cd-stress, higher plants synthesize sulfur rich peptides, phytochelatins (PCs). Cadmium, after being taken up in cells, can be detoxified by Cd-binding proteins, such as phytochelatins or metallothionins (Cobbett, 2000; Hall, 2002). PC biosynthesis is closely dependent on sulfur metabolism (Cobbett, 2000; Leustek *et al.*, 2000), synthesized from different isoforms of glutathione (Grill *et al.*, 1985). In plants PCs play a crucial role in Cd detoxification (Howden, *et al.* 1995). It complexes cations (Cd^{2+}), and make them less harmful to the plant cells (Weigel and Jager, 1980). It has been observed in many plants that Cd induces PCs synthesis (Zenk, 1996). PCs form complexes with Cd^{2+}, reducing the activity of the metal in the cytosol (Cobbett, 2000). Cd-PC complexes are then compartmentalized into the vacuole, probably by means of an ATP-binding cassette-type transporter localized in the tonoplast (Salt and Rauser, 1995).

In fact, Cd exposure induces the activity of enzymes involved in the sulfate reductive assimilation pathway and glutanthione content (GSH) biosynthesis. Nussbaum *et al.* (1988) showed that Cd^{2+} accumulation into maize (Zea mays) seedlings was related to an increase in the activity of both ATP-sulfurylase and adenosine 5′-phosphosulfate reductase, the first enzymes in the sulfate assimilation pathway. Other works reported on the induction of enzyme activities involved in GSH biosynthesis, such as γ-glutamyl-Cys (γEC) synthetase and glutathione synthetase, indicating a cellular response to a transient GSH depletion during PC biosynthesis (Ruegsegger and Brunold, 1992).

Glutathione contains sulfur, an important complex stabilizer via disulfide bonds. The stability of the PC-Cd complex can be enhanced by additional sulfur ions (Oritz, *et al.*, 1992). Cd-induced sulfate uptake was associated to both a decrease in the contents of sulfate and glutathione and synthesis of a large amount of PCs. Glutathione plays a role in quenching reactive oxygen species; the depletion of glutathione in response to Cd exposure (Scheller, *et al.*, 1987) may lead to damage via reactive oxygen species (ROS) (DeVos and Schat, 1981). Recently, we conducted a hydroponics experiments and observed that GSH content increase with increase in Cd level, while increasing S level in medium has significantly interact the Cd toxicity in rice plants. In addition, higher S dose alleviated the oxidative stress, leading to a reduced MDA content and less growth inhibition by Cd toxicity, which shows that the alleviation of Cd toxicity by S is S-level dependent. Furthermore, the higher Cd and MDA content was consistently accompanied by higher SOD activity and higher S levels caused the marked increase in glutathione content and reduction in SOD activity, indicating the exact effect of S and glutathione in alleviating oxidative stress in rice cultivars.

Effect of N form on Cd uptake and accumulation

Nitrogen (N) is an important component of many important structural, genetic and metabolic compounds in plant cells. Plant N status is highly dependent on N fertilization (Hernandez *et al.*, 1997). N in soils can occur as organic N and mineral N ($NH4^+$ and NO_3^-) (Majerowicz and Kerbauy, 2002). NO_3^- is the most abundant source of N, in the most soils assimilated by higher plants (Ajakaiye, 1981), while a lot of the crops grow well in mixture of NH_4^+ and NO_3^- (Frechilla *et al.*, 1999). In plants availability of N source is related to the efficiency of N utilization, and depends both on the photosynthetic and respirations processes and also on the partitioning of N and biomass (Frechilla *et al.*, 1999).

Nitrate assimilation can be affected by the presence of Cd in the growing medium of higher plant. The absorption of NO_3^- from the nutrient solution reduced dramatically when the plants were subjected to long term Cd exposure (Herandez *et al.*, 1997; Khater *et al.*, 1991; Petrovic *et al.*, 1990). In the most environmental conditions, Cd enters first the roots, and consequently damage plants. It can also reduce the absorption of nitrate and its transport from roots to shoots, by inhabiting the nitrate reductase activity in the shoots (Hernandez *et al.*, 1997).

N is also a major component of chlorophyll and amino acids, the building blocks of proteins. Increase in N supply can stimulates plant growth and productivity (Joel *et al.*, 1997), as well as photosynthetic activity (Makino *et al.*, 1992) through increased amounts of stromal and thylakoid proteins in leaves (Bungard *et al.*, 1997). In Phaseolus, N deficiency reduced in vitro Rubisco activities and electron transports (von-Caemmerer and Farquhar, 1981), while in vivo activities of Rubisco and electron transport remains constant under different N supplies (Makino *et al.*, 1992). Evans and Terashima, (1987) found a decrease in the ratio of Rubisco activity of N deficient spinach leaves. On the other hand, inhibition of photosynthesis by Cd toxicity decreases the amount of photosynthetic pigments per unit area (Siedlecka and Krupa, 1996). The presence of Cd in plants induces water stress symptoms such as a decreased stomatal conductance, transpiration rate and relative water content in leaves (Kastori *et al.*, 1992; Chen and Huerta, 1997). Therefore, proper N supply may overcome the adverse effects caused by Cd toxicity in crop.

Cadmium can influence N metabolism, but the effect may be direct and/or indirect (Kastori *et al.*, 1997). However, in the most previous experiments involved, the use of Cd concentrations, were higher than those found in heavily polluted soils (Larsson *et al.*, 1998). To study the effect of N forms on Cd toxicity in rice, we conducted another nutrient solution experiment to compare the effect of NH_4NO_3, $(NH_4)_2SO_4$, and $CaNO_3$ on Cd toxicity (Hassan *et al* 2005d). We found that the inhibition of growth and photosynthetic traits under Cd stress varied with applied N forms in the nutrient solution, i.e. $Ca(NO_3)_2$ > NH_4NO_3 > $(NH_4)_2SO_4$ in the order of damage severity with significant differences among three N treatments in Cd and N concentrations of the plants subjected to Cd stress. Moreover, a substantial difference was noted among N forms in their effect on Cd and N uptake, with $(NH_4)_2SO_4$-fed plants had less Cd and more N uptake than both $Ca(NO_3)_2$- and NH_4NO_3-fed plants, suggesting potential antagonist effect between Cd and ammonium-N, and synergist between Cd and nitrate-N. Heavy metals cause nutrient deficiency and even change the concentrations of basic nutrients in plant tissues (Siedlecka, 1995). Therefore, better understanding that how heavy metals toxicity disturbs macronutrient metabolisms is needed.

Acknowledgement

We are deeply indebted to The British Council for its support to this project (SHA/992/310).

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(Received: Feb. 4, Accepted Apr. 22, 2006)