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Effects of exogenous salicylic acid on growth characteristics and biochemical content of wheat seeds under arsenic stress

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Abstract

The present study illustrates the phytotoxic effect of As on wheat seedlings and pre-application of salicylic acid in alleviating toxic effect of arsenic. Wheat seedlings treated with different concentrations (50-400µM) of arsenic decreased the germination rate (34.7% and 86.9%), root and coleptile length, fresh and dry weight of roots and coleoptile, chlorophyll (67%) and protein content (27.1%), while increased proline and MDA content. However, pretreatment with 1mM saliycilic acid partially alleviated the toxic effect of arsenic on germination parameters and significantly reduced the proline (181.2%) and MDA (80%) content thereby increasing chlorophyll and protein content in As stressed wheat plants ($p<0.01$ or $p<0.05$). The data suggests that saliycilic acid reduced the damaging effects generated by As and enhanced the tolerance of wheat plants to arsenic toxicity.

Key words

Arsenic toxicity, Chlorophyll, Germination, Proline, Salicylic acid, Wheet

Introduction

Arsenic (As) is a toxic metalloid widely distributed in the environment. The source of arsenic in groundwater is primarily geogenic through dissolution of arsenic compounds absorbed onto pyriteores into water by geochemical factors. Earlier, inorganic As compounds such as calcium arsenate, lead arsenate, sodium arsenate and many others were used as insecticides/pesticides, for debarking trees, in cattle and sheep dips to control ticks, fleas, lice and also in controlling aquatic weeds. However, application of inorganic As compounds in agriculture has gradually reduced since 1960s due to greater understanding of As toxicity and awareness regarding food safety and environmental contamination (Smith *et al.,* 1998). Groundwater arsenic contamination is a widespread problem due to leaching of naturally occuring arsenic into drinking water (Garg and Singh, 2011). This has caused a global epidemic of As poisoning, especially in Bangladesh and West Bengal (India), with skin lesions, cancers and other symptoms (Mondal *et al.,* 2006). Several studies carried out in different plant species have reported that As toxicity hampers normal growth of plants, interfers with normal metabolic functions, induces oxidative stres by generating reactive oxygen species and eventually results in plant death (Carbonell-Barrachina *et al.*, 1998; Abedin and Meharg, 2002).

Salicylic acid (SA) is a well known naturally occurring signaling molecule that affects various physiological and biochemical activities of plants. It is an endogenous plant growth regulator and has been found to regulate growth and productivity of plants. SA not only helps in establishing and signaling defense response against various pathogenic infections, but also play an important role in mediating plant response to some abiotic stresses such as salinity, temperature, UV radiation, ozone and heavy metal stress (Hayat *et al.,* 2010). Recenly, SA has been found to alleviate heavy metal induced toxicity in barley (Metwallyet et. *al.*, 2003), rice (Panda and Patra, 2007) and maize seedlings (Krantev *et al.,* 2008). Most of the studies have reported the role of SA in provoking resistance to heavy metal in plant seedlings, however not much has been emphasised on its effect on seed germination. Seed represents the most protective stage in the life cycle of plants and is well-protected against various environmental stresses. However, soon after imbibition and protrusion of embryonic axis, seeds in general become stress sensitive (Liu *et al.,* 2005).

In light of the above, the present study was carried out to examin the ameliorative effect of SA on As induced toxicity on growth and biochemical parameters of wheat plants.

Materials and Methods

Seeds of wheat (*Triticum aestivum* L.) were used for experiment. Application of salicylic acid (SA) was 1mM. Eight different concentrations of arsenate were used at concentrations 50-400 µM (8 different concentrations). All preparations were made in distilled-deionized water. For experiment, 30 seeds were soaked in different test concentrations in plastic beakers, for 4 hrs at 24 °C, and further germinated in petri dishes for 72 hr in a plant growth cabinet. Germination rate was noted every 24 hr. A 1-mm radicle emergence from seeds was considered as seed germination. However, root elongation and coleoptile growth of germinated seeds were analyzed at 72 hr of incubation (Munzuroglu and Zengin, 2006), a duration required for studying physiological and biochemical parameters. The roots of the seedlings were washed with deionized water, and the seedlings were divided into eighteen groups, each consisting of 10 seedlings. Eight groups were placed into jars containing different concentrations of arsenate (50-400 µM), eight groups into jars containing 1mM SA+ different concentrations of arsenate (50-400 µM), one group into jar containing only 1mM SA and one group into jar containing Hoagland solution that served as control and left for ten days under laboratory conditions. After 10 days seedlings were analyzed for various growth and biochemical parameters. Root and coleoptile fresh weight were determined. For dry weight determination, root and coleoptile were separated from seeds and oven-dried at 70°C for 72 hr before weighing (Kacar, 1972).

