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Influence of Salicylic Acid on *Bromus tomentellus* Germination and Initial Growth Properties under Cadmium Stress

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ABSTRACT

Biotic and abiotic stresses affect germination and the growth of plants. Heavy metals such as cadmium (Cd) can decrease seed germination and the growth of plants. Salicylic acid (SA) is able to reduce the negative effect of heavy metals. The current study was carried out to investigate the effect of SA on germination and on initial growth properties of *Bromus tomentellus* under Cd stress. Therefore, *B. tomentellus* seeds were pretreated with 0, 100, 200 and 300 mg/L of SA for 10 h and then placed under stress with Cd at 0, 10, 20 and 30 mg/L. Results indicated that Cd reduced germination percentage from 57% in the control to 40% at 30 mg/L Cd solution without SA pretreatment. Speed of germination was increased to 200 mg/L SA and then decreased. Cd also caused a decrease on it by increasing Cd levels. Results also indicated that Cd reduced plumule length from 5.2 cm in the control to 1.7 cm at 30 mg/L Cd solution without SA pretreatment and radicle length was reduced from 2.4 cm in the control to 0.3 cm at 30 mg/L Cd solution without SA pretreatment. The highest values of plumule and radicle lengths were at 200 and 300 mg/L of SA under Cd stress, respectively.

Keywords: heavy metals, percentage and speed of germination, radicle and plumule lengths
Abbreviations: Cd, cadmium; **SA**, salicylic acid

INTRODUCTION

Cadmium (Cd) is a strong environmental pollutant with high toxicity to animals and plants. It is released from metals, phosphate fertilizers (Nriagu and Pacyna 1988; Popova *et al*. 2008) and rock mineralization (Baker *et al.* 1990). Cd is easily taken up by plant roots and can be loaded into the xylem for transport into leaves. The high mobility of this metal in a soil-plant system makes its entrance to the food chain easier (Nogawa *et al.* 1987). It induces complex changes in plants at genetic, biochemical and physiological levels. The most obvious are: a) the reduction of tissue and organ growth (Liu *et al.* 2003; Choudhury and Panda 2004; Dražić *et al.* 2006; Houda *et al*. 2008); b) ethylene production caused by membrane degradation (Vassilev *et al.* 2004); and c) changes in the content of reactive oxygen species and activity of the antioxidant system (Skórzyńska-Polit *et al.* 2003). In addition, the toxic effect of Cd includes a decrease in the uptake of nutrient elements (Sandalio *et al.* 2001), inhibition of photosynthesis through effects on chlorophyll metabolism and structure of chloroplasts (Gadallah 1995), the activity of both photosystem II and the enzymes of photosynthetic carbon metabolism (Atal *et al.* 1993; Siedlecka *et al.* 1998) and also increases lipid peroxidation, hydrogen peroxide content and superoxide radical production (Choudhury and Panda 2004). Cd also produces alterations in the functionality of membranes by inducing changes in their lipid composition (Hernández and Cook 1977) and this can affect some enzymatic activities associated with membranes such as H^+- ATPase (Fodor *et al.* 1995).

The principal mechanisms of a plant's response to Cd stress include phytochelatin-based sequestration and compartmentalization, as well as additional defence mechanisms, based on cell wall immobilization, plasma membrane exclusion, induction of stress proteins, etc. The degree to which higher plants are able to take up Cd depends on its concentration in the soil and on its bioavailability, modulated by the presence of organic matter, pH, redox potential, temperature and the concentrations of other elements. In particular, the uptake of Cd ions seems to compete for the same carrier with nutrients such as potassium, calcium, magnesium, iron, manganese, cooper, zinc and nickel (Sanita di Toppi and Gabbrielli 1999). One of the plant hormones that can reduce the negative effects of Cd is salicylic acid (SA).

