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Effect of selenium induced seed priming on arsenic accumulation in rice plant and subsequent transmission in human food chain

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ABSTRACT

The south-east Asian countries are facing a serious threat of arsenic (As) toxicity due to extensive use of As contaminated groundwater for rice cultivation. This experiment was configured to assess the consequences of rice seed priming with selenium (Se) and cultivation in As free and As contaminated soil. The experiment was arranged in a factorial complete randomized design having two factors viz. seed priming and soil As stress with total twenty-five treatment combinations replicated thrice. Seed priming with Se promotes growth, yield under both As free and As stressed conditions. Se supplementation considerably enhanced the tiller numbers, chlorophyll content, plant height, panicle length and test weight of rice by 23.1%, 23.4%, 15.6% and 30.1%, respectively. When cultivated in As spiked soil and compared with control, Se primed plant enhance growth and yield by reducing As translocation from root to aerial parts, expressed as translocation factor (TF). A reduction of $TF_{root\ to\ shoot}$ (46.96%), $TF_{root\ to\ husk}$ (36.78–38.01%), $TF_{root\ to\ grain}$ (39.63%) can be seen among the Se primed plants than unprimed plants both cultivated in similar As stress. Besides these, a noteworthy reduction in estimated daily intake (EDI) and cancer risk (CR) were also noticed with the consumption of cooked rice obtained after cooking of brown rice of Se primed plants than their unprimed counterparts.

1. Introduction

Paddy cultivation in Ganga- Meghna- Brahmaputra basin, located across India and Bangladesh gets affected due to arsenic (As) contaminated soil and groundwater. The well-recognized threat associated with paddy cultivation in those areas is the subsequent transmission of As into food chain through consuming As laden rice as cooked rice. The situation gets even more worsened when As enriched rice cooked along with As contaminated water (Santra and Samal, 2013). Agricultural fields including paddy fields are considered as the source for tons of As gets shipped away into the food chain year after year through irrigation the of As rich groundwater and cultivation in As laden soil (Ali, 2003; Neumann et al., 2011). In the environment especially in the agroecosystem, As use to exists mainly as arsenate (As^{V}) and arsenite (As^{III}) in soil and groundwater (use for irrigation). Besides these As^{III} and As^{V} in the soil environment, the presence of organic As species like methylarsonic acid or dimethylarsinic acid in fractional amount have been also reported (Jia et al., 2012, 2013). *Kharif* (monsoon) and *boro* (winter), the two main cropping season has been found to be dominated by two different As species i.e. As^{V} and As^{III}, respectively (Moulick et al., 2016a, b). Speaking of a toxicological aspect of As to plants or cultivated crops species, it depends upon chemical forms (organic/inorganic), concentration etc. Plants used to take up As^{V}, As^{III} species by employing different means like phosphate transporters, aquaporin channels{NIPs or nodulin26-like intrinsic proteins} respectively (Asher and Reay, 1979; Ma et al., 2008). On the other hand, organic As were absorbed by plants (rice) using aquaporin Lsi1 (Li et al., 2009). After taking entry into the plant's system, As^{V} executed it's toxicity by replacing phosphate (PO_4^{3-}) due to having close resemblance with phosphorus, whereas, As^{III} sticks with the sulphahydryl group (-SH) of peptides and interfere with its function (Finnegan et al., 2012).

In the paddy field, under submerged / waterlogged soil condition with lower soil pH (acidic), As^{V} gets reduced in to AS^{III}. Irrespective of the nature of As species (As^{V} / As^{III}) plant species gets exposed, once inside the plant system As^{III} species dominates over other As species (Pickering et al., 2000, 2006). When plants exposed to As in lower concentration gets deposited within the nucleus and cause damage and interfere with replication of nucleic acid and further, upon relocating to leaves photosynthetic machinery gets damaged with pronounced reduction in chlorophyll content by interrupting chlorophyll biosynthesis (Moulick et al., 2017; Mishra et al., 2016, 2014, 2013; Xu et al., 2007; Bhattacharya et al., 2013). Moreover, injurious consequences of As on

