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Super absorbent polymer mitigates deleterious effects of arsenic in wheat

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ABSTRACT

In order to investigate the possibility of reducing adverse effects of arsenic (As) in wheat by applying super absorbent polymer (SAP), a glasshouse experiment was conducted in Varamin, Iran in 2015 growing season. Four levels of As (0, 50, 100 and 150 mg kg^{-1} of soil) and three levels of SAP (0, 5 and 10 g kg^{-1} of soil) were investigated in a completely randomized design, arranged in factorial with three replicates. The results indicated that As significantly decreased 1000-grain weight, grain yield and total chlorophyll content, whereas, increased superoxide dismutase (SOD) and catalase (CAT) activity as well as As accumulation in wheat shoots and roots. By contrast, SAP application could increase total chlorophyll content and 1000-grain weight and mitigate SOD and CAT activity as well as As accumulation in the shoots and roots. In general, the results suggest that SAP application could reduce harmful effects of As by alleviating oxidative stress.

1. Introduction

Soil contamination with heavy metals as a result of human activities such as mining, metallurgical processes and application of fertilizers, pesticides and fungicides in agriculture is a serious threat for ecosystems and human health. Modern industrial activities lead to environmental pollution, especially heavy metals contamination. A significant percentage of acidic soils and arable land have potential to be contaminated by heavy metals (Dixi et al. 2001). Arsenic is an environmental toxin that is found naturally in all type of soils (Cullen and Reimer, 1989; Smedley and Kinniburgh, 2002). Human food contamination by As is a global concern that is not restricted by economic boundaries. Rice grain has been recognized as major source of human As outside of contaminated drinking water (Meharg et al. 2009). Arsenic is a non-essential element for plants and is generally toxic to them. Obviously, roots are the first tissue to be exposed to As, where the metalloid inhibits root extension and proliferation. Upon translocation to the shoots, As can severely inhibit plant growth by slowing or arresting expansion and biomass accumulation, as well as compromising plant reproductive capacity through losses in fertility, yield and fruit production (Garg and Singla, 2011). Various heavy metals generate reactive oxygen species (ROS) directly through Haber-Weiss reactions or the overproduction of ROS could be an indirect consequence of heavy metal toxicity (Wojtaszek, 1997; Mithofer et al. 2004). Several physiological processes are susceptible to as toxicity. Cellular membranes become damaged in plants exposed to as, causing electrolyte leakage (Singh et al. 2006). Membrane damage is often accompanied by an increase in malondialdehyde, a product of lipid peroxidation, pointing to the role of oxidative stress in as toxicity. Arsenic exposure induces antioxidant defense mechanisms (Khan et al. 2009). When soils are contaminated with heavy metals, the clean-up is one of the most difficult tasks for environmental engineering. For remediating sites contaminated with inorganic pollutants, several techniques have been developed. Super absorbent polymer application positively influence crop production, improve soil physical properties and can be used to reduce heavy metal hazards in plants. Applying sorbents, including zeolite and super absorbent, should result in immobilizing heavy metals and restoring the ionic balance and ratio of nutrients within a soil environment (Kinraide, 2007; Pyrzynska, 2007; Zielazinski and Wyszkowska, 2005; Gambus and Rak, 2005). Super absorbent polymers hold a large amount of poly functional groups (amino and imino groups) that can effectively adsorb heavy metal ions (Huang et al. 2011). In such polymers, chelating functionalities are present in the polymeric side chains or are embedded in the backbone. The choice of the type of ligand, ligand density, structure and solubility of the polymer, as well as pH, governs the metal ion affinity, retention efficiency and selectivity. The number of surface adsorbing groups limits the sorption process. In the case of SAPs, metal ions can easily enter the polymeric network and, hence, these polymers are expected to exhibit a higher sorption capacity (Roy et al. 2011).

The aim of this study was to determine As distribution in shoot and root of wheat grown under manually contaminated growing medium, and also to understand if SAP application could be a strategy for immobilizing As, thus reducing its deleterious effects in wheat.

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Table 1
Analysis of variance on wheat attributes as affected by AS and SAP.

S.O.V	df	Grain yield	1000-grain weight	Shoot As	Root As	Chlorophyll	SOD	CAT
As	3	**	**	**	**	**	**	**
SAP	2	ns	ns	**	**	**	**	**
As × SAP	6	ns	ns	**	**	ns	**	**
C.V (%)	9.69	2.58	3.34	2.50	1.72	2.72	5.25	

*,** and ns significant at 0.05, 0.01 percentage and no significant

2. Materials and methods

The experiment was conducted in a glasshouse in Varamin, Iran, in 2015. The experimental design was a completely randomized design arranged in 4×3 factorial scheme, with three replicates. Four levels of As (0, 50, 100 and 150 mg kg^{-1} of soil) and three levels of SAP (0, 5 and 10 g kg^{-1} of soil) were considered as the first and second factors, respectively. Thirty six 20 liter plastic pots were filled with free draining peat-vermiculite (2:1 volume ratio). The different levels of As and SAP were mixed into the peat-vermiculite mixture prior to potting. Surface-disinfected (surface sterilized by 1% sodium hypochlorite solutionfor 5 min and rinsed in double distilled water) wheat seeds (*Triticum aestivum* L c.v Pishtaz) were sown in the pots (10 seeds in each pot) and placed in a glasshouse equipped with cool white fluorescent lamps as complementary light. Glasshouse air temperature was 22/20º C, during the 16/8 h light/dark photoperiod. Photosynthetically active radiation (PAR) at the top of the canopy was 400 μmol m^{-2} s^{-1}, during the light photoperiod (measured by a LI-COR quantum sensor (LI-COR Environmental, Lincoln, Nebr.). Relative humidity in the glasshouse was 70%. Plants were hand watered daily until saturated with freshly prepared nutrient solution (100% Hoagland solution, pH 6). As Pishtaz cultivar is a spring type, there was no need to be vernalized. At grain filling stage, flag leaves were collected and immediately frozen in liquid nitrogen and stored at -80º C freezer until further laboratory analyses. At the maturity stage, intact plants were uprooted and grains were collected and weighted. Grain yield per plant was determined. All the leaves and roots were dried for 48 h, at 85º C, in a laboratory oven, for determining As content.

