Influence of arsenate on seed and seedling growth of *Triticum aestivum*

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Abstract

Damaging effect of arsenate(V) was investigated on seed germination and seedling growth of wheat (*Triticum aestivum* L.). Experiment was conducted in Petri plates lined with filter papers moisturized with 3 mL of each of four concentrations of As(V) i.e. 0.25, 0.5, 0.75 and 1 ppm and kept for 72 hours in a completely randomized design at 25 ± 2 °C in growth chambers. Increasing concentrations of As(V) significantly suppressed germination by 25-50%, length and dry weight of shoot by 50-95% and that of root by 60-96% over control. GI (germination index) and RGR (relative germination index) were significantly reduced while RAIR (relative arsenic injury rate) was significantly increased with increasing the concentrations of As(V). It was concluded that As(V) has potential to drastically reduce seed and seedling growth of wheat within concentration range of 0.25-1 ppm.

Keywords: Arsenic, cereal, heavy metal, seedlings growth, wheat.

Introduction

Study of plant response to heavy metal pollution is important for the management of healthy ecosystem (Ali *et al*., 2007). It has been stated that of three toxic metals viz., arsenic (As), chromium(Cr) and copper(Cu), As is of the most concern because of its carcinogenic nature (Fayiga *et al*., 2007) . Whereas, its extensive prevalence in the soil and water has increased potential agricultural and environmental hazard worldwide, and become a serious problem for safe food production during past two-decades (Zhang *et al*., 2009; Abdollahi *et al.,* 2011; Nawazish *et al.,* 2012; Akhtar and Shoaib, 2012). Volcanically derived sediment, sulphide minerals, and metal oxides are natural sources (Mailloux *et al.,* 2009), while various industrial wastes, mining activities, sewage sludge and pesticides are man-made sources of As (Eisler, 2004). National Standard for maximum acceptable concentration (NEQS) of As in drinking water is 0.05 ppm that value is identical in several countries including India and Bangladesh based on an earlier WHO (1971) advice.

Plants respond differently to As toxicity (Su *et al*., 2010). Various researches have reported presence of As (0.007 to 7.50 mg kg^{-1}) growing in contaminated areas (Roychowdhury *et al.,* 2002; Dahal *et al*., 2008; Bhattacharya *et al*., 2010). Smith *et al*. (2009) have documented that relatively high concentration (30 mg As kg^{-1}) of As in radish. Pigna *et al*. (2010) reported increased level of As in wheat with increasing its concentration in soil and water. Finnegan and Chen (2012) stated that As is generally non-essential and toxic to plant and sensitive plant species could die upon exposure. Kundu *et al.* (2012) found higher concentration of As in roots than other part of wheat plant. Srivastava and Sharma (2013), observed alteration in the normal growth and development of black gram plants due to As stress.

In Pakistan, high levels of As has been documented in groundwater of areas lying along the Indus River (Multan, Bahwalpur and Rahim Yar Khan) (Mandal and Suzuki, 2002; Ahmad *et al.,* 2004) and River Ravi (Sheikhupura, Kasur, Sargodha and Jhang lying) (PCSIR, 2000). Likewise, in a study jointly conducted by UNICEF (2004), As was found @ 10 µg L^{-1}, 10-50 µg L^{-1} and 50 µg L^{-1} in 60%, 31% and 9% of the total ground water samples taken from 32 districts of the Punjab. In previous reports (Dawn, 2000, 2004), urgent attention was directed towards deteriorating water quality in villages close to Lahore, Sahiwal, Kasur and Multan due to As accumulation. So far, Javied *et al*. (2009) in their findings has declared the catastrophic situation in irrigation water, cultivated and uncultivated areas of the Multan due to As containing untreated waste of Pak-Arab factory. Abbas *et al*. (2010) detected the accumulation of some heavy metals including As in different vegetables grown in the Sindh. Baig *et al*. (2011) in their research findings highlighted the increased danger of growing food crops (wheat, maize and sorghum) in the agricultural land continuously irrigated by As contaminated ground water in Khairpur.

There is urgent need to address As relevant problems on crops growing in Pakistan. Current investigation was conducted *in vitro* to assess the influence of different concentrations of As on seed germination and seedling growth of wheat.

Materials and Methods

Natriumarsenate ($Na_3AsO_4.7H_2O$ MERCK, Germany) was used for preparation of 1000 ppm solution of As(V). Further desired dilutions (0.25, 0.5, 0.75 and 1 ppm) were prepared by diluting solution with double distilled deionized water. Healthy seeds of wheat var. Sehar-2006. were surface sterilized with 1% solution of sodium hypochlorite for 1 minute followed by repeated washing with distilled water.

