



## Effect of water management and silicon on germination, growth, phosphorus and arsenic uptake in rice



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### ABSTRACT

Silicon (Si) is the 2nd most abundant element in soil which is known to enhance stress tolerance in wide variety of crops. Arsenic (As), a toxic metalloid enters into the human food chain through contaminated water and food or feed. To alleviate the deleterious effect of As on human health, it is a need of time to find out an effective strategy to reduce the As accumulation in the food chain. The experiments were conducted during September–December 2014, and 2016 to optimize Si concentration for rice (*Oryza sativa* L.) exposed to As stress. Further experiment were carried out to evaluate the effect of optimum Si on rice seed germination, seedling growth, phosphorus and As uptake in rice plant. During laboratory experiment, rice seeds were exposed to 150 and 300  $\mu\text{M}$  As with and without 3 mM Si supplementation. Results revealed that As application, decreased the germination up to 40–50% as compared to control treatment. Arsenic stress also significantly ( $P < 0.05$ ) reduced the seedling length but Si supplementation enhanced the seedlings length. Maximum seedling length (4.94 cm) was recorded for 3 mM Si treatment while, minimum seedling length (0.60 cm) was observed at day7 by the application of 300  $\mu\text{M}$  As. Silicon application resulted in 10% higher seedling length than the control treatment. In soil culture experiment, plants were exposed to same concentrations of As and Si under aerobic and anaerobic conditions. Irrigation water management, significantly ( $P < 0.05$ ) affected the plant growth, Si and As concentrations in the plant. Arsenic uptake was relatively less under aerobic conditions. The maximum As concentration (9.34 and 27.70 mg kg DW<sup>-1</sup> in shoot and root, respectively) was found in plant treated with 300  $\mu\text{M}$  As in absence of Si under anaerobic condition. Similarly, anaerobic condition resulted in higher As uptake in the plants. The study demonstrated that aerobic cultivation is suitable to decrease the As uptake and in rice exogenous Si supply is beneficial to decrease As uptake under both anaerobic and aerobic conditions.

### 1. Introduction

Rice is one of the major food crops in Southern Asia where groundwater contaminated with As is used for the irrigation of paddy fields (Dixit et al., 2016). It has been estimated that 1000 t of As per year are added to the agricultural soils as a result of applying As loaded ground water for irrigation purposes (Duxbury and Panaullah, 2007). Bioavailability of As to plant is governed by biological, chemical and physical processes and their interactions altering metal speciation and behavior in soil-plant systems (Bakhat et al., 2017). Concentration of As in plants depends on plants root ability to uptake and transport it from soil to roots/shoots. The most widely adopted conditions to cultivate the rice in field are water submerged conditions. Anaerobic conditions

of the paddy fields facilitate the reductive dissolution and release of the adsorbed arsenate (As<sup>V</sup>) in the soil stoma water (Bakhat et al., 2017). Furthermore, anaerobic conditions in paddy soils usually lead to the reduction of As<sup>V</sup> into more mobile arsenite (As<sup>III</sup>) (Takahashi et al., 2004; Punshon et al., 2016).

Rice is an efficient crop in As uptake in comparison to other cereal crops (Bhattacharya et al., 2009; Su et al., 2009). Studies showed that As concentrations in rice plant depends on As presence in soil and/or irrigated groundwater in addition to other factors governing the As mobility and uptake in plant-rhizosphere. In rice, As is taken up by plant roots using macro-nutrient transporters; As<sup>V</sup> via the phosphate while As<sup>III</sup> through Si transporters (Ma et al., 2008). Arsenate is a chemical analog of phosphate and shares the uptake pathway in rice

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with the same transporters (Chen et al., 2017). Therefore, antagonistic effects of phosphate on As<sup>V</sup> uptake and vice versa are probable in rice plant. Silicon can influence As<sup>III</sup> uptake competitively as As<sup>III</sup> shares the same uptake pathway as Si (Sanglard et al., 2016). The rice Si transporter LSi1 is permeable to As<sup>III</sup> and it acts as As<sup>III</sup> influx transporter. Silicon transporter LSi2 is an efflux transporter of Si which transports Si/As<sup>III</sup> from exodermis to endodermis and towards the stele of the root cells. Redox status (governed by soil moisture contents) of paddy fields is another important factor controlling the As bioavailability to rice plant. Changing soil redox status through water management in paddy fields has been supposed to be one of sustainable solution to reduce As accumulation in rice. A remarkable effect of aerobic conditions turns the speciation pattern towards As<sup>V</sup> in comparison to As<sup>III</sup> which has prominently higher solubility, plant availability, and toxicity (Takahashi et al., 2004).

Therefore to eliminate the As associated risks, integrated approaches are needed to cultivate the rice especially in those areas that are facing higher contamination of As in ground water or in soil. Among these strategies aerobic rice production with judicious fertilization of nutrient can be a solution to tackle this specific problem (Bakhat et al., 2017). Aerobic rice cultivation is a revolutionary way of rice production and requires only 50% of the water required for irrigated rice production for achieving yield levels of 4–6 Mg t ha<sup>-1</sup> (Anil et al., 2014). In addition to water saving, uptake of As can be decreased by plant through aerobic rice production. Understanding of the combined effects of rice cultivation under aerobic condition with exogenous Si is critical strategy to eliminate the elevated uptake of the toxic metalloid. Therefore, present study was conducted; 1) to investigate the effect of two rice cultivation method on As uptake in rice and, 2) to determine the effect of optimum Si on rice germination, phosphorus and As uptake in rice.