Chlorophyll pigment from treated and control leaves were extracted in 80% acetone following the method of Arnon (1949). Chlorophyll a and b content were calculated by the equation given by Graan and Ort (1984). Proline content in leaf tissues were estimated by acid ninhydrin method (Bates *et al.*, 1973). Lipid peroxidation was measured in terms of malondialdehyde (MDA) content by TBARS method (Heath and Packer, 1968). Protein was extracted from the fresh leaves following the method of Larson and Beevers (1965), while protein content was determined according to the method of Lowry *et al.* (1951). Statistical analysis was carried out by SPSS program. In order to detect the significance of differences ($p<0.01$ or $p<0.05$) of variables, a multiple comparison (LSD) test was performed. All values were expressed as mean ±SE. The experiment was carried out in triplicate.

Results and Discussion

Wheat seedlings treated with different concentrations of As inhibited seed germination, coleoptile and root length in a dose dependent manner. Seed germination after 72 hr of As treatment was reduced by 34.7%, 51.7%, 62.1% and 86.1% in 100, 200, 300 and 400 µM As. However, pretreatment of seeds with 1 mM SA induced seed germination by 24.1%, 34.4%, 58.5% and 86.1% in 100, 200, 300 and 400 µM As treated seeds, respectively (Fig. 1).

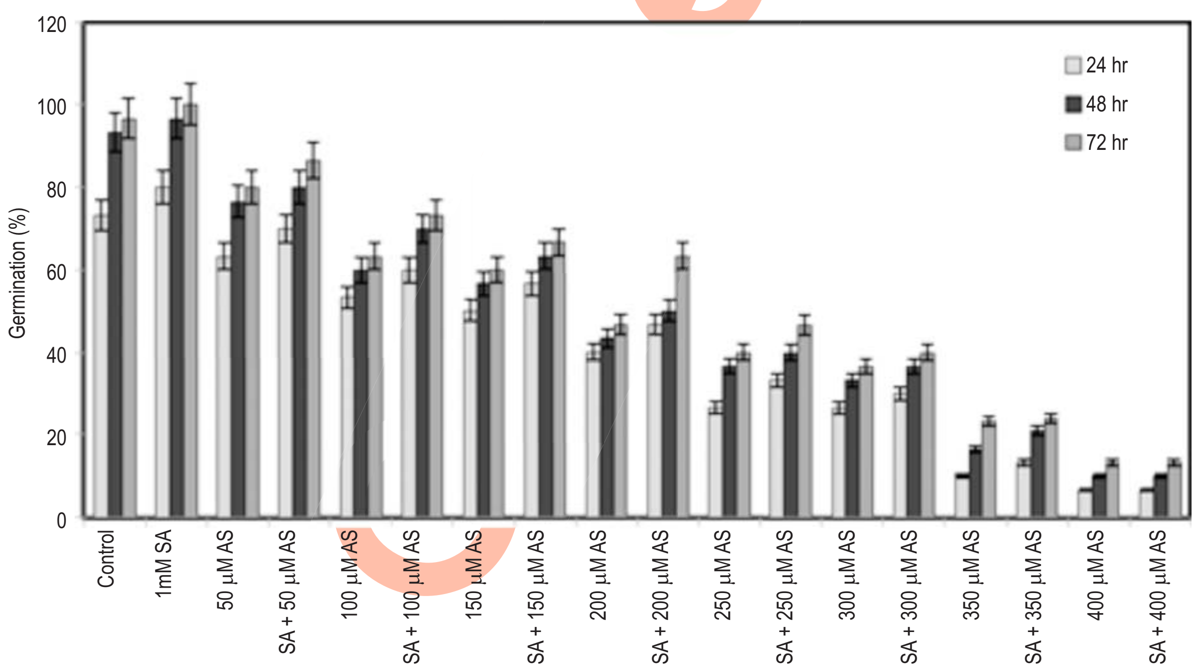

Fig. 1 : Germination rate in wheat seedlings treated with different doses of arsenic alone and in combination with salicylic acid. Values are mean of three replicates ±SD