SA is an endogenous growth regulator of phenolic nature which participates in the regulation of physiological processes in plants (Raskin 1992). These include effects on ion uptake, membrane permeability, etc. (Barkosky and Einhelling 1993). In addition, SA interacts with other signalling pathways including those regulated by jasmonic acid and ethylene (Ding and Wang 2003). SA induces an increase in the resistance of seedlings to osmotic stress (Borsani *et al.* 2001), low or high temperature by activation of glutathione reductase and guaiacol peroxidase (Kang and Saltveit 2002), and toxic action of heavy metals (Mazen 2004) by activation of systemic acquired resistance (Metraux *et al.* 1990). It decreases the toxic level of Cd by lowering lipid production, lessening the production of hydrogen peroxide and reducing the generation of superoxide radical. Choudhury and Panda (2004) indicated that, in rice (*Oryza sativa*) 24 h of Cd treatment inhibited root dry biomass and root elongation while presoaking root in SA for 16 h protected the plant against Cd, with only minor changes in root dry biomass and root elongation with a minimal accumulation of Cd. They also noted that SA had a potentiating effect by regulating Cd-induced oxidative stress in roots. Popova *et al.* (2008) also noted that pea (*Pisum sativum*) seed pretreatment with 500 μM SA alleviated the negative effect of Cd on growth, photosynthesis, carboxylation reactions, thermoluminescence characteristics and chlorophyll content and

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led to a decrease in the oxidative injuries caused by Cd.

Bromus tomentellus (poaceae family) is an Irano-Turanian plant which grows in Alborz and Zagros mountainous regions of Iran and is also distributed in South Eastern Europe and temperate Asia (Caucasus and Western Asia). It grows in soil that is neither saline nor alkali, and in deep to shallow soils with moderate texture. This grass is one of the most important grasses in mountainous rangelands used to reclaim and improve degraded rangelands. It reproduces sexually through seed production. It has 90% vigor that decreases rapidly over time reaching 50% after 5 years and 30% after 10 years in storage conditions (Moghimi 2005).

Due to the importance *B. tomentellus* in reclaiming rangelands and the high level of Cd in some of its habitats, the objective of this study was to investigate if pretreatment of seeds with SA could improve the germination of *B. tomentellus* under Cd stress.

MATERIALS AND METHODS

B. tomentellus seeds were collected from Taleghan rangelands in 2009. The seeds were sterilized with 0.1% sodium hypochlorite (NaOCl) (Merck, Darmstadt, Germany) and then washed three times with distilled water. Then the seeds were pretreated in SA (Merck) at three concentrations (100, 200 and 300 mg/L) for 10 h at 25°C; distilled water was used as the control. The seeds were then washed using distilled water. Thereafter, 25 seeds were placed in each Petri dish. The seeds were germinated under Cd stress on Watman No. 1 filter paper (Whatman International Ltd., Springfield Mill, Maidstone, UK) in a germinator at 25°C. Four levels of Cd (0, 10, 20 and 30 mg/L) were tested.

The number of germinated seeds whose plumule was longer than 2 mm was counted during a 10-day period. Germination percentage and speed, and length of roots and shoots were measured. Germination percentage (Camberato and McCarty 1999) and speed were measured by the following formulae:

$$GP = \frac{\sum G}{N} \times 100 \qquad (1)$$

where GP = germination percentage, G = number of germinated seeds and N = total number of seeds.

$$GR = \sum_{i=1}^{n} \frac{S_i}{D_i} \qquad (2)$$

where *Si* is the number of germinated seed at each counting, *Di* is the number of day until *n* counting and *n* is the number of counting.

The study was planned as a completely randomized design. The experiments were carried out with four replications and the data obtained was analyzed using analysis of variance (ANOVA). Means were compared at the 5% level of significance using Duncan's multiple range test with statistical software MSTAT-C version 2.00.

RESULTS

The simultaneous application of SA and Cd caused significant differences in germination percentage, and plumule and radicle length of *B. tomentellus* seedlings (**Table 1**).

Germination percentage and speed

The mutual effect of SA and Cd indicated that the percentage of seeds which were exposed to different concentrations of Cd was significantly different from the control ($P < 0.05$). Cd reduced germination percentage at all Cd treatments compared to the control. For example, germination percentage was reduced from 57% in the control to 40% at 30 mg/L Cd solution without SA pretreatment. SA increased the germination percentage so that in each of the three SA concentrations an increase in germination percentage was observed. In addition, SA improved germination percentage under both stressed and non-stressed conditions. The highest increase in germination percentage was when 200 mg/L SA was used as a pretreatment under non-stressed conditions (i.e., without Cd) (**Fig. 1**).