## 2. Materials and methods

Highly pure analytical-grade reagents and chemicals were purchased and used for the solutions preparation. Standard stock solution of As was prepared by dissolving sodium arsenite (Na<sub>2</sub>AsO<sub>2</sub>, M.W. 129.91, BDH, England) and sodium arsenate (Na<sub>2</sub>HAsO<sub>4</sub>·7H<sub>2</sub>O; MW: 312.01, BDH, England) in ultra-pure water while solutions of required concentrations were prepared by further dilution of this stock solution. Silicon solution was prepared using sodium trisilicate solution (Sigma Aldrich).

### 2.1. Optimization experiment

Optimization experiments were performed to determine the growth response of rice variety KSK-133 against As stress in presence of various levels of Si. Sand prewashed with acid (growing media) was filled in the pots and seedlings were transferred. Nutrient solution was applied as described by Zhu et al. (2009) to fulfill the nutritional requirements. After one week of transplantation, to make most effective use of the situation or resources, four sets of experiments were arranged in a completely randomized design with different concentrations of Si and As. These treatments were as: Set 1) 0 mM Si (control), 0.25 mM Si, 1 mM Si, 2 mM Si, 3 mM Si; Set 2) 0 μM As (Control), 50 μM As, 100 μM As, 150 μM As, 300 μM As; Set 3) 0 mM Si + 0 μM As (Control), 0.5 mM Si + 150 μM As, 1 mM Si + 150 μM As, 3 mM Si + 150 μM As; Set 4) 0 mM Si + 0 μM As (Control), 0.5 mM Si + 300 μM As, 1 mM Si + 300 μM As, 3 mM Si + 300 μM As. The nutrient solution was applied fortnightly to compensate the nutrient depletion in the growth medium. After two months of treatments application, the plants were harvested and washed with deionized water. Afterward, these were separated into root and shoot and fresh weights of the fractions were recorded using analytical balance (AS 220R Radweg, Europe).

**Table 1**

Physicochemical properties of soil used for the experiment. Values are the means of three replicates ± Standard Error.

Characteristics	Values
Soil Texture	Silt loam
Electrical conductivity (dS m <sup>-1</sup> )	1.88 ± 0.06
pH <sub>H<sub>2</sub>O</sub> extract	7.78 ± 0.04
Organic matter (%)	0.60 ± 0.05
Available phosphorous (mg kg <sup>-1</sup> soil)	6.44 ± 0.10
Available potassium (mg kg <sup>-1</sup> soil)	123.34 ± 9.79
Saturation percentage	37.27 ± 1.47

### 2.2. Laboratory experiment to determine effect of Si supplementation on seed germination and seedling growth

In this experiment, seeds in the petri plates lined with filter paper were soaked with the solution of 0 mM Si + 0 μM As, 0 mM Si + 150 μM As, 0 mM Si + 300 μM As, 3 mM Si + 0 μM As, 3 mM Si + 150 μM As, 3 mM Si + 300 μM As. Total six treatments with three replicates were performed during the experiment. In this experiment, germination percentage, root and shoot length were recorded.

### 2.3. Pot trial and experimental set up

Rice variety KSK-133 nursery was grown in pots. After the establishment, the seedlings were transplanted to soil pots. Soil was taken from the research farm of the COMSATS Institute of Information Technology, Vehari campus. The soil was thoroughly mixed and sieved using a 4 mm mesh to remove plant parts and other debris. Soil was processed for physico-chemical properties (Table 1). The soil was alkaline in nature with low in available phosphorus and organic matter contents. Afterwards, 7 kg of soil were filled in pots and basal doses of fertilizers nitrogen, phosphorus, and potassium @170 kg, 90 kg and 60 kg per hectare, respectively were added. Nitrogen was added in three split doses (At 1st day of nursery transplantation (DAP), 30 DAP and 45 DAP) while full dose of potassium and phosphorus was added at the pot filling stage. The treatment were arranged as completely randomized design with four repeats.

#### 2.3.1. Seedlings transplantation

In each pot, five seedlings were transplanted and thinned in to two after establishment of the seedlings. After thinning, the pots were divided into two groups. Twenty four pots were kept under flooded conditions to maintain anaerobic environment with treatments; T<sub>1</sub>-Si0As0, T<sub>2</sub>-Si0As150, T<sub>3</sub>-Si0As300, T<sub>4</sub>-Si3As0, T<sub>5</sub>-Si3As150 and T<sub>6</sub>-Si3As300. Other twenty four pots were kept moist with lesser amount of water to maintain aerobic conditions with the Si and As treatments; T<sub>7</sub>-Si0As0, T<sub>8</sub>-Si0As150, T<sub>9</sub>-Si0As300, T<sub>10</sub>-Si3As0, T<sub>11</sub>-Si3As150 and T<sub>12</sub>-Si3As300.

#### 2.3.2. Plants harvesting and growth attributes measurements

Three months after the treatments application, plant height and number of tillers per plant were recorded. Plants were harvested, washed with deionized water and separated into roots and shoots. The fresh weights of roots and shoots were measured by using weighing balance. The samples were kept in oven at 78 °C till constant weight and then the dry weights were recorded.

#### 2.3.3. Chemical analysis

The plant samples (root and shoot) were acid digested with nitric and perchloric acid as reported by Miller (1998). Briefly, one gram plant sample was taken in conical flask, kept overnight after adding 5 mL concentrated HNO<sub>3</sub> and 5 mL HClO<sub>4</sub>. Next day again 5 mL of concentrated HNO<sub>3</sub> were added and plant material was digested on hot plate by increasing the temperature slowly to 120 °C for 2 h and

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