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Full Length Article

Salicylic Acid Modifies Growth Performance and Nutrient Status of Rice (*Oryza sativa*) under Cadmium Stress

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Abstract

To investigate the toxic effects of Cd on seedling growth parameters and mineral nutrients, and the impact of SA in the alleviation of Cd toxicity in four basmati rice genotypes (Basmati-198, Basmati-370, Basmati-2000 and Kashmir Basmati), the pot experiment was conducted in sand-filled pots under normal temperature (28±2ºC). After germination, the seedlings were subjected to 0, 100, 500, 1000 and 1500 µM of Cd concentrations. The results showed reductions in different seedling growth attributes and the mineral nutrients at different Cd regimes. When SA was applied alone in the medium, no change in root Cd or slight reduction in shoot Cd revealed the protective effect of SA against subsequent Cd toxicity that might be ascribed to the inhibition of Cd uptake. However, with the elevating Cd stress, SA reduced the root or shoot Cd contents, improved the seedling growth attributes and the mineral nutrients exhibiting the ameliorating impact to Cd toxicity. Variable genotypic responses were observed for different seedling growth traits and the status of mineral nutrients. Under elevating Cd levels, Basmati-198 showed less Cd accumulation revealing reduced toxic effects of Cd on seedling growth traits and the mineral nutrients as compared to other rice genotypes. © 2014 Friends Science Publishers

Keywords: Rice (*Oryza sativa* L.); Salicylic acid; Plant growth; Mineral nutrients

Introduction

The presence of Cd in the environment caused serious threats to plant life as well as to human health and animals (Nawarot *et al*., 2006). In nature, plants take Cd directly from the soil solution. Plants being the primary producers in the food chain accumulate Cd in the edible parts and thus serve as a source of cadmium intake for humans and animals (Lopez-Millan *et al*., 2009). Higher amounts of Cd cause several diseases and disorders in humans (Nishijo *et al*., 2006).

The incorporation of the Cd in plants occurs via non-specific pathways. Being a non-redox metal and toxic to plants, Cd stimulates the inhibition of growth leading to the death of the plant. High concentration of Cd also induces oxidative stress by producing free radicals that may damage the tissues of plants (Zhang *et al*., 2005; Shekhawat *et al*., 2008). It causes reduction in photosynthesis, reduces the contents of chlorophyll and disturbs the plant water and nutrient uptake balance (Mobin and Khan, 2007; Razinger *et al*., 2008).The literature reports that inhibition of plant growth is due to the direct effect of Cd on the nucleus or interaction with hormones where as in the upper parts (aerial) of the plants it is due to photosynthesis inhibition (Laspina *et al*., 2005). Moreover, it causes chlorosis, plant growth inhibition, deficiency of nitrogen and phosphorus, condensed transport of manganese and accelerates plant senescence (Mishra *et al*., 2006). In plant tissues Cd also induces damaging effects on the micro-and macro-elements stability (Lopez-Millan *et al*., 2009).

Salicylic acid, a plant growth regulator and found in crystalline form, is naturally present in several plants (Raskin *et al*., 1990) and considered an endogenously produced growth regulator due to its phenolic nature. In plants it takes part in the regulation of several plant physiological processes like growth, development, production of heat and ethylene, nitrate metabolism, flowering and also responds to environmental stresses (Hayat *et al*., 2007). It plays an important role in the germination of seeds and fruit yield (Klessig and Malamy, 1994), as well as anion uptake (Harper and Balke, 1981). SA also acts as a non-enzymatic antioxidant and as a growth promoter by regulating various physiological processes in plants like photosynthesis (Fariduddin *et al*., 2003; Arfan *et al*., 2007; Murtaza and Rehana, 2013).

Moreover, SA being a water soluble antioxidative compound plays a role in abiotic stress tolerance for example under drought stress in wheat (Sakhabutdinova *et al*., 2003). Recently it has been observed that external application of SA can raise plant’s tolerance to abiotic stresses such as salinity (Gunes *et al*., 2007), drought (Azooz and Youssef, 2010), osmotic (Al-Hakimi, 2006) and heavy metal stress (Moussa and El-Gamel, 2010). Pretreatment of seeds with SA reduces the toxicity of Cd in rice (Guo *et al*., 2009), maize (Krantev *et al*., 2008), soybean (Drazic and Mihailovic, 2005) and barley (Metwally *et al*., 2003).