Table 1 Variance analysis of effect of salicylic acid and cadmium on properties of *Bromus tomentellus*.

VS	df	Germination	Germination speed	Root length	Shoot length
SA	3	51.2^{**}	26.8^{**}	149.2^{**}	22.6^{**}
Cd	3	81.9^{**}	47.9^{**}	96^{**}	105.9^{**}
Cd×SA	9	4.5^{**}	1.5^{ns}	40^{**}	16.8^{**}
Error	48	14.4	0.05	0.01	0.09
CV	-	6.9	13.5	15.1	11

**; Significant difference between treatments at 1%, ns: not significant; VS: variable source, SA: salicylic acid, Cd: cadmium, CV: coefficient of variation

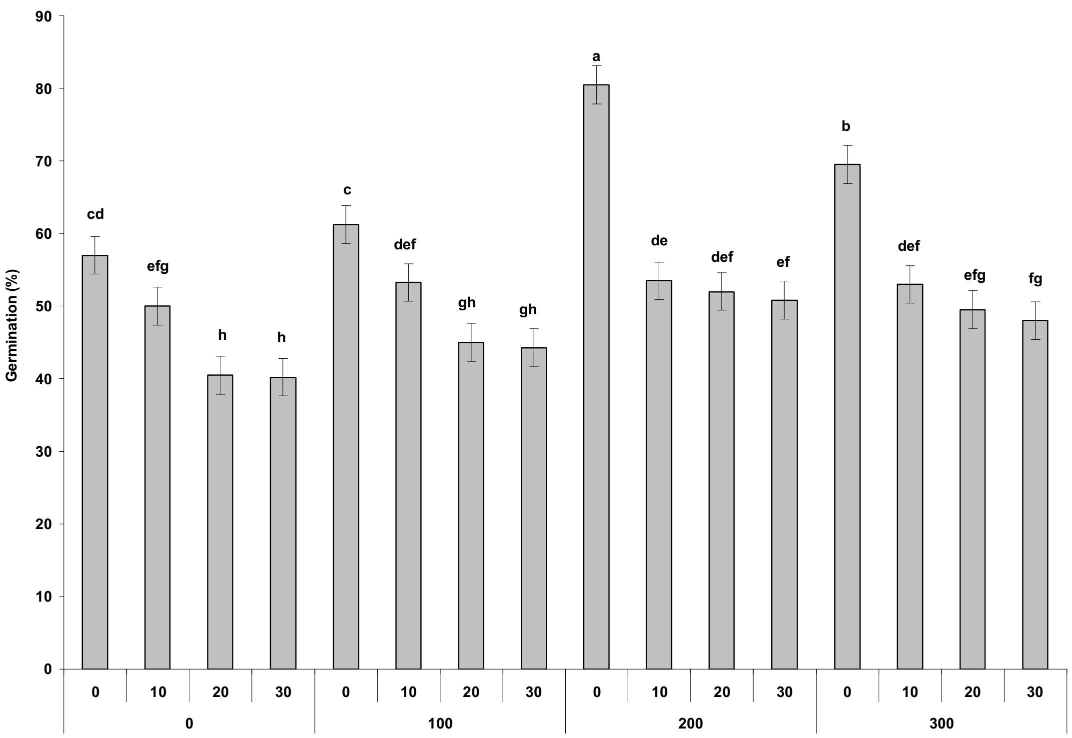

Fig. 1 Interaction of salicylic acid and cadmium on germination percentage of *Bromus tomentellus*.

