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Research article

Evaluation of effectiveness of seed priming with selenium in rice during germination under arsenic stress

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ABSTRACT

Due to extensive use of arsenic (As) contaminated ground water in rice cultivation As toxicity has become a growing concern to rice growers of south east Asian countries. The presence of As in soil and irrigation water causes impaired crop growth and development. Selenium (Se) at lower concentration (1.0 mg L^{-1}) is reported to be stimulatory on crop growth and it has also an antagonistic behavior with As. With this rationale the present study was conducted to investigate into the potentiality of seed priming technology with Se to ameliorate the As stress on rice seed germination and seedling growth. The seed germination percentage, seedling growth, total phenolics, proline and malonaldehyde content as well as total As uptake pattern of rice seedlings grown under As stressed condition were measured. The As induced toxicity markedly reduced the germination percentage by 70%, whereas, Se supplementation through seed priming enhanced the rice seed germination by 9% and root and shoot length vis-a-vis seedling biomass accumulation by 1.3, 1.6 and 1.4 fold respectively. The inhibitory effect of As stress was more on root growth than that of shoot. The toxicity due to arsenite stress was higher than the arsenate stress. Seed priming with Se enhanced seed germination and seedling growth by reducing As uptake, suppressing the oxidative damage through increase in antioxidants accumulation in rice seedlings. Seed primed with 0.8 mg Se L^{-1} was more effective in improving rice seed germination and seedling growth, compared to 1.0 mg Se L^{-1}.

1. Introduction

Rice is one of the most important cereal crops and a principal source of food for more than half of the world's population. More than 90% of the world's rice is grown and produced in Asia (FAO, 2014). Nowadays, the south east Asian countries are facing serious threat to arsenic (As) toxicity as a result of extensive use of As contaminated ground water in rice cultivation specially in winter (*boro*) season. Occurrence of it in ground water beyond permissible limit (0.01 mg L^{-1}) has become a serious threat for mainly China, India and Bangladesh (WHO, 1992). In the studied area, As concentration in ground water (used for irrigation) and agricultural soil ranges from 0.05 to 0.67 mg L^{-1} and 7.83–14.9 mg kg^{-1} respectively (Das et al., 2016). Entry of it in the plant body and subsequent transfer to the rice grain and finally to the consumers' plate is of a big issue at this juncture (Santra and Samal, 2013). Due to As contamination in ground water and/or food chain (beyond permissible limit), a big portion of the total population of this region is highly vulnerable to various skin lesions, gangrene in leg, skin, lung, bladder, liver, and renal cancer (Santra and Samal, 2013). In this region, rice is mainly grown under anaerobic situation by manual transplanting of 3–6 week-old seedlings into puddled soil (wet tillage) and with continuous flooding (Ghosh et al., 2016). The soil pH of rice field is slightly acidic in nature. Under monsoon (*aman*) season inorganic arsenic (iAs) is predominant in arsenate (As^{V}) state, whereas, arsenite (As^{III}) is dominant in winter season. Plant exposed to As stress with higher concentration (from irrigation and/or soil), in any part of their life cycle, resulted in particular stress responses ultimately shuffling the physiological and biochemical machinery of plant system like inhibition of germination (Liebig, 1966), limited plant height (Barrachina et al., 1995) and restricted root growth (Tang and Miller, 1991).

So far, several methods have been reported to diminish As toxicity in rice plant and subsequent less accumulation in grain. From the agronomic point of view, rice cultivation under aerobic

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situation can effectively reduce As accumulation in rice plant (Xu et al., 2008). Duxbury and Panaullah (2007) found that rice cultivation in raised bed and furrow irrigation system effectively reduces the As accumulation in rice grain. Proper irrigation management during rice cultivation can markedly check As transmission from soil to grain (Talukder et al., 2011). Application of silicon and phosphetic fertilizer in higher amount can also improve rice plant growth, yield and yield attributes of winter and monsoon season rice by restricting As accumulation in plant (Fan et al., 2013; Talukder et al., 2011). The main challenge for crops grown in As contaminated sites is to restrict the entry of As in plant system, especially in rice; as As^{V} and As^{III} shares common transport pathways alike phosphate and silicate respectively (Zhao et al., 2009).

Seed priming has been proved to be an effective method in imparting stress tolerance to plants. It is the stimulation of a particular physiological state in plant system by treating with natural and/or synthetic compounds to the seeds before germination (Jisha et al., 2013). During last few years, seed priming has been emerged as a cost effective as well as promising strategy in biotic and abiotic stress management without imposing any genetic/transgenic alteration. It improves the germination percentage and also ensures uniform seedling establishment of crops (Basra et al., 2005).

