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Full Length Article

Exploring the Role of Salicylic Acid to Attenuate Cadmium Accumulation in Radish (*Raphanus sativus*)

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Abstract

A field experiment was carried out to assess the role of exogenously applied salicylic acid (SA) on radish (*Raphanus staivus* L.) plants grown in artificially cadmium (Cd)-contaminated soils. For this purpose, SA (300 mg L^{-1}) was applied as foliar spray on control and Cd-stressed (0.5 mM and 1.0 mM $CdCl_2$) plants at vegetative stage and its influence on Cd toxicity at vegetative and mature stages was examined. A progressive increase in the uptake of Cd and its accumulation in radish plant parts was observed with increase of $CdCl_2$ concentration in the soil, which retarded root growth while stimulated the shoot growth. Reproductive stages like inflorescence raceme length, number of branches of inflorescence raceme and number of siliques per plant were enhanced due to presence of Cd in the soil. Foliar application of SA substantially retarded Cd uptake in radish plants thereby improved root vegetative characters resulting in amelioration of Cd-toxicity. Moreover, SA improved shoot characters, contributed to weight gain by mature siliques and positively influenced inflorescence and raceme characters.

Keywords: Cadmium; Salicylic acid; Radish; Cd accumulation

Introduction

The addition of toxic heavy metals to the terrestrial communities as a result of anthropogenic activities is a serious concern as it threatens plant and animal life. Cadmium (Cd) is one such environmental toxicant, which persists and prevails as toxic heavy metal among animals and plants (di Toppi and Gabbrielli, 1999; Zulfiqar *et al.*, 2012). Being regarded as one of the most aggressive metal (Hegedûs *et al.*, 2001), Cd is discharged excessively from industrial source as a by-product (Mengel *et al.*, 2001). Furthermore its release is associated with the application of Cd containing phosphate fertilizers and use of sewage sludge (Grant, 2011) in the agricultural lands for irrigation purpose or as a fertilizer. Exposure to Cd stress initiated modification in physiological functioning of plants, like reduction of photosynthetic activity, inhibition of growth, imbalanced nutrient uptake and water relations, eventually contributing to death (di Toppi and Gabbrielli, 1999). Cd limits crop production and transit its way to food chain by being sequestered and accumulated in edible portion of plants (Michalska and Asp, 2001). The uptake and tolerance of Cd metal varied significantly among different plant species (Metwally *et al.*, 2005; Wahid and Ghani, 2008).

Radish is a vegetable with edible root portion. It is a source of two medicinally important compounds i.e. isothiocyanates and peroxidases (Curtis, 2003). Radish hypocotyls are edible and are exposed to heavy metals because of having direct contact with contaminated soil (Teklić *et al.*, 2008). There are studies that emphasize the impacts of Cd on various aspects of radish at seedling stage (Vitoria *et al.*, 2003; Chen *et al.*, 2003); however, the evidence is scarce when considering its impact on vegetative and mature stages under field conditions.

Different antioxidants are used exogenously in order to mitigate the negative effects of Cd. One such compound is salicylic acid (SA), which is known to have alleviated Cd-induced toxicity in barley roots (Metwally *et al.*, 2003). In addition to its role in abiotic stress tolerance (Klessig and Malamy, 1994; Alvarez, 2000), more recently its role as a signaling molecule which is necessary to amplify or sustain Cd generated oxidative stress was reported (Zawoznik *et al.*, 2007; Guo *et al.*, 2007, 2009). SA showed ameliorative and positive effect on crop growth and yield due to its role in uptake of nutrients (Glass, 1974), regulation of stomata (Larqué-Saavedra, 1979; Arfan *et al.*, 2007), photosynthesis (Khan *et al.*, 2003; Arfan *et al.*, 2007) and plant water relations (Barkosky and Einhelling, 1993). Therefore, the purpose of this study was to improve growth and yield attributes of radish and to draw a relationship between Cd uptake and SA application.

Materials and Methods

Exogenous application of SA on radish variety 40-Days under Cd stress was investigated in a field experiment at

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Govt. College University Faisalabad, Pakistan. Seeds were attained from Vegetable Research Institute, Ayub Agricultural Research Institute, Faisalabad, Pakistan. Faisalabad, Pakistan. Morphological, reproductive stage and biochemical studies were conducted at Environmental Botany Lab, GCU, Faisalabad.