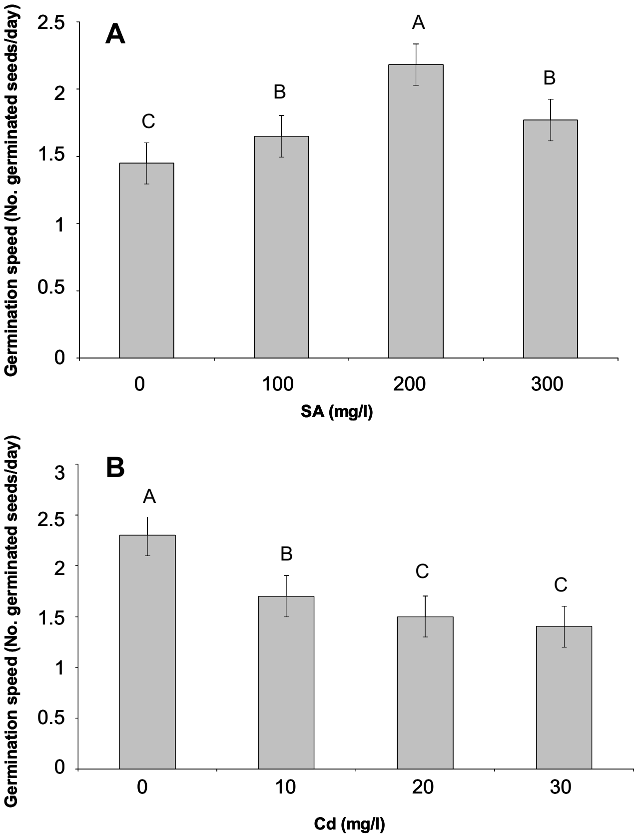

Fig. 2 Effect of salicylic acid (A) and cadmium (B) on germination speed of *Bromus tomentellus.*

Cd reduced germination speed (**Fig. 2B**) with significant differences between the control and different Cd levels ($P < 0.05$). SA increased germination speed relative to the control. The fastest germination occurred at 200 mg/L SA (**Fig. 2A**).

Plumule and radicle length

The interaction effect of SA and Cd significantly affected plumule length ($P < 0.05$). Longest plumules were observed under non-stressed conditions. Cd decreased the plumule length of *B. tomentellus* seedlings, although a mutual effect of SA and Cd indicated that SA improved plumule length under Cd stress. By comparing SA levels, the highest increase occurred at 200 mg/L SA under Cd stress (**Fig. 3**).

The interactive effect of SA and Cd significantly affected radicle length ($P < 0.05$). The longest radicles were observed in the control. SA did not affect radicle length in non-stressed conditions but it increased radicle length of *B. tomentellus* seedlings under Cd stress. The highest increase occurred at 300 mg/L SA. Cd decreased the radicle length of *B. tomentellus* seedlings, the highest decrease occurring under Cd stress without SA pretreatment (**Fig. 4**).

DISCUSSION AND CONCLUSIONS

The response to Cd stress in higher plants is a complex phenomenon. Cd evokes a response of parallel and/or consecutive events, rapid physiological and slow morphological processes, in which every mechanism could be at the same time a cause and effect of metabolic changes, directly or indirectly related to the management of Cd stress (Dražić *et al.* 2006). Cd decreases plumule and radicle length, and germination percentage and speed of *B. tomentellus*. The highest decrease occurred in radicle length under Cd stress without SA pretreatment. Growth reduction under Cd toxicity conditions was observed for several species tested, including *Cucumus sativus* (Abu-Muriefah 2008), *Lemna polyrrhiza* (John *et al.* 2008) and *Glycyrrhiza uralensis* (Zheng *et al.* 2010). The negative effect of Cd may be due to changes in nitrogen metabolism (Boussama *et al.* 1999), inhibition of photosynthesis (Gadallah 1995; Januškaitienė 2010), increase of lipid peroxidation, hydrogen peroxide content or superoxide radical production (Choudhury and Panda 2004) and alteration of membranes (Hernández and Cook 1977) and enzymatic activities (Fodor *et al.* 1995).