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rice plant like inhibition of germination and seedling growth (Moulick et al., 2016a), reduces tiller number, biomass and yield of rice (Abedin et al., 2002). Which ultimately leads to higher accumulation of As in rice grain and finally in cooked rice (Moulick et al., 2016a, b; Santra and Samal, 2013; Bhattacharya et al., 2013). The As content in rice grain is mainly governed by the ability of rice plant's (variety) to accumulate As into their root zone and subsequent transfer or translocate of As to grain trough plant system (Bhattacharya et al., 2010).

Several attempts have been evaluated as the possible method to restrict the entry of As into grain and subsequently into cooked rice portion. Among the studied methods, organic matter amendment (Rahaman et al., 2011), water management in SRI (systemic rice intensification) - cultivation practice (Thakur et al., 2014), application of phosphorus (as phosphate) and farmyard manure (Mukhopadhyay and Sanyal, 2002), co-application of organic matter and zinc (Zn) in varying amounts to soils (Das et al., 2008), aerobic cultivation (Xu et al., 2008), intermittent ponding (Stroud et al., 2011), water deficit irrigation practice (Mukherjee et al., 2017) etc. were evaluated to reduce As load in rice grain. Beside these practices, developing new rice variety with lower As uptake or translocation potentials (Norton et al., 2009) were also reported.

Seed priming is the stimulation of a particular physiological state in plant system by treating with natural and/or synthetic compounds to the seeds before sowing (Jisha et al., 2013) and it has also been proved to be an effective method in imparting stress tolerance to plants. During last few years, several authors reported about the positive outcomes of seed priming as a promising strategy in biotic and abiotic stress management without imposing any genetic/ transgenic alteration.

The positive role of selenium (Se) from human and animal as an essential trace mineral is well recognized (Zeng, 2002). Two leading organizations like United States Department of Agriculture and Agency for Toxic Substances and Disease Registry (ASTDR) have set a benchmark value of 55 $\mu g\ day^{-1}$ and ≥ 900 $\mu g\ day^{-1}$ as recommend dietary allowances and toxic levels respectively, for Se (Levander, 1991; ASTDR, 2003; Jukes, 1983). Though the role of Se for plant species is still under consideration but current reports suggest that optimal supplementation of Se (in early stage) is beneficial for plants through improvement of photosynthesis and antioxidative responses. Reactive oxygen species or ROS (generates in the chloroplast or in mitochondria) forms even if plants are cultivated under optimal or normal conditions (both invitro and invivo conditions). According to the opinions of Zhang et al. (2014), Jiang et al. (2015) and Kaur and Nayyar (2015) supplementation of Se improves productivity in common buckwheat (*Fagopyrum esculentum moench*), mungbean (*Vigna radiata* L. *Wilczek*) and in rice (*Oryza sativa, L.*) respectively. Furthermore, authors like Pilon-Smits and LeDuc (2009) and White and Broadley (2009) found that supplementation with Se significantly improves growth irrespective of the presence of any stressor (i.e. under stressed or absence of stress). Reports also suggest that supplementation of Se can alleviate (when applied in low doses) drought and salinity induced stress mainly diminishing ROS through modulating various enzymatic and nonenzymatic antioxidative machinery (s) in rape seed seedlings (*Brassica napus*) (Hasanuzzaman et al., 2011; Hasanuzzaman and Fujita, 2011); wheat (Nawaz et al., 2013, 2015); tomato (*Solanum lypersicon*) (Diao et al., 2014); maize (*Zea mays* L.) (Jiang et al., 2017) besides these, minimize extreme temperature induced damages in spring wheat (Iqbal et al., 2015); sorghum (*Sorghum bicolor* L.) plants (Abbas, 2012, 2013) also found. On the other hand, evidence reveals about the ability of Se to execute antagonism against metalloids and metals like As, antimony and other heavy metals have been recognized (Feng et al., 2013; Cao et al., 2013; Zhao et al., 2013; Han et al., 2015). Recently, Moulick et al. (2016a), (2017) reported about positive consequences of priming rice seeds with Se in promoting germination and seedling growth under both AS^{III} and As^{V} stress condition in in-vitro condition in non-soil and soil based assay. So far there is no such evidence available that describe the impact of rice seed priming with Se on growth and yield, and As uptake pattern in mature rice plant, grown under As contaminated/ spiked soil either from invivo (field study) or from invitro (controlled study in the pot) investigations.