Root length decreased significantly by 28.8%, 59.4%, 79.4% and 86.8% in 100, 200, 300 and 400 µM As treated seeds (Fig. 2A). However, 1 mM SA pretreatment considerably reduced the inhibitory effect of As on root length by 24.3, 48.9, 70 and 83.4% in 100, 200, 300 and 400 µM As treated seeds. As induced inhibitory effect on coleoptile length ($p<0.05$) (Fig. 2B) by 12.9, 47.8, 65.7 and 72.9% in 100, 200, 300 and 400 µM treated seeds, while in 1 mM SA pretreated seeds decrease was only 1.6, 39.7, 62.4 and 69.6%, respectively. Root fresh and dry weight decreased significantly by 27.1, 34.5, 57.6, 75.2% (Fig.3A) and by 12.4, 26.5, 47.2, 69.6% (Fig 3B) in 100, 200, 300 and 400 µM As treated seeds. While in SA pretreated seeds, root fresh and dry weight decreased by 19.3, 24.9, 53.3, 72.5% (Fig.3A) and 4.8, 20.4, 44.1, 68.4% (Fig.3B) in 100, 200, 300 and 400 µM As treated seeds, respectively. Similarly, fresh weight of coleoptile was reduced by 18.7, 33.8, 50.8 and 69.8% and dry weight by 10.1, 24.7, 46.7 and 67% in 100, 200, 300 and 400 µM As treated seeds (Fig. 4A). Combined treatment of 1 mM SA with different As doses however, reduced coleoptile fresh weight by 11.2, 27.7, 47.2 and 67.8% and dry weight by 2.9, 19.9, 41.3 and 66.8%, respectively (Fig. 4B).

Chlorophyll content (a+b) in leaves of wheat seedlings decreased significantly with increase in As concentration with respect to control. Maximum reduction of 67% in chlorophyll content was noted in 400 µM As treated seeds. However, 1 mM SA pretreatment prevented reduction in chlorophyll content in As stressed wheat seedlings (Fig. 5A). As shown in Fig. 5B and C, wheat seedlings treated with different concentrations of As significantly increased proline and MDA content in a dose-dependent manner. Maximum increase in proline (181.2%) and MDA (80%) content was noted in 400 µM As treatment. In contrast, proline and MDA content increased under different combined treatment of As+1 mM SA treatment (Fig. 5B and C) showing maximum reduction of 147.9% in proline and 49.5% in MDA content in 400 µM As +1m MSA treatment.

As compared to control seedlings, protein content in As treated seedlings decreased in a dose dependent manner showing maximum reduction of 27.1% at 400 µM As treated seeds (Fig. 5D). Whereas combined treatement of 1 mM SA with different concentrations of As significantly lowered (24.5%) protein content in wheat seedlings as compared to individual As