SA could improve radicle length of *B. tomentellus* with the greatest increase occurring at 300 mg/L SA under all levels of Cd stress. SA also increased plumule length so that the highest increase occurred at 200 mg/L SA under Cd stress. SA-induced stimulation of growth has been noticed in other plant species (Sharikova *et al.* 2003), as well as its effect in suppressing the effects of abiotic stresses such as temperature stress (Kang and Saltveit 2002; Tasgin *et al.* 2003) and heavy metal stress (Metwally *et al.* 2003). Popova *et al.* (2008) noted that seed pretreatment with 500

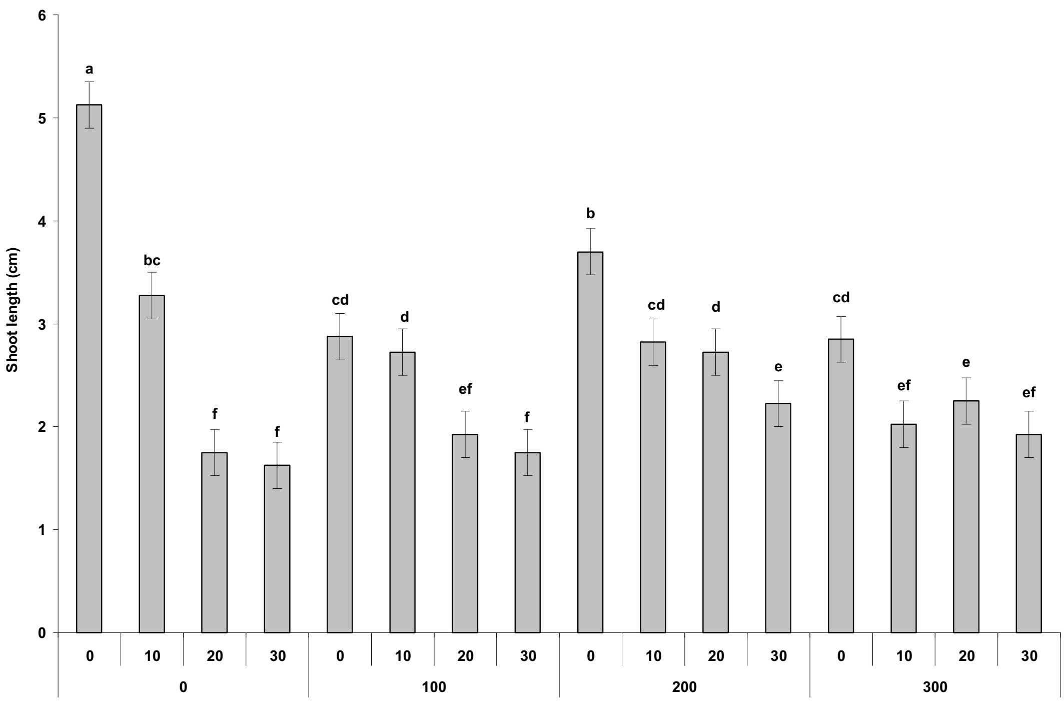

Fig. 3 Interaction of salicylic acid and cadmium on shoot length (plumule) of *Bromus tomentellus.*