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Rice (*Oryza sativa* L.) is one of the staple food crops and the diet of more than one third of the population throughout the world (Konwar and Jha, 2010). Among several factors affecting the rice yield and growthare the heavy metal contaminated soils. The heavy metals accumulate in agricultural lands due to the application of fertilizers, different types of manures and sludge (McLaughlin *et al*., 1999). All heavy metals are toxic to higher plants disturbing enzymes and other metabolic processes leading to reduced growth and yield (Wang *et al*., 2003). A wide range of genotypes in their ability to accumulate Cd exists in rice (Liu *et al*., 2003) and several researchers had reported the genotypic differences in response to seedling growth and Cd toxicity/stress (Shao *et al*., 2004; Wu *et al.,* 2006; Cheng *et al*., 2008; Du *et al.,* 2009). Keeping in view the toxic effects of Cd on rice, the present study was designed with the objectives to assess the role of SA in the alleviation of toxic effects of Cd in relation to Cd accumulation, seedling growth parameters and the contents of mineral nutrients and the genotypic responses to Cd toxicity.

Materials and Methods

Experimental Details

The experiment was conducted in the growth chamber under controlled conditions of light and temperature (28±2ºC) in the Dept. of Botany, University of Agriculture, Faisalabad. Seeds of basmati rice genotypes (Basmati-370, Basmati-198, Basmati-2000 and Kashmir Basmati) were obtained from Nuclear Institute of Agriculture and Biology (NIAB).

Cd regimes were prepared in the form of Cadmium chloride ($CdCl_2$). Ten grains of each genotype were sown in small plastic pots containing river sand under five Cd treatments (control, 100, 500, 1000 and 1500 µM) and two SA levels (control and 0.1 mM). Different treatment combinations (0 µM Cd + 0.0 mM SA), (100 µM Cd + 0.0 mM SA), (500 µM Cd + 0.0 mM SA), (1000 µM Cd + 0.0 mM SA), (1500 µM Cd + 0.0 mM SA), (0 µM Cd + 0.1 mM SA), (100 µM Cd + 0.1 mM SA), (500 µM Cd + 0.1 mM SA), (1000 µM Cd + 0.1 mM SA) and (1500 µM Cd + 0.1 mM SA) were maintained throughout the experiment. After germination, the seedlings were irrigated at an alternate day interval with half strength of Hoagland's solution (Hoagland and Arnon, 1950) along with the corresponding Cd treatment combination for 15 days. After harvesting the experiment, different seedling growth parameters and contents of mineral nutrients were measured.

Growth Determination

Shoot and root lengths, shoot and root fresh weights, and their dry weights were recorded. The root stress tolerance index (STI) was calculated as follows:

$$STI = \frac{\text{Average length of root (treated)}}{\text{Average length of root (control)}} \times 100$$

Determination of Mineral Nutrients

The dried ground material (0.5 g) of shoots, and roots were digested in concentrated HNO_3 (5 mL) at 100^{o}C temperature and then raised the temperature at150^{o}C in digestion tubes and then made volume of the extracted up to 50 mL in the volumetric flask. Filtered the extract and used it for the determination of mineral nutrients concentrations. The dissolved amount of sodium (Na), potassium (K) and calcium (Ca) were determined by using flame photometer (Model: PFPI-7, Jenway, UK) and magnesium (Mg), manganese (Mn), iron (Fe) and cadmium (Cd) were determined with atomic absorption spectrometer (Model: Analyst-3100 Perklin Elmer, USA).

Determination of Phosphorus (P)

Br-reagent was prepared by dissolving 25 g of ammonium molybdate in 400 mL of distilled water. 1.25 g of ammonium metavenadate was dissolved in 300 mL of distilled water, then 250 mL HNO_3 and cooled down. Mixed both solutions and maintained volume up to 1000 mL. Took 2 mL of solution obtained by digesting plant material in concentrated HNO_3 (as described above) and mixed with 2 mL of Br-reagent. Kept for half an h and then took optical density (O.D.) at 460 nm (Hitachi-U 2001).

Statistical analysis

A three-way analysis of variance of data for all parameters was computed by using a computer software COSTAT (Cohort software Berkeley, California). The least significant differences between means were calculated using Duncan's New Multiple Range test ($P \leq 0.05$).

Results

Shoot and Root Lengths

Shoot and root lengths significantly reduced with the increase in Cd concentration in the growth medium as compared to control (Fig. 1).The extent of reduction was observed more in root length as compared to shoot length. Among the genotypes, Basmati-198 attained the maximum shoot and root lengths in the control as well as under Cd stress. The application of SA improved the shoot as well as root lengths at all Cd concentrations. Among the genotypes, more reduction in shoot and root lengths was noted in Kashmir Basmati followed by Basmati-370, Basmati-2000 and Basmati-198.