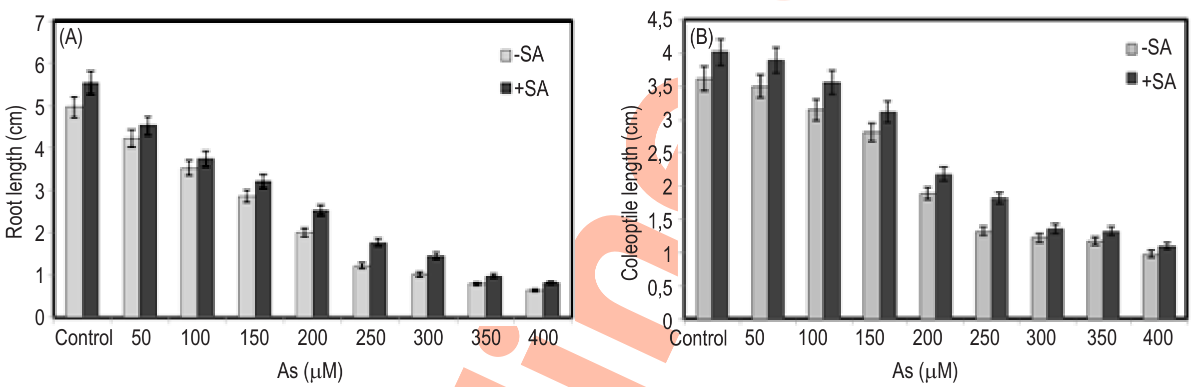

Fig. 2 : Root length (A) and Coleoptile lenght (B) of As treated wheat seedlings with (+SA) with and without (-SA) salicylic acid pre-treatment. Vertical bars indicate mean ±SE

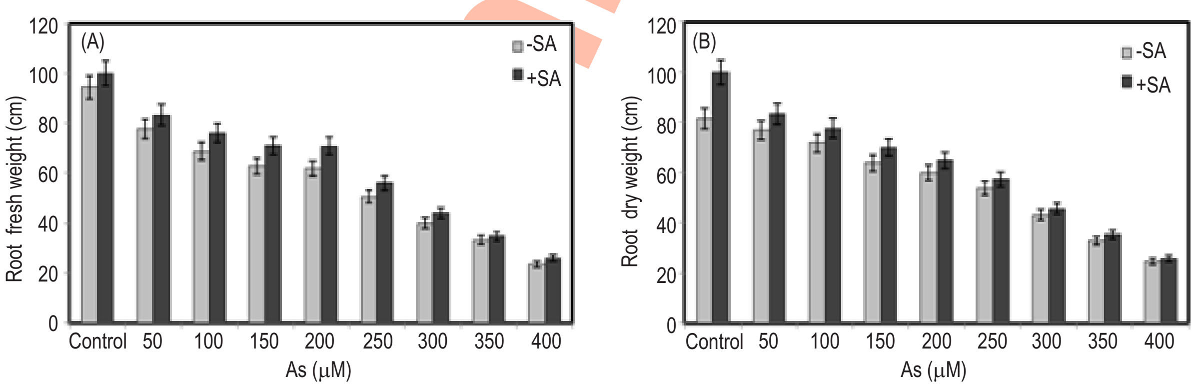

Fig. 3 : Root fresh weight (A) and root dry weight (B) of As treated wheat seedlings with (+SA) with and without (-SA) salicylic acid pre-treatment. Vertical bars indicate mean ±SE

treatment. Seed germination is one of the most sensitive process to metal pollution because of lack of defense mechanisms and hence is an important consideration while studying the effect of heavy metals on seedling growth (Liu *et al.,* 2005). In the present study, SA pretreatment ameliorated As phytotoxicity in wheet seedlings. The results of the present study are consistent with the previous reports regarding As toxicity in plants. Mishra and Choudhuri (1996) reported that SA (Raskin, 1992) alleviated the inhibitory effect of Pb and Hg on seed germination of *Oryza sativa*. In addition, treatment with exogenous SA caused H_2O_2 accumulation in *Arabidopsis*, tobacco and mustard (Rao *et al.,* 1997).

Yadav *et al.* (2014) reported decrease in all the growth parameters, viz., fresh mass, shoot length and root length of *Helianthus annu* L. var. DRSF-113 seedlings due to As toxicity. Similarly 30% decrease in biomass production and increase in anthocyanin concentration was observed in As treated *Azolla caroliniana (*Rofkar *et al.,* 2014).

A significant reduction in root length was observed as compared to shoot length due to the fact that plant roots are the first site of contact with these toxic arsenic species. Reduced root length growth in response to arsenic treatment has been reported by Hartley-Whitaker *et al.* (2001) and Liu *et al*. (2005) *Holcus lanatu* and *Triticum aestivum*. Peralta *et al.* (2001) tested the effect of five heavy metals on root and plant growth of alfalfa and reported that exogenous application of SA ameliorated the damaging effect of these heavy metals. Drazic *et al.* (2006) reported that pretreatment with low concentrations of salicylic acid enhanced root and shoot growth in alfalfa plants, which was otherwise inhibited due to Cd toxicity. Choudhury and Panda (2004) investigated the ameliorative role of SA on Cd induced oxidative stress in roots of *O. sativa*. Their study revealed that Cd treatment led to resulting in concomitant accumulation of Cd loss of root elongation, growth and biomass of roots, thereby, generating oxidative stress in plants. However, SA pretreatment decreased the toxic effects generated by Cd as observed by low MDA content, lesser production of H_2O_2, reduction in generation of superoxide radicals and stability of membranes.