Twenty-five surface sterilized seeds were placed on Petri dishes (90 mm diameter) with single layer of filter paper (Whatman No. 42) moisturized with 3 mL of metal solution in different concentrations i.e. 0.25, 0.5, 0.75 and 1 ppm. Seeds in control treatments were treated with 3 mL of distilled water. Petri plates were kept in a completely randomized design at 25 ±2 °C in growth chambers with 10 hrs light period daily. Each treatment was replicated four times. The root and shoot lengths (cm) and fresh and dry weight (g) of germinated seeds were evaluated at 4^{th} day of incubation. For control treatments, distilled water was used by repeating above procedure.

Percentage germination, germination index (GI), relative germination rate (RGR) and relative arsenic-injury rate (RAIR) were calculated for each treatment using following formulas (Li, 2008).

$$\text{Germination rate} = \frac{\text{Total No. of germinated seeds}}{\text{Total No. of seeds}} \times 100$$

$$\text{Germination index} = \frac{\text{Total No. of germinated seeds}}{\text{No. of days required to germinate}}$$

$$\text{RGR} = \frac{\text{Germination \% in arsenate concentration}}{\text{Germination \% in control}}$$

$$\text{RIR} = \frac{\text{Germination \% in control - Germination \% in arsenate}}{\text{Germination \% in control}}$$

Results and Discussion

Germination was observed at all applied concentration of As(V). However, rate of germination was significantly suppressed by 25% at metal concentration of 0.25 and 0.5 ppm over control. At these two concentrations germination rate was non-significantly differ from each other. Whereas, significantly greater decline of 40-52% in germination rate was recorded due to metal concentration of 0.75 and 1 ppm over control (Table 1). GI and RGR was significantly reduced by 0.75-0.5, while RAIR was significantly increased by 0.25-0.53 with increasing the concentrations of As(V) from 0.25 to 1 ppm (Table 1). The germination percentage may reflect the reaction rate of plant seeds to their living environment and germination index (GI) reflects seed quality (Chun *et al*., 2007). Similar to present investigation, Abedin *et al.* (2002), Chun-Xi *et al.* (2007) and Bhattacharya *et al.* (2012) reported negative effect of arsenate on germination in rice, pea, bean and wheat seeds with increase in concentration. Reduction in germination could be due to direct exposure of the radical to metal toxicity.

Root growth parameters found to exhibit the maximum sensitivity against As(V) as compared to rest of plant parameters investigated in present study. Thus, there was statistically gross decline of 82-96% in root length with increasing metal concentration from 0.25 to 1 ppm over control. The root fresh and dry weight was significantly decreased by 50-60% at 0.25 ppm and by 70-80% at both 0.5 and 0.75 ppm as compared to control. However, the maximum reduction of 95% in root fresh and dry weight was recorded due to the highest dose of 1 ppm over control (Fig. 1 A-C). Shoot length, fresh and dry weight was significantly suppressed by 60-95%, 30-80% and 50-90%, respectively with increase in metal concentration from 0.25-1 ppm over control (Fig. 1 A-C). A number of studies have reported reduction in root and shoot growth and biomass under arsenic stress (Hartley-Whitaker *et al*., 2001; Mokgalaka-Matlala *et al*., 2008; Piršelová, 2011). The inhibition was stronger in the root than in the shoot (Wang *et al*., 2002). Inhibition of root elongation is considered one of the frequently observed symptoms of metal toxicity (Wang *et al*., 2003) and it could be attributed to fact that plant roots are generally the first tissue to be exposed where the metalloid inhibits root extension and proliferation (Garg and Singla, 2011). When metal translocated to shoot it severely inhibit plant growth by slowing or arresting expansion and biomass (Finnegan and Chen, 2012) possibly by reducing number of merismatic cells and disturbing functioning of important enzymes in this region. Disturbance in enzymatic activity could result in production and accumulation of reactive oxygen radical (ROS). The ROS probably surpass the elimination ability of the active oxygen free radical in plants. Therefore, the imbalance between active oxygen free radical could create stress condition and whole wheat seedling probably poisoned by As (Chun-Xi *et al*., 2007). Exclusive literature supports the evidence that excessive arsenic toxicity to plant is probably due to series of physiological and biochemical alterations in plant (Han *et al.*, 2002; Liu and Zhang, 2007; Liu *et al.*, 2007; Zhang *et al.*, 2009).

The present study concludes that Sseed and seedling growth of *T. aestivum* were significantly influenced by increasing As concentration in the range of 0.25-1 ppm. Thus, widespread use of As-contaminated irrigation water would cause issues of food security, food safety and degradation of the environment. There is urgent need to address As relevant problems on crops growing in Pakistan.