Shoot and Root Fresh Weight

Shoot and root fresh weights decreased as Cd concentration increased as compared to control (Fig. 2). More reduction in root fresh weight was observed than shoot fresh weight. Kashmir Basmati had the least shoot and root fresh weights among the genotypes in control and under Cd stress. Shoot

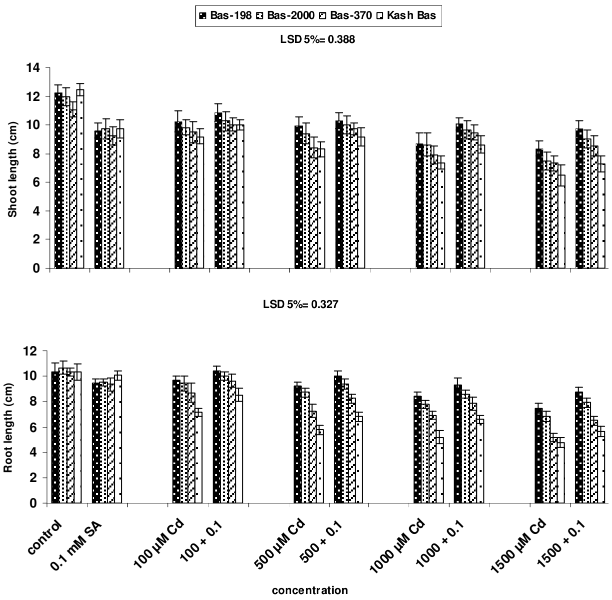

Fig. 1: Interactive effects of SA and Cd on shoot and root lengths of four basmati rice genotypes. Error bars are shown

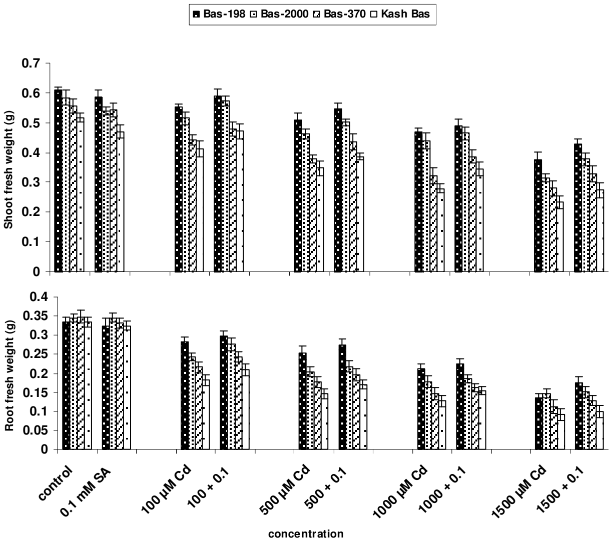

Fig. 2: Interactive effects of SA and Cd on shoot and root fresh weights of four basmati rice genotypes. Error errors are shown

and root fresh weights decreased more in Kashmir Basmati than other genotypes. Less reduction was observed in shoot and root fresh weight of Basmati-198 followed by Basmati-2000 and Basmati-370 under Cd stress. SA reduced Cd toxicity at all levels of Cd treatments and the improvements were observed in shoot and root fresh weights.

Shoot and Root Dry Weight

Shoot and root dry weights showed declines with the increments in the concentration of Cd in the growth medium as compared to control (Fig. 3). Root dry weight declined more than shoot dry weight. More reductions in shoot and root dry weights were observed in Kashmir Basmati followed by Basmati-370 and Basmati-2000 whereas the least reduction was noted inBasmati-198. The addition of SA in the Cd-containing medium improved the shoot and root dry weights in all genotypes than with the Cd treatments.

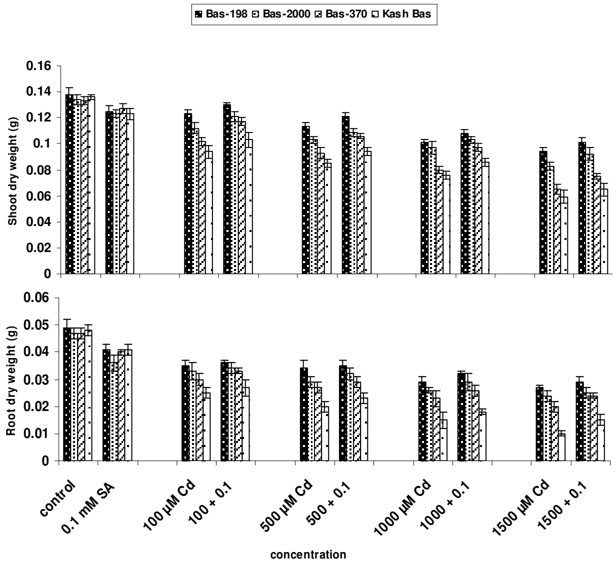

Fig. 3: Interactive effects of SA and Cd on shoot and root dry weights of four basmati rice genotypes. Error bars are shown

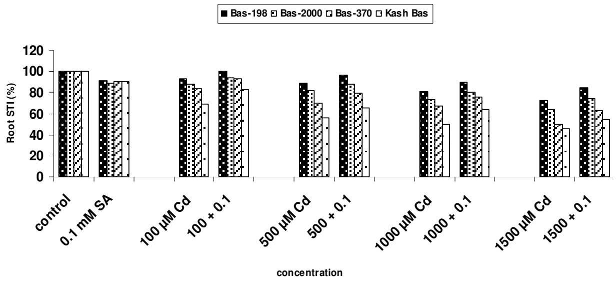

Fig. 4: Interactive effects of SA and Cd on root stress tolerance index (STI) of four basmati rice genotypes