Field Preparation

Experiment was conducted in field by digging the soil up to 90 cm depth. Main plot was divided into 3 sub plots, each with an area of 8.1 m^2 and a polythene sheet was laid down beneath to isolate each sub plot. The sub plots were filled with soil (EC_e 2.24 and pH 6.68). A split plot statistical design was used with three replications to each experimental unit. Sub plot were further divided into four ridges of 2.4 m length and 0.75 m width. Viable seeds were grown in the water imbibed soil and after germination, plants were allowed to establish for 10 days. A 10 cm plant to plant distance was maintained by thinning practice. Three levels of Cd were applied at a concentration of control, 0.5 and 1.0 mM (Vitoria *et al.,* 2003). For stress application sub plots were randomly selected.

Spray Treatment

The foliar application of SA (300 mg L^{-1}) followed 10 days after stress induction (DSI) and the plants were sprayed until drenching. Two controls were set for this experiment as positive control plants were sprayed with water while the negative control plants were left unsprayed. Before spraying, the soil surface was covered and interference of SA with the rooting medium was prevented. Tween-20 (0.1%) was used as surfactant to ensure penetration of spray.

Growth Analysis

Growth attributes were recorded at vegetative stage. Total plant leaf area was calculated by drawing all the leaves on graph paper. Leaf area index (LAI) was calculated with the following formula as described by Amanullah *et al.* (2007).

$$LAI = \text{Leaf area per plant } (\times) \text{ Number of plants per } m^{-2}$$

Cd Analysis

The concentration of Cd in the radish plant parts was quantified after digestion of dry plant material as described by Netondo *et al.* (2004). The Cd content in the digested samples was determined with the aid of Atomic Absorption Spectrophotometer (Analytik Jena, Model - nov, A 400, Germany). Cd accumulation in the radish plant parts was calculated by the following formula.

$$\text{Accumulation} = [\text{Cd concentration } (\times) \text{ DW of plant (g)}].$$

Cd distribution percentage to the total uptake among radish plant parts was also considered.

Reproductive Stage Studies

Inflorescence raceme length, number of branches of inflorescence raceme, number of leaves at reproductive stage, number of silique per plant and weight of 10 mature siliques were recorded at maturity.

Statistical Analysis

The data obtained for each attribute was subjected to ANOVA by using COSTAT statistical software. The Duncan's Multiple Range test (DMR) at 5% level of probability was used to assess the difference among mean values.

Results

Plant Growth and Biomass

Influence of Cd stress levels on both shoot and root length was recorded significant ($P \leq 0.05$). Maximum shoot length was found in 1 mM Cd level (25.7% more than control and 23.8% than 0.5 mM Cd stress) but in contrast highest value of root length was recorded in control plants. The decrease in root length was 30 and 31.6% in 1 mM and 0.5 mM $CdCl_2$ level respectively. SA treatment imparted significant effects on both the attributes and was almost 11% more effective than unsprayed and water sprayed plants (Table 1). Similarly as in shoot length, fresh weight and dry weight shoot of increased up to 27% in 1 mM Cd stressed plants from other two levels.

The SA application was found to be significantly effective in improving fresh and dry weight of shoot in comparison with other two treatments ($P \leq 0.05$). On the other hand, highest values for root fresh weight were observed in control plants (Table 1) and the dry weight of roots followed the similar pattern. Plants grown in 1 mM $CdCl_2$ treated soil exhibited significantly more values of LAI as compared with the other two levels. The effectiveness of spray treatments on LAI was non-significant ($P \leq 0.05$) (Table 1).

Cd Concentration

The amount of Cd in radish plants increased significantly with inclining levels of Cd in the soil. Cd concentration in radish tops was high in unsprayed and water sprayed plants as it ranged from 3.062 to 67.047 mg kg^{-1} and 3.118 to 69.425 mg kg^{-1} respectively but in contrast SA foliar application resulted in reduced Cd contents ranging between 5.379 to 28.988 mg kg^{-1}. In comparison with unsprayed and water sprayed plants respectively, application of SA significantly ($P \leq 0.05$) reduced Cd uptake in radish above ground parts and roots (Fig. 1a).