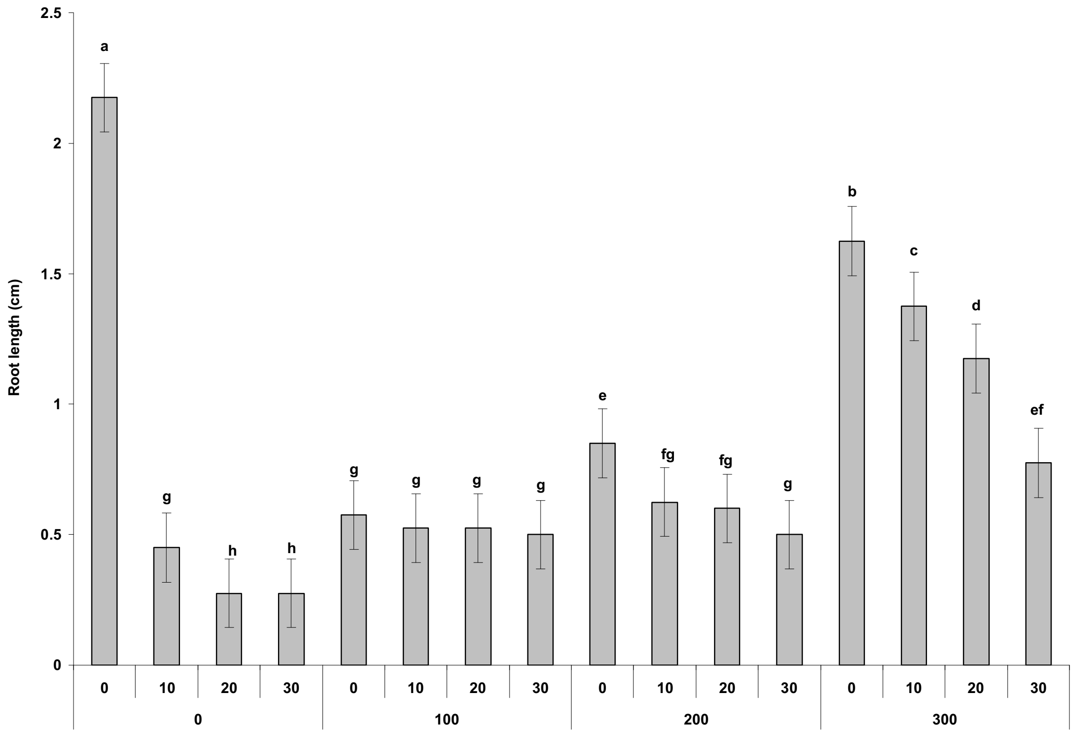

Fig. 4 Interaction of salicylic acid and cadmium on root length (radicle) of *Bromus tomentellus*.

μM SA alleviated the negative effect of Cd on growth, photosynthesis, carboxylation reactions, thermoluminescence characteristics and chlorophyll content and led to a decrease in the oxidative injuries caused by Cd in common pea (*Pisum sativum* L. cv. 'Ran'). The protective action of SA in the presence of heavy metals is linked with cell membrane stabilization (Mishra and Choudhuri 1999), change in hormonal balance (Sharikova *et al.* 2003) and Cd ion inactivation (Metwally *et al.* 2003). Mazen (2004) reported that SA significantly decreased lipid peroxidation and delayed senescence in Cd-treated *Arabidopsis.* These effects of SA might be achieved by SA-induced protein synthesis and stimulation of antioxidants. SA also caused an increase in the level of plant hormones (such as auxin and cytokinin) and proline (Sharikova *et al.* 2003).

Presoaking seeds for 10 h with different levels of SA before exposure to Cd had a beneficial effect on root and shoot length, percentage and speed of germination, and led to a decreased oxidative injury caused by Cd. Although SA participates in the development of stress symptoms, it is also needed for adaptation to and the induction of stress tolerance. Most abiotic stresses increase the concentration of SA in the plant, which points to its involvement in stress signaling. SA is a direct scavenger of hydroxyl radical and an iron-chelating compound, thereby inhibiting the direct impact of hydroxyl radicals as well as their generation via the Fenton-reaction (Dinis *et al.* 1994).

The amount of Cd in the radicle was higher than in the plumule; consequently, radicle length was severely reduced during the emergence of leaflets. Burning and drying which was found on radical late experiment also confirmed this result. Zheng *et al.* (2010) stated that under Cd stress, hypocotyls and radicles lengths of *Glycyrrhiza uralensis* reduced significantly by Cd toxicity treatments. They stated the rate of decline in radicle lengths was more pronounced under Cd stress compared to hypocotyl length whose highest decrease was observed at 0.4 mmol/L Cd. Cd usually accumulates in the roots, because this is the first organ exposed to heavy metals in the soil, but it is also translocated into the shoots. It seems that pretreatments of seeds with different levels of SA had a higher effect on the radicle than on the plumule under Cd stress. Obviously, radicle length under Cd stress increased in proportion to the increase in SA concentration so that the highest growth was observed at 300 mg/L SA.

In summary, SA can reduce the toxic effects of Cd via antioxidant defense mechanisms, change of hormonal balance, increasing some plant hormones such as auxin and cytokinin, aggregating proline, increasing photosynthesis and the stability of membranes, while inactivating and removing toxic metals such as Cd from metabolic processes.

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