Root Stress Tolerance Index (STI)

All the genotypes showed reductions in stress tolerance index (STI) of root length at all the Cd treatments as well as at the treatments of combined SA and Cdas compared to control (Fig. 4). With the increments in Cd, the root STI reduced but differential genotypic improvements were observed when SA was applied with different Cd treatments. The highest STI was observed in genotype Basmati-198 (84.0) followed by Basmati-2000 (76.8) and Basmati-370 (67.8) whereas the lowest STI (55.4) was observed in Kashmir Basmati.

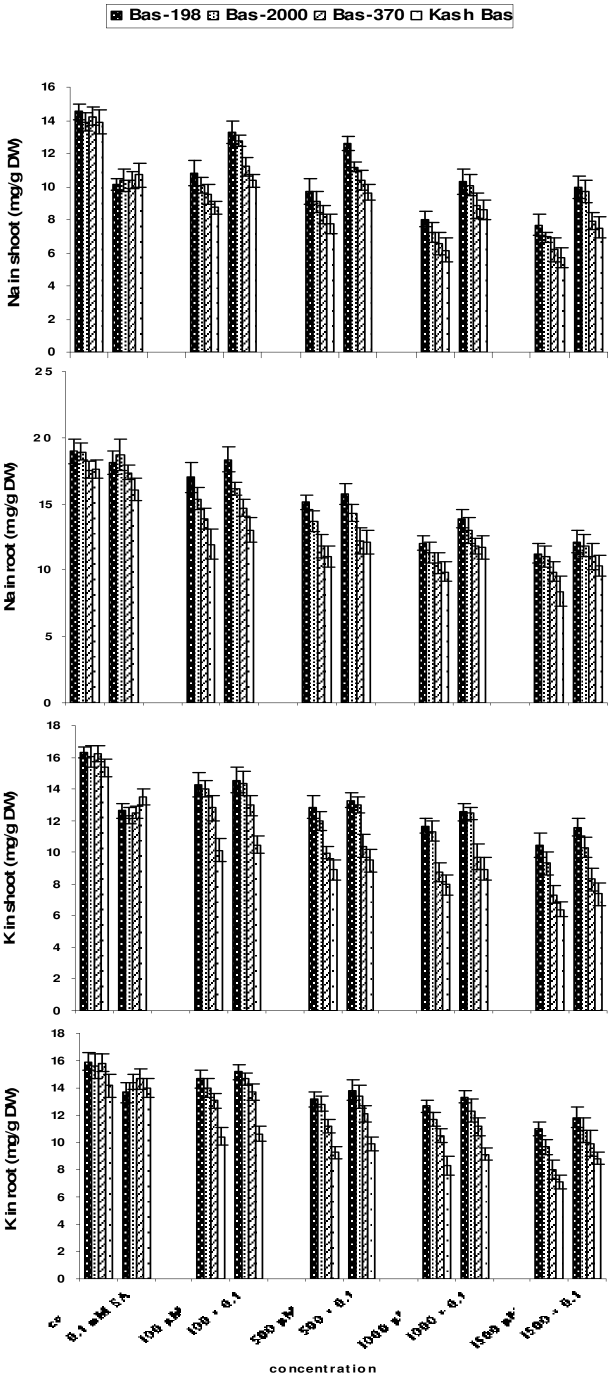

Fig. 5: Interactive effects of SA and Cd on Na and K contents in both shoot and root of four basmati rice genotypes. Error bars are shown