The dried leaves and roots samples were separately digested by HNO_3 and $HClO_4$ in tubes placed on an A1 block brought gradually to 205º C. As was determined using atomic absorption spectrophotometry method, using a VARIAN ES-710 atomic absorption spectrophotometer (Instrument Manual Digesdahl Digestion Apparatus, 1999).

Chlorophyll was extracted in 80% acetone from the leaf samples (Arnon 1949). Extracts were filtrated and total chlorophyll content was determined by spectrophotometer at 645 nm and 663 nm. The content of chlorophyll was expressed as mg g^{-1} of fresh weight using following formula.

$$\text{Total chlorophyll} = [20.2(\text{D645}) + 8.02(\text{D663})]\frac{V}{1000W}$$

Catalase activity was estimated by the Cakmak and Horst (1991) method. The reaction mixture contained 100 μl of crude extract, 500 μl of 10 mM H_2O_2 and 1,400 μl of 25 mM sodium phosphate buffer. Catalase activity was estimated by recording the absorbance reduction at 240 nm, for 1 min, using a spectrophotometer.

Superoxide dismutase activity was determined by measuring the ability of the enzyme extract to inhibit the photochemical reduction of nitro blue tetrazolium (Giannopolitis and Ries 1977). The reaction mixture contained 100 μl of l μM riboflavin, 100 μl of mM L-methionine, 100 μl of 0.1 mM EDTA (pH 7.8), 100 μl of 50 mM Na_2CO_3 (pH 10.2), 100 μl of 75 μM nitroblue tetrazolium, 2,300 μl of 25 mM sodium phosphate buffer (pH 6.8) and 200 μl of crude enzyme extract, in a final volume of 3 ml. Glass test tubes that contained the reaction mixture were illuminated with a fluorescent lamp (120 W), and identical tubes that were not illuminated served as blanks. After illumination for 15 min, absorbance was measured at 560 nm. One unit of SOD activity was defined as the amount of enzyme that caused 50% inhibition of photochemical reduction of nitro blue tetrazolium.

All data were analyzed from analysis of variance using the GLM procedure in SAS (SAS Institute Inc., 2002). The assumptions of the variance analyses were tested by checking if the residuals were random, homogenous, with a normal distribution and a mean of about zero. The significance of differences among means was carried out using Duncan's multiple range test at $p < 0.05$.

3. Results and discussion

According to analysis of variance, the main effect of As was significant on all measured traits (Table 1). In addition, SAP effect was significant for all measured traits except for grain yield and 1000-grain weight (Table 1). The interaction between As and SAP was not significant on grain yield, 1000-grain weight and total chlorophyll content (Table 1).

The lowest grain yield and 1000-grain weight were obtained when wheat plants were exposure to 150 mg As (Table 2). Growth reduction is the most common response to stress conditions, such as heavy metals contamination. Reduction in yield components might be due to direct toxic effects of As on biochemistry and physiological processes in the wheat plant. For instance, As affects phosphate uptake and utilization as As is a phosphate analogue (Meharg and Hartley-Whitaker, 2002). In addition, As causes chlorosis, inhibition of growth and finally death, when soil As concentration is high (Gulz et al. 2005). Furthermore, the maximum grain yield and 1000-grain weight were obtained when 10 g SAP was applied (Table 2). Increased water and nutrients absorption may explain the increased grain yield and 1000-grain weight after the application of SAP.

Similar results have been found by Ullah (2016), who have reported that high levels of As reduce photosynthesis, water uptake, nutrient uptake and finally grain weight. The positive effect of SAP in increasing

Table 2
Main effect of As and SAP on some wheat attributes.

Factors	Grain yield (g per plant)	1000-grain weight (g)	Shoot As (mg kg^{-1})	Root As (mg kg^{-1})	chlorophyll (mg g^{-1} FW)	SOD (ΔA mg pro min^{-1})	CAT (ΔA mg pro min^{-1})
As (mg kg^{-1})							
0	12.31a	40.63a	1.18d	2.16d	1.90a	142.76d	66.65d
50	11.15a	38.85b	5.94c	8.03c	1.86b	290.93c	112.09c
100	9.30b	36.66c	8.51b	10.66b	1.81c	427.93b	122.04b
150	7.70c	35.14d	10.91a	13.58a	1.71d	487.90a	146.44a
SAP (g kg^{-1})							
0	9.68a	37.30b	7.25a	9.44a	1.75c	360.79a	127.30a
5	10.21a	37.92ab	6.55b	8.40b	1.83b	332.76b	109.55b
10	10.46a	38.25a	6.10c	7.99c	1.88a	318.59c	98.57c

Treatment means followed by the same letter within each common are not significantly different ($P < 0.05$) according to Duncan's multiple range test