Cd Accumulation and Distribution in Radish

Cd accumulation was also increased as a result of elevated

Table 1: Morphological attributes of radish (*R. sativus*) variety viz. 40-Days under increasing levels of Cd stress as influenced by exogenous application of control and SA 300 mg L^{-1}

	Length (cm)		RL/SL Ratio	LAI	Fresh weight (g)		Dry weight (g)	
Foliar Spray	Shoot	Root			Shoot	Root	Shoot	Root
Effect of SA on Control Plants								
No Spray	21.5^{bcd}	19.8^{b}	0.97^{b}	4.53^{c}	25.4^{bc}	22.7^{a}	2.15^{b}	1.25^{a}
Water	21.8^{bcd}	20.1^{b}	0.93^{b}	4.48^{c}	25.2^{bc}	22.9^{a}	2.18^{b}	1.22^{a}
SA (300 mg L^{-1})	19.7^{cd}	24.9^{a}	1.27^{a}	5.09^{bc}	23.4^{bc}	20.2^{ab}	2.09^{b}	1.12^{a}
Effect of SA on 0.5 mM $CdCl_2$ stressed plants								
No Spray	16.9^{d}	16.2^{cd}	0.91^{b}	4.72^{c}	21.4^{c}	9.9^{c}	1.8^{7b}	0.65^{bc}
Water (control)	17.3^{d}	16.3^{cd}	0.95^{b}	4.76^{c}	21.8^{ab}	9.7^{c}	1.91^{b}	0.67^{bc}
SA (300 mg L^{-1})	23.1^{bc}	14.5^{d}	0.64^{c}	4.25^{c}	30.2^{ab}	18^{b}	2.63^{ab}	1.03^{a}
Effect of SA on 1mM $CdCl_2$ stressed plants								
No Spray	24.8^{ab}	14.3^{d}	0.57^{c}	6.83^{a}	29.5^{ab}	6.9^{c}	2.41^{ab}	0.52^{c}
Water (control)	25.0^{ab}	14.1^{d}	0.57^{c}	6.90^{a}	29.9^{ab}	6.8^{c}	2.44^{ab}	0.54^{c}
SA (300 mg L^{-1})	29.4^{a}	17.5^{c}	0.60^{c}	6.47^{ab}	36.7^{a}	10.3^{bc}	3.13^{a}	0.97^{ab}

levels of $CdCl_2$ in the soil while SA applied radish plants exhibited prominent reduction in Cd uptake (Fig. 1b). At highest Cd contaminated level (1 mM) total Cd accumulation was 168.56 mg $plant^{-1}$ in unsprayed and 173.58 mg $plant^{-1}$ in water sprayed plants; however, application of SA significantly reduced these values to 90.59 mg $plant^{-1}$ (Table 2).

Reproductive and Yield Attributes

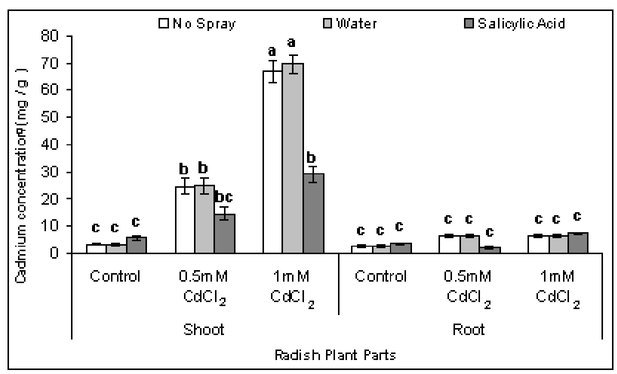
Inflorescence raceme length was significantly affected by Cd stress levels however SA foliar application at vegetative stage did not show much of its influence in this regard. The plants grown in stress exhibited more inflorescence raceme length with respect to control and all the stress levels demonstrate significant deviations (Fig. 2a). The number of branches of the inflorescence raceme was significantly affected by spray treatments as the control plants had an edge over SA applied plants. In addition, the impact of stress levels on number of branches was also significant (Fig. 2b). Plants grown in 0.5 mM $CdCl_2$ showed maximum of number of leaves (30.6%) than control (Fig. 2c). Unsprayed and water sprayed plants exhibited more number of siliques per plant than SA treated ones. Furthermore, the plants grown in 1 mM level of stress had 26.7% and 30.1% more siliques than 0.5 mM and control plants respectively (Fig. 2d). In contrast, SA treated plants showed approximately 17% more weight of 10 mature siliques as compared with control treatments. Similarly weight of mature siliques was more in stressed levels than control plants (Fig. 2e).