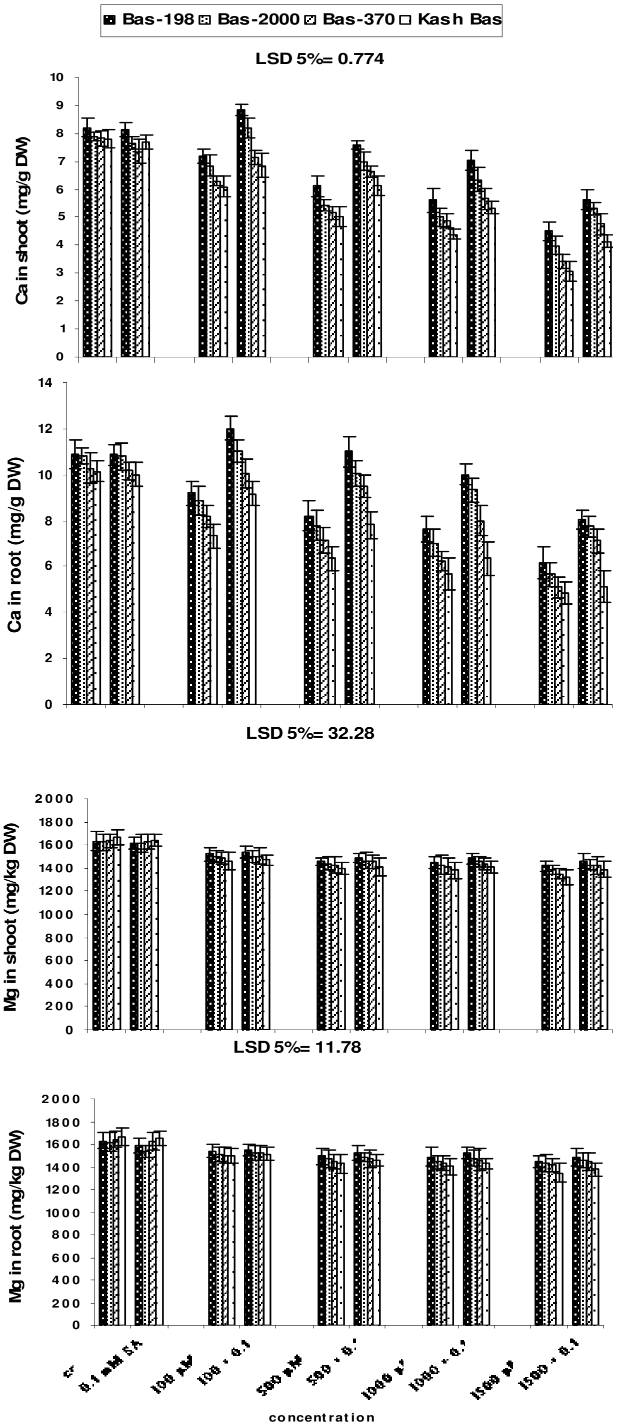

Fig. 6: Interactive effects of SA and Cd on Ca and Mg contents in both shoot and root of four basmati rice genotypes. Standard errors are shown

Mineral Nutrients

The mineral nutrients (Na, K, Ca, Mg, and P) in all genotypes gradually decreased both in shoot and root with the increase in Cd concentrations in the growth medium as compared to control (Fig. 5-7). Basmati-198 showed lesser reduction in the contents of these nutrients than in other genotypes. The extent of reduction was more in shoots than in roots. The application of SA in combination with the elevating Cd improved the contents of nutrients both in

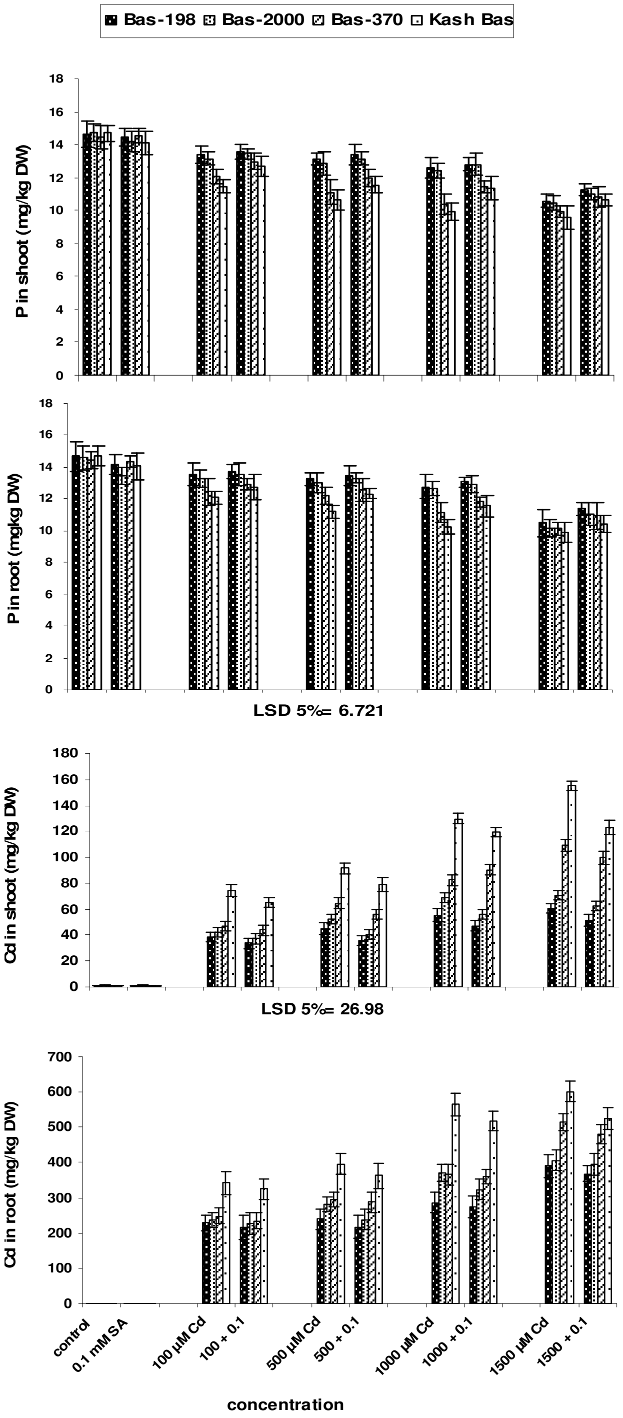

Fig. 7: Interactive effects of SA and Cd on P and Cd contents in both shoot and root of four basmati rice genotypes. Error bars are shown