Results of the present study revealed that arsenate adversely inhibited the fresh and dry weight of root and coleoptile in wheat seeds. *L. leucocephala* seedlings showed gradual decrease in dry weight with increase in Pb and Cd concentrations (Shafio *et al.,* 2008). Maheshwari and Dubey (2009) reported that SA pre-application significantly relieved the retarding effect of high concentration of Ni on growth, fresh weight and leaf dry weight in rice seedlings. Kazemi *et al.* (2010) and El Tayep *et al.* (2006) reported that dry weight of roots and shoots decreased in *Brassica napus* and *Helianthus annus* due to Ni and Cu treatment, however SA pretreatment relieved the toxic effects of Cu on the growth parameters of *Helianthus annus*. Rofkar and Dwyer (2013) showed that high concentration of arsenic (25 mg L^{-1}), plant biomass, leaf area, and total chlorophyll were all lower than values in control plants.

In the present study, chlorophyll content decreased significantly with increase in As concentrations which might be due deterioration of chlorophyll pigments that eventually led to decrease in photosynthetic efficiency in plants and ultimate reduction in plant growth (Upadhyay and Panda, 2005). Dehabadi *et al.* (2013) showed that toxic effects of As was reflected by reduction in growth parameters and photosynthetic pigments.

Proline accumulation in response to As toxicity and its alleviation due to exogenous application of SA has been reported in several plants (Mishra and Dubey, 2006; Matysik *et al.,* 2002). Proline accumulation in plants is usually observed under various stressed conditions. Although, its mechanism is not fully understood, proline accumulation is suggested to be an adaptive response against various abiotic stresses. SA inhibited As-induced increase of MDA in this study, indicated that SA alleviated the As induced oxidative stress in wheat. Benhamdi *et al.* (2014) reported that MDA level in (*Hedysarum pallidum* and *Lygeum*

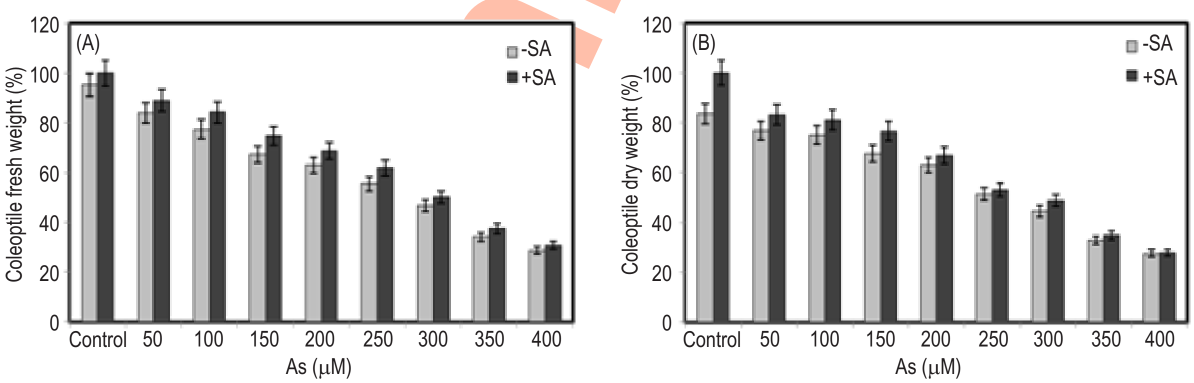

Fig. 4 : Coleoptile fresh weight (A) and coleoptile dry weight (B) of As treated wheat seedlings with (+SA) with and without (-SA) salicylic acid pre-treatment. Vertical bars indicate mean ±SE