We carried out this experiment with the intention to further enrich our understanding of: (a) influence of Se seed priming on As accumulation pattern by different plant parts on rice; (b) effect of seed priming technology with Se on growth and yield of rice crop and (c) estimate the positive impact of Se seed priming on human health risk associated by consuming rice, grown in As contaminated soil.

2. Materials and methods

2.1. Seed priming treatment

For this particular experiment high yielding rice variety *IET-4094*, popularly known as *Khitis* (with medium and slender grain morphology) was obtained from Regional rice Research Station Chinsurah, West Bengal, India to ensure the absence of any previous history of As contamination. Surface sterilization (with 0.1% $HgCl_2$ solution for 2 min) and seed priming treatments were carried out using sodium selenite solution (Anhydrous Na_2SeO_3 salt, purchased from HIMEDIA; molecular weight 172.94, ≥ 99% purity) of five different strength (0.0, 0.5, 0.75, 1.0 and 1.25 mg Se L^{-1}) for 24 h in absence of light according to the protocol of Moulick et al. (2016a, b).

2.1.1. Seed bed preparation

For preparing seed bed large PVC trays (28 cm length × 20 cm width × 20 cm deep) were used and filled up with garden soil. Before sowing of rice seed, nitrogen, phosphorus and potassium were applied in 1:1:1 ratio (on dry weight basis) in all the trays and irrigated with tap water (Se content < 0.001 mg/L and As content < 0.0003 mg/L). The seed beds were maintained 10 days after transplanting in order to replace damaged seedlings within seven days of transplanting (though no such replacement required).

2.1.2. Pot preparation and experimental layout

Bottom sealed earthen pots (40 cm diameter × 40 cm depth) were selected for this experiment. Soil (adjacent to Department of Environmental Science greenhouse, University of Kalyani, having As & Se concentration < 0.0003 mg Kg^{-1} and < 0.001 mg Kg^{-1} respectively) from the depth of 15 cm were taken, then the large aggregates were broken by wooden hammer and passed through 2.0 mm stainless steel sieve to obtain a homogeneous mass. For each earthen pot, 5.0 kg soil was poured two days before of transplanting. The available metal (As, Se) content in soil and irrigation water were also analyzed as per methodology of Moulick et al. (2017); whereas, soil physiochemical and textural properties were determined according to the methodology described by Trivedy and Goel (1986) and Kettler et al. (2001), respectively (given in Table 1). A day before transplanting, the pots were spiked with As of different levels (0.0, 10.0, 15.0, 20.0 and 25.0 mg As Kg^{-1} soil) as a solution of sodium arsenate (sodium arsenate salt; MW-312.01, obtained from MERCK, Germany) and pots were kept under with cover. For irrigation purpose tap water (As & Se concentration < 0.0003 mg/L and < 0.001 mg/L respectively) used to ensure As and Se exposure to rice plant only from arsenic spiked soil and Se primed seedlings only (except for unprimed seedlings). Irrigation was carried in such a way that ensure 3–4 cm constant standing water throughout, but irrigation stops ten days before harvesting. The entire experiment was arranged according to factorial complete randomized design (fCRD) having two factors (i) seed priming with Se (five different doses) and (ii) soil As stress (five different As dose) with total twenty-five treatment combinations replicated thrice in seventy five earthen pots.