shoot and root. Likewise, Mn and Fe contents decreased in the Cd-containing growth medium as compared to control (Fig. 8) whereas improvements were observed in these nutrients in both shoot and root of all the genotypes when SA was applied in combination with Cd.

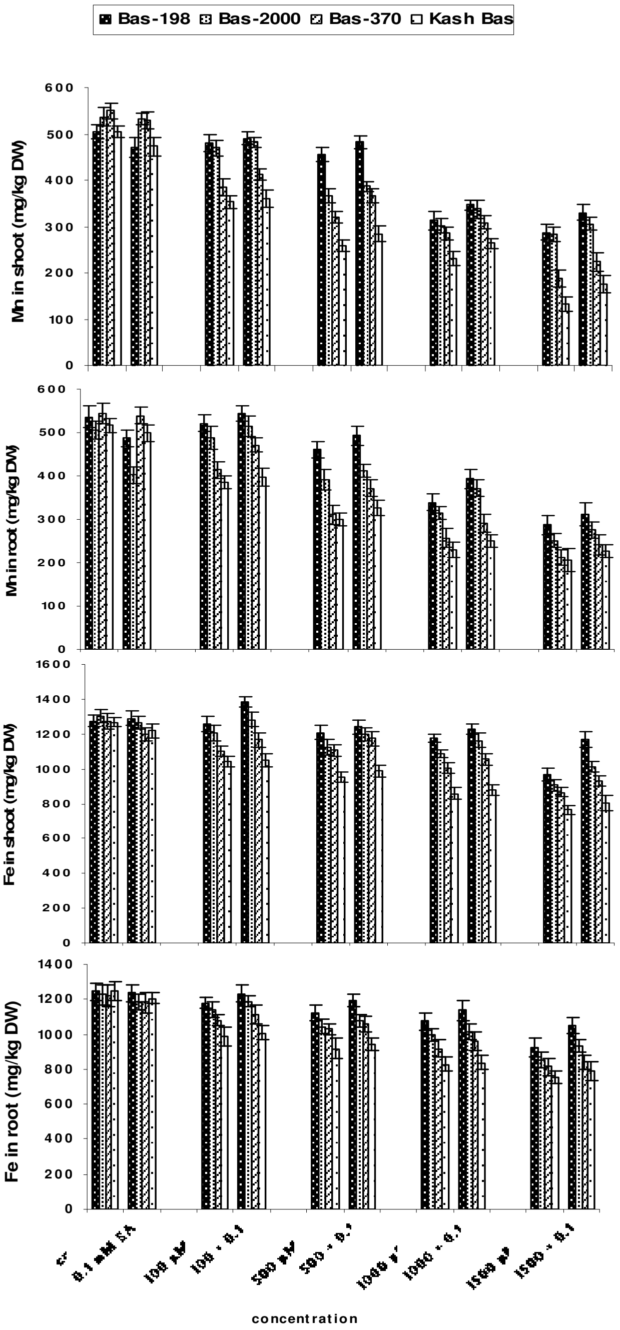

Fig. 8: Interactive effects of SA and Cd on Mn and Fe contents in both shoot and root of four basmati rice genotypes. Error bars are shown

Cd Contents

Cd contents significantly increased both in shoot and root as the Cd concentration increased in the medium as compared to control in all genotypes (Fig. 7). Comparatively, higher Cd contents were observed in roots than in shoots. The roots of Kashmir Basmati accumulated higher Cd contents than in

Table 1: Correlation coefficients among different growth parameters and mineral nutrients in basmati rice genotypes