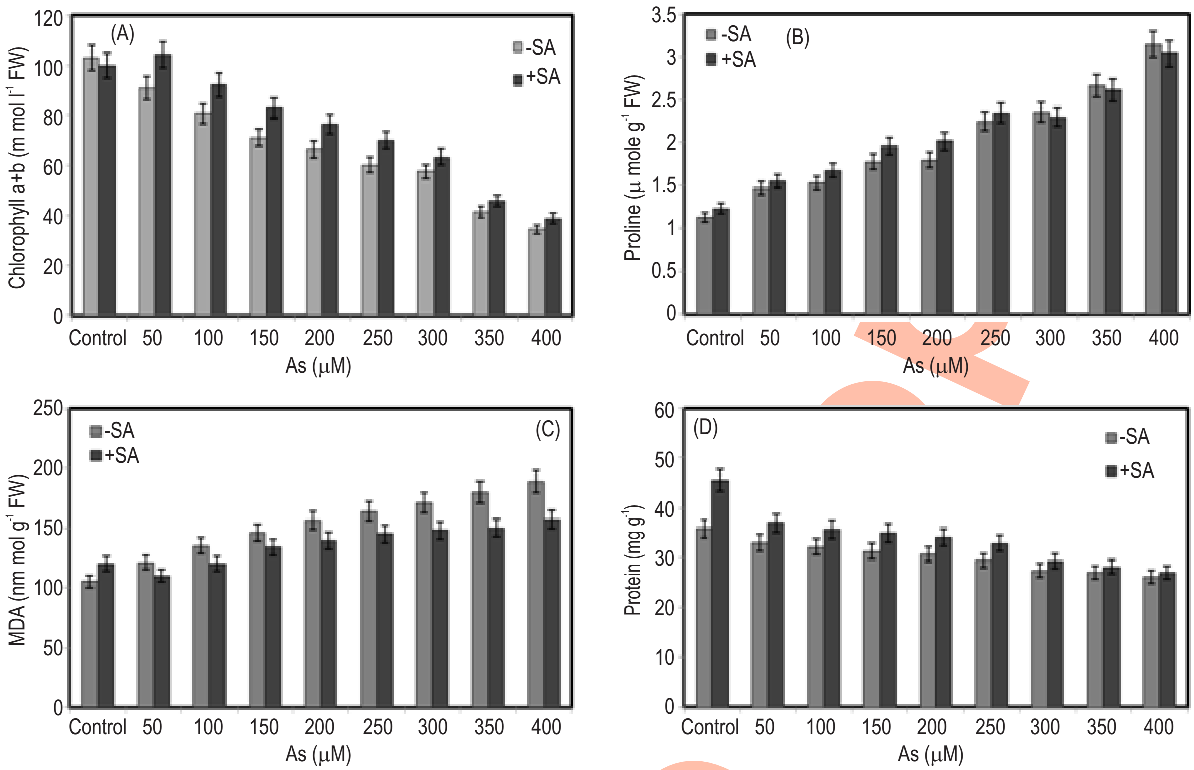

Fig. 5 : Chlorophyll (a+b) content [μmol l^{-1} f.wt.] (A); Proline content [μmol g^{-1} f.wt.] (B); MDA content [nmol g^{-1} f.wt.] (C); Protein content [mg g^{-1}] (D) of untreated SA (–SA) and SA-pretreated (+SA) in the leaves wheat seedlings at various As concentrations. Vertical bars indicate mean ±SE

spartum), increased significantly with increase in soil Sb and As concentrations.

SA treatment has been found to reduce MDA concentrationin many plants under heavy metal stress (Wang *et al.,* 2011; Popova *et al.*, 2009). Decreased oxidative damage may partially be ascribed to the antioxidant attribute of SA and to the role of SA in activation of antioxidant responses (Popova *et al.*, 2009).

In the present study, SA pretreatment inhibited MDA content in As stressed plants indicating reduced oxidative stress in wheat seedlings. Similar results have earlier been reported in *Vallisneria natans* and *Pisum sativum* (Wang *et al.,* 2011; Popova *et al.,* 2009). Increased oxidative damage may be partially ascribed to the role of SA in activating antioxidant response (Popova *et al.,* 2009). These results suggests that exogenous application of SA improved plant tolerance towards As phytotoxicity.

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