The essentiality of selenium (Se) for plant is still under consideration, but the beneficial role of Se in providing protection to plants against environmental stress is well recognized. Se supplementation to plant system can stimulates plant growth under ultra violet ray induced oxidative stress and also helps to delay the aging by enhancing antioxidative defense mechanism (Xue et al., 2001). Se can also mitigate the adverse effect of salinity stress (Hasanuzzaman et al., 2011). However, Se concentration more than 1.0 mg L^{-1} has been proved to be toxic in case of higher plants like mungbean, but the lower dose less than 1.0 mg L^{-1} has beneficial effect on crop growth (Malik et al., 2012). Se can also play an antagonistic behavior against a wide range of heavy metals, like As, cadmium, chromium trivalent, chromium hexavalent, antimony, mercury and lead by inhibiting their uptake and/or translocation in plant system under *in vitro* condition (Feng et al., 2013). Zhang et al. (2012) found that application of Se as soil amendment or fertilizer, can effectively limit the bio-accessibility, absorption, translocation and bioaccumulation of mercury (Hg) in the aerial part of rice plant due to the formation of insoluble Hg–Se complex in the rhizospheres. With all these facts keeping in background the present study was carried out with the focal objective of evaluating the impact of seed priming technology with Se supplementation on seed germination and seedling growth of rice under As stress.

2. Materials and methods

2.1. Experimental design and seed priming

The experiment was conducted at Environmental Biology Laboratory, Department of Environmental Science, Kalyani University, Nadia, West Bengal, India in completely randomized design with eighteen treatment combinations, replicated five times. The treatments comprised of two rice varieties of contrasting grain morphology (*Kranti* and *IR-36*), three levels of As stress (control, 8 mg L^{-1} As^{III} and 8 mg L^{-1} As^{V}) and three levels of seed priming (control, 0.8 mg Se L^{-1} and 1.0 mg Se L^{-1}). The two tested varieties viz. *Kranti* (short and bold seed) and *IR-36* (long and slender seed) were obtained from ICAR-Directorate of Weed Research, Jabalpur, Madhya Pradesh, India to ensure absence of any previous history of As contamination. The seeds of *Kranti* and *IR-36* had initial moisture content (dry weight basis) of 10.5 and 9.9%, and the length to width ratio was 2.78 ± 0.04 and 3.11 ± 0.02, respectively. Prior to seed priming, the seeds of tested rice varieties were surface sterilized with 0.1% mercuric chloride (w/v) solution for 1 min and washed thoroughly. After that, seeds were taken into a glass beaker and soaked in Se solution [Anhydrous Na_2SeO_3 salt, purchased from HIMEDIA; molecular weight (MW) 172.94; ≥99% purity] of desired concentration (0.8 mg Se L^{-1} and 1.0 mg Se L^{-1}) in 1:5 seed to priming solution ratio. The top of the beakers were covered with a clean paper and then kept for 24 h in absence of light (25 ± 1 °C). After 24 h the seeds were withdrawn from the solution and dried by keeping in between two layers of filter paper followed by under bright sunlight till it become completely dry i.e. ±10% of initial weight. The dried seeds were then kept in airtight plastic zipper pouch at 25 ± 1 °C for two days (Basra et al., 2005). For each treatment twenty seeds were kept in between two layers of filter papers in a glass petri plate having 90 mm diameter for 5 and 10 days (d). The seeds were treated with 10 mL of double distilled water for without As stress (control), 10 mL of As^{III} (sodium-m-arsenite salt; MW 130, obtained from MERCK) and As^{V} (sodium arsenate; MW-312.01, obtained from MERCK) solution for As stress. The petri plates were kept inside an incubator at 26 ± 0.5 °C under aphotic condition. Seeds were considered as germinated only when radical length were found to be ≥ 2 mm, after 5 d and 10 d seedlings were washed in distilled water and used for total phenolics, proline, malondialdehyde (MDA) and total As content.

2.2. Final germination percentage, root and shoot length, and seedling dry biomass determination

On 5 d and 10 d, ten seedlings were washed with double distilled water (thrice) and surface dried by using tissue paper. The root and shoot length were measured by keeping them on a clean glass plate with measuring scale. Final germination percentage (FGP) was computed by using formula of Ellis and Roberts (1981), seedling dry weight was as per the formula of Kharb et al. (1994).

2.3. Determination of total phenolics, lipid peroxidation and proline content

Total phenol contents were estimated by following the methodology of Malik and Singh (1980). Total phenols were extracted from 200 mg of fresh rice seedlings separately in 80% (v/v) ethanol and estimated by Folin-Ciocalteau reagent. The absorbance of the reaction mixture was measured at 650 nm wavelength and using catechol as standard. The lipid peroxidation was carried out following the method of Heath and Packer (1968) and measured in terms of MDA content by using thiobarbituric acid reacting substances (TBARS). Proline content in intact rice seedlings was carried out according to the method described by Bates et al. (1973).

2.4. Determination of total arsenic content in rice seedlings

Dried rice seedlings (0.5 g) were digested with perchloric acid (70%), concentrate nitric acid and sulphuric acid (ACS grade MERCK), according to the method described by Das et al. (2004), along with pure reagent blank in triplicate and Standard Reference Material (SRM) of rice flour (Item No.1568A), obtained from National Institute of Science and Technology (NIST), USA. Total As content of rice seedlings was analyzed by adopting the protocol given by Welsch et al. (1990) using flow injection hydride generation atomic absorption spectrometer (FI-HG-AAS, Perkin Elmer A Analyst 400). The certified value of SRM was 0.29 ± 0.03 and our obtained value was 0.279 ± 0.007, respectively (96.2% recovery).