	Shoot Length	Root Length	Shoot Fresh Weight	Root Fresh Weight	Shoot Dry Weight	Root Dry Weight	Shoot K	Root K	Shoot Mg	Root Mg	Shoot Mn	Root Mn	Shoot Fe	Root Fe	Shoot P	Root P	Shoot Na	Root Na	Shoot Ca	Root Ca	Shoot Cd
Root Length	0.776**																				
Shoot Fresh Weight	0.791**	0.848**																			
Root Fresh Weight	0.671**	0.770**	0.766**																		
Shoot Dry Weight	0.743**	0.838**	0.869**	0.789**																	
Root Dry Weight	0.647**	0.801**	0.734**	0.736**	0.767**																
Shoot K	0.821**	0.892**	0.865**	0.773**	0.840**	0.786**															
Root K	0.729**	0.833**	0.878**	0.773**	0.845**	0.721**	0.884**														
Shoot Mg	0.769**	0.824**	0.785**	0.853**	0.829**	0.812**	0.822**	0.795**													
Root Mg	0.766**	0.864**	0.820**	0.860**	0.856**	0.837**	0.850**	0.815**	0.955**												
Shoot Mn	0.772**	0.882**	0.908**	0.872**	0.888**	0.800**	0.892**	0.891**	0.862**	0.896**											
Root Mn	0.750**	0.834**	0.857**	0.771**	0.813**	0.729**	0.857**	0.815**	0.808**	0.828**	0.888**										
Shoot Fe	0.724**	0.860**	0.888**	0.732**	0.819**	0.765**	0.874**	0.869**	0.767**	0.803**	0.882**	0.805**									
Root Fe	0.741**	0.861**	0.873**	0.759**	0.847**	0.729**	0.849**	0.868**	0.818**	0.849**	0.893**	0.836**	0.888**								
Shoot P	0.738**	0.833**	0.866**	0.845**	0.830**	0.762**	0.824**	0.820**	0.831**	0.846**	0.870**	0.787**	0.807**	0.826**							
Root P	0.761**	0.829**	0.860**	0.808**	0.823**	0.766**	0.836**	0.846**	0.828**	0.851**	0.866**	0.815**	0.845**	0.831**	0.860**						
Shoot Na	0.867**	0.778**	0.817**	0.664**	0.771**	0.658**	0.816**	0.788**	0.746**	0.776**	0.811**	0.783**	0.741**	0.781**	0.755**	0.758**					
Root Na	0.783**	0.836**	0.899**	0.846**	0.861**	0.761**	0.864**	0.842**	0.858**	0.879**	0.938**	0.856**	0.835**	0.860**	0.865**	0.853**	0.801**				
Shoot Ca	0.775**	0.784**	0.857**	0.680**	0.812**	0.686**	0.798**	0.791**	0.755**	0.789**	0.850**	0.825**	0.796**	0.823**	0.783**	0.793**	0.879**	0.847**			
Root Ca	0.767**	0.821**	0.884**	0.713**	0.853**	0.705**	0.812**	0.845**	0.772**	0.808**	0.867**	0.821**	0.846**	0.842**	0.811**	0.844**	0.863**	0.859**	0.930**		
Shoot Cd	-0.792**	-0.848**	-0.882**	-0.895**	-0.873**	-0.823**	-0.858**	-0.852**	-0.924**	-0.940**	-0.932**	-0.831**	-0.853**	-0.868**	-0.896**	-0.887**	-0.786**	-0.927**	-0.822**	-0.859**	
Root Cd	-0.717**	-0.811**	-0.823**	-0.872**	-0.845**	-0.819**	-0.808**	-0.814**	-0.899**	-0.929**	-0.893**	-0.773**	-0.829**	-0.831**	-0.846**	-0.846**	-0.708**	-0.861**	-0.746**	-0.793**	0.949**

other genotypes. When SA added alone in the medium, no change in mean Cd contents of roots or slight reduction in shoots was observed. The Cd contents in shoot and root of all genotypes decreased when SA was applied in combination with the elevating Cd treatments as compared to Cd-stressed conditions without SA.

Correlations among Different Seedling Growth Parameters and Mineral Nutrients

All possible combinations of the seedling growth parameters and the mineral nutrients within and among each other showed significantly positive associations whereas Cd contents in shoots as well as in roots showed significantly negative correlations with all the seedling growth parameters and the mineral nutrients (Table 1). The Cd contents in roots showed significantly positive correlation with the Cd contents in the shoots.

