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Toxicity of arsenic on germination and seedling growth of indigenous aromatic rice varieties of India

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Abstract
Arsenic (As), at present, a major problem in agricultural production. The concomitant increase in As concentration in rice belt areas affects early establishment and production of rice. So, it is necessary to choose a proper variety. The objective of the study was to evaluate the germination performance and initial growth habits of indigenous rice varieties in response to the different As concentration in the growing solution. Therefore, the effects of As (0, 50, 100, 150, 200 µ M/L) on germination percentage, speed of germination, hypocotyls and radicle length, fresh and dry weight of root and shoot and their percentage reduction were determined. Increasing As concentration caused a decrease in both germination percentage and speed of germination except