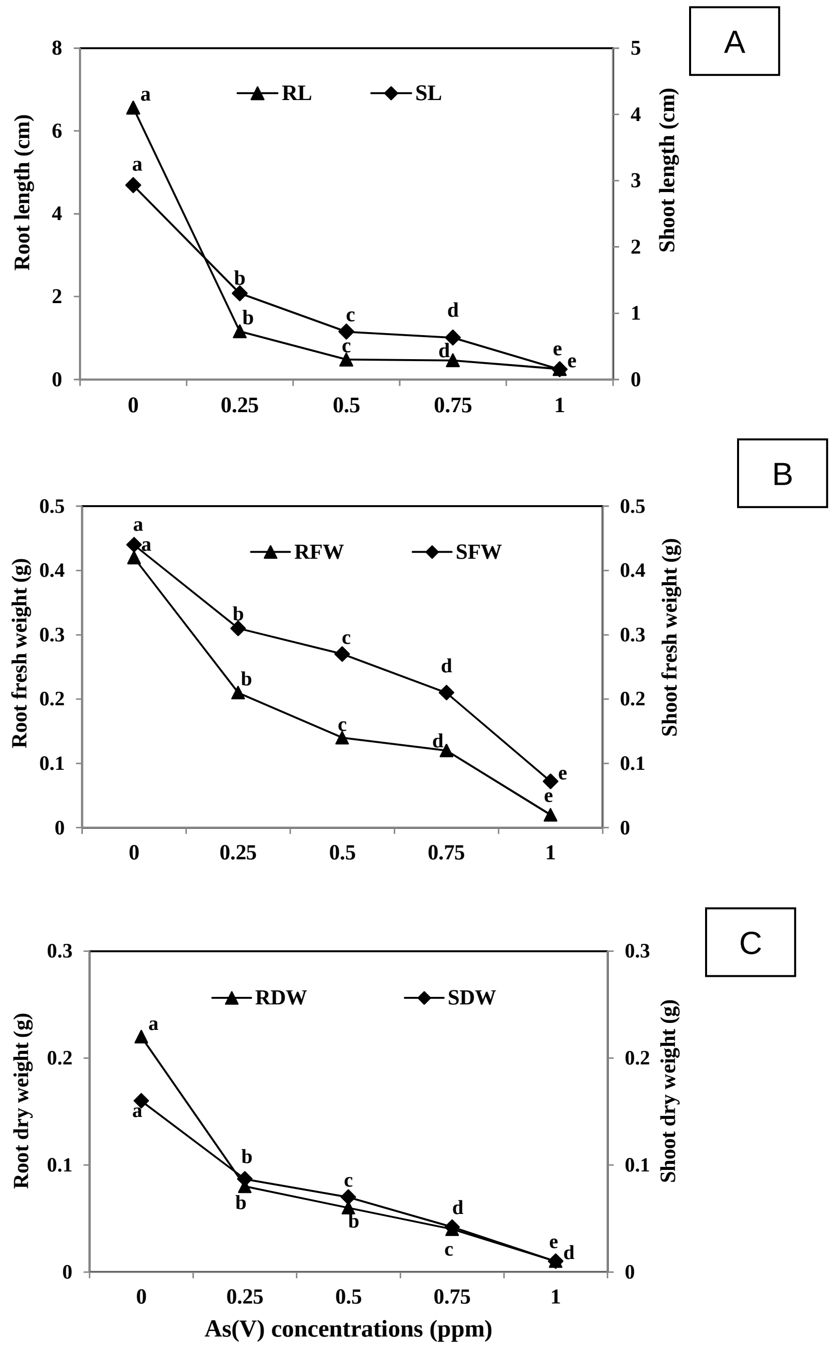

Fig. 1: Effect of As(V) on seedling growth and biomass in wheat. Values with different letters show significant difference ($P \leq 0.05$) as determined Duncan's Multiple Range Test.

Table 1: Effect of As(V) on percentage germination, germination index relative germination rate and injury rate in *Triticum aestivum.*

As(V) concentration (ppm)	Germination (%)	Germination Index (GI)	Relative germination rate (RGR)	Relative arsenic-injury rate (RAIR)
0.25	75±1.41a	0.75±0.01a	0.75±0.01a	0.25±0.01c
0.5	75±1.41a	0.75±0.01a	0.75±0.01a	0.25±0.01c
0.75	67.5±2.12b	0.65±0.01b	0.67±0.01b	0.33±0.01b
1	47.5±0.71c	0.45±0.02c	0.47±0.02c	0.53±0.01a
LSD	3.4487	0.016	0.0163	0.011

Data are the mean values of three replicates. In a column values with the different letters show significant difference ($P\leq0.05$) as determined by Duncan's Multiple Range Test.

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