Discussion

After entering the plant system, Cd accumulated in the roots and caused disruptions in the status of mineral nutrients (Drazic *et al*., 2004; Singh and Brar, 2002) as a competition with other nutrients through the same carrier in the membrane (Welch *et al*., 1999). Reductions in growth due to Cd stress had been described directly relating to the inhibition in the length of apex and mitotic activity (Fusconi *et al*., 2006). SA had been recognized playing its role in the regulation of growth, development processes of plants and their responses to environmental stresses (Senaratana *et al*., 2000) inducing plants defense to most of the abiotic stresses including Cd toxicity (Pal *et al*., 2005). In the present studies, no effects of SA treatment alone on root Cd or slight reduction in shoot Cd as compared to control revealed that the protective effect of SA against subsequent Cd toxicity in roots or shoots was likely due to the inhibition of Cd uptake, thus occurring the possibility of formation of stable SA-Cd complexes, which might help in the reduction of Cd toxicity after SA application. Shoot and root lengths, shoot and root fresh weights, and their weights decreased with the increments in Cd concentration in the growth medium as that reported in *Raphanus sativus* (Raza and Shafiq, 2013), *Oryza sativa* (Choudhury and Panda, 2004), mungbean (Wahid *et al*., 2007) and *Zea mays* (Perveen *et al*., 2011). More adverse effects of Cd toxicity were observed on roots than on shoots implying that roots rapidly absorbed heavy metal in different plant species (Cd) (Piotrowska *et al*., 2010; Bah *et al*., 2011). On the other hand, the addition of SA in Cd-containing growth medium enhanced the growth parameters (shoot and root lengths, shoot and root fresh, and their dry biomasses) similar to the results reported by Krantev *et al*. (2008) in maize (*Zea mays*). Our results may be integrated with the results that emphasized the enhancement of growth parameters with the application of SA under Cd-stressed conditions in *Zea mays* (Krantev *et al*. 2006). The positive associations within all the seedling growth parameters in this study revealed that increases in these traits might improve the biomass (roots and shoots) helpful in plant growth whereas Cd stress might hinder the growth (negative correlation of Cd with all traits).

Drastic effects of Cdtoxicity had been reported on the concentration of mineral nutrients in different plant species (Goncalves *et al*., 2009) and Kim *et al*. (2002) described that Cd entered the root cells of rice through Mg and Ca transporters, and these transporters had the ability to inhibit the transport of Cd. The depressions in the concentration of Ca had been described in higher plants due to the occurrence of polyvalent Cd cations in the medium (Marschner, 2002). Potassium had been considered the essential ion to plant life in regulating cell osmotic balance during stress periods and in the activity of a number of enzymes (Epstein and Bloom, 2005; Taiz and Zeiger, 2010). Phosphorus had multifaceted roles in plant metabolism and development whereas its deficiency led to stunted growth and purple pigmentation of leaves (Epstein and Bloom, 2005). In this study, the increasing Cd concentrations showed drastic effects on the contents of mineral nutrients (Na, K, Ca, Mg, P, Mn and Fe) in shoots as well as in roots like that in wheat (Shukla *et al*., 2003). More reductions in the concentration of shoot Mg and Fe cations under Cd stress observed in this study supported the view that reduced concentrations of Fe and Mg caused chlorosis because these elements had been the essential cofactors of polypeptide enzymes of photosystems (PS-I and PS-II), while the Mn cations had the competing ability with the Cd for uptake in plants. The beneficial effects of SA on seedling growth under Cd stress could be realized in the maintenance of optimal mineral nutrients (Taiz and Zeiger, 2010).

In rice seedlings, the application of SA enhanced the concentration inducing transport of the cations (Ca, K, Fe, Mg, Mn, Na, P) from growth medium to the shoots and roots. This enhancement in the concentration of cations might be ascribed to the SA-induced H^+-ATPase activity (Gordon *et al*., 2004) responsible to increase the absorption of these cations under Cd toxicity (Belkhadi *et al*., 2010). Enhanced concentrations of micro- and macro-nutrients by the addition of SA in the leaves and roots of Maize (Tuna *et al*., 2007) and increases in Na and K concentrations in wheat (Kaydan *et al*., 2007) had been reported. In literature, the SA ability to alleviate the Cd toxicity (Choudhury and Panda, 2004; Krantev *et al*., 2006), and synchronize the metabolic capacity and defense mechanisms in plants (Singh *et al*., 2010) had been described. Negative correlations of Cd with all the mineral nutrients under study suggested that Cd toxicity might hinder the translocation of these nutrients coinciding with the findings of Goncalves *et al*. (2009) in potato. The positive associations among the cations in the present studies indicated the possibility of alleviating Cd toxicity and the enhancements in the concentration of these cations might help in the improvement of the seedling growth and development.

Based on root or shoot Cd contents, seedling growth parameters and STI values, and the status of mineral nutrients, differential genotypic responses were observed. Basmati-198 showed lesser root or shoot Cd contents, higher STI values, and lesser reductions in growth parameters and nutrient assimilations as compared to other genotypes under elevating Cd toxicity exhibiting tolerance whereas Kashmir Basmati was prone to Cd toxicity.

In conclusion, Present studies revealed that Cd toxicity caused detrimental effects on basmati rice seedlings affecting the uptake and distribution pattern of certain mineral nutrients consequently responsible for the disturbances in status of mineral nutrients and the depressions in growth parameters. No effects of SA treatment alone on root Cd or slight reduction in shoot Cd as compared to control revealed the protective SA effects against subsequent Cd toxicity that might be ascribed to the inhibition of Cd uptake in roots or shoots. However, SA addition in the medium under elevating Cd stress improved these parameters revealing the ameliorative response of SA to Cd toxicity. Further studies are suggested to clarify the mechanisms involved in the detoxification of Cd in basmati rice that could further provide information for the selection of Cd-tolerant plants.

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