Discussion

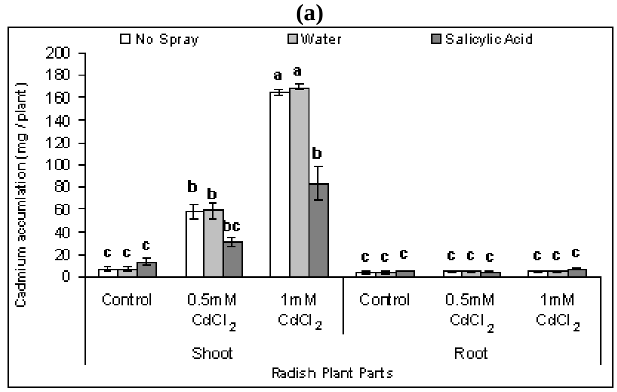
The present study was conducted to evaluate the impact of Cd and SA on radish under field conditions. Reduction in root length, fresh and dry weights of root was recorded with increasing Cd concentration in the soil. A decrease in the root attributes of *Oryza sativa* roots under Cd stress has been reported (Choudhury and Panda, 2004; Wahid *et al.*, 2007). Basically Cd interferes with cellular redox

Table 2: Cd distribution in shoot and root of *R. sativus* under incline cadmium stress levels (Percent of the total uptake)

Cadmium distribution (%) in shoot			
	Control	0.5 mM $CdCl_2$	1 mM $CdCl_2$
No Spray	63.87	91.94	97.21
Water	64.73	92.74	97.65
Salicylic Acid	72.39	88.89	92.29
Cadmium distribution (%) in root			
No Spray	34.65	7.19	2.19
Water	35.26	7.26	2.35
Salicylic Acid	27.60	11.10	7.70



(a)



(b)

Fig. 1a, b: Concentration and accumulation of Cd in shoot and root of *R. sativus* under incline Cd stress levels

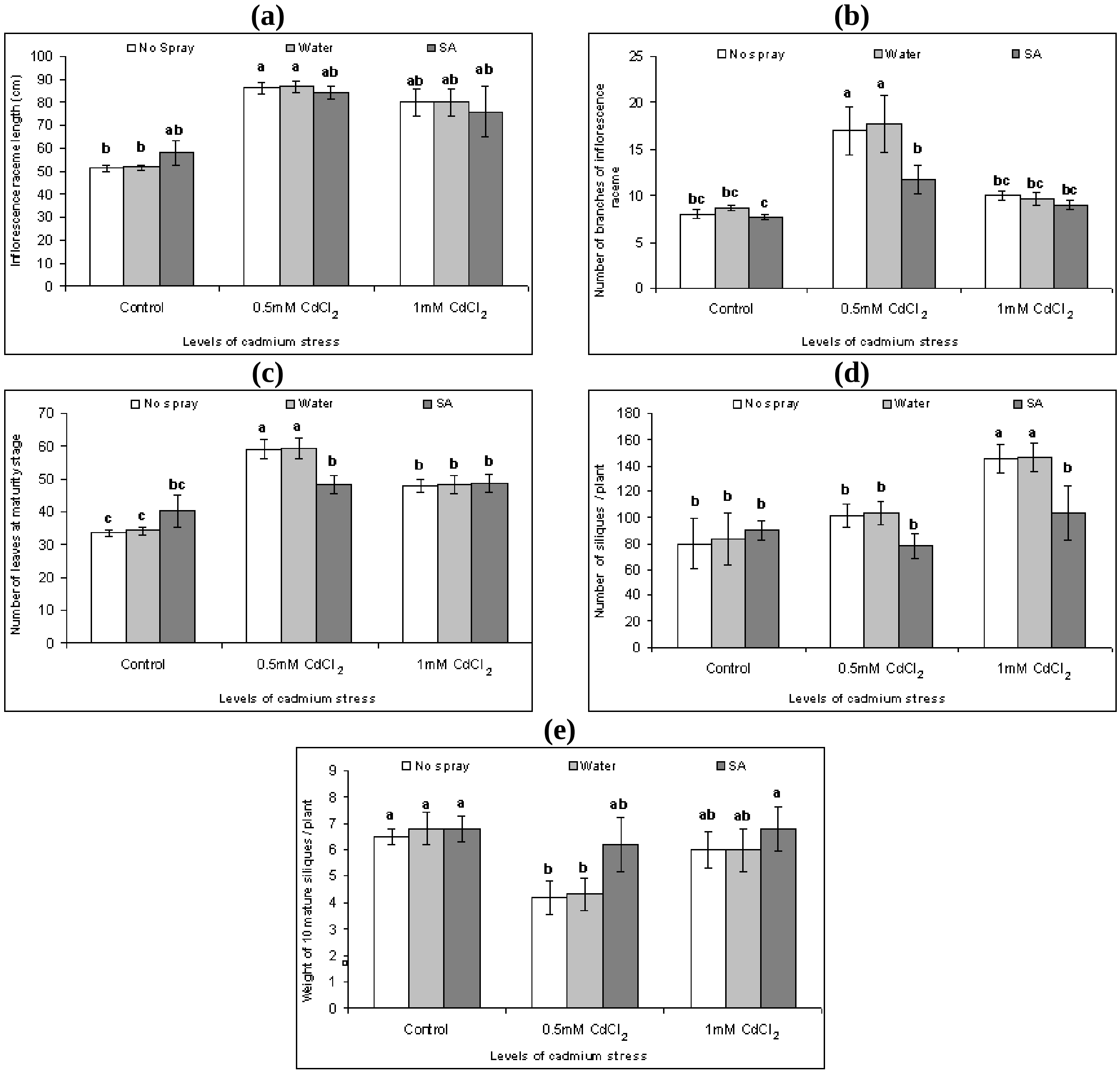


Fig. 2: Influence of incline Cd stress on (a) inflorescence raceme length, (b) number of branches of inflorescence raceme, (c) number of leaves at mature stage, (d) number of siliques per plant and (e) weight of 10 mature siliques

environment therefore generating oxidative stress in roots (Romero-Puertas *et al.*, 2004). Furthermore in response to Cd, an increase in the composition of endodermal suberin and lignin was reported by Schreiber *et al.* (1999) in maize roots. In radish lateral roots, thickening of pericycle and cortical cell walls adjacent to endodermis was reported under Cd-stress (Lux *et al.*, 2010). We attribute this decrease in root characters to the combination of Cd generated oxidative stress, cell wall thickening and deposition of these relatively impermeable substances in root endodermis as a barrier in order to reduce Cd uptake, thus affecting root growth attributes. SA foliar application resulted in improvement of root growth parameters in the Cd-contaminated levels that can be linked with the findings of Metwally *et al.* (2003).

However, contrary to this, enhancive effect of Cd was evident in shoot attributes. The possible stimulatory effects of Cd concentrations on leaf area and biomass accumulation were also reported by Zhang *et al.* (2002) and Ivanov *et al.* (2003). Our results can be integrated with these findings that emphasize on the promontory effects of Cd. Exogenous SA application resulted in the enhancement of shoot growth attributes including length, fresh and dry weight both in normal and Cd stressed condition. This beneficial effect of SA is attributed to its role in improvement of nutrient uptake (Glass, 1974), water relations (Barkosky and Einhelling, 1993), photosynthesis and eventually plant growth (Khan *et al.*, 2003; Wahid *et al.*, 2007).

Above all, increasing Cd levels in the soil resulted in its increased uptake and accumulation in radish plant parts. The greater proportion of the Cd was found in radish above ground parts. SA application resulted in the prominent decrease in Cd content of stress grown plants therefore mitigated Cd toxicity. In agreement with our findings many reports documented that SA application can ameliorate heavy metal toxicity (Zhou *et al.*, 2007, 2009). Likewise, SA application reduced Cd uptake and attenuated toxicity in *Cannabis sativa* (Shi *et al.*, 2009; Ahmad *et al.*, 2011). Similar reports were documented by Pál *et al.* (2002) and Yang *et al.* (2003). The differential accumulation of Cd in plant parts is one of the SA potential physiological effects (Choudhury and Panda, 2004).

Inflorescence raceme length, its number of branches and number of siliques increased due to Cd stress. Although weight of 10 mature siliques was not much affected, thou it also was slightly more in stress grown plants. These results are in agreement with the findings of Bosiacki (2009) who found yield of marigold plants were positively influenced by Cd at a dose of 10 mg Cd dm^{-3}. Additionally a marigold cultivar named as “Mann in Mond” showed higher inflorescence yield at 1000 mg Pb dm^{-3} as compared to its control. The effect of SA foliar spray on reproductive stages was insignificant in comparison with control. Although weight gain by 10 mature siliques in SA applied plants was significantly elevated due to more number of seeds.

In conclusion, Cd exposed radish plants (40-Days) displayed changes at morphological and reproductive level, while differential accumulation of Cd in radish plant parts was observed in response to SA. The application of SA resulted in reduce Cd accumulation in radish plants thus promoted beneficial effects on radish vegetative growth characters, while reproductive stages were triggered positively by presence of Cd in the growing medium. These results can be explained on the basis of two possible reasons based on Cd competition with nutrients and possible interference with enzymes at molecular level. We emphasize there is a need to find out the physiological basis of these enhancements at vegetative and reproductive stage caused by Cd metal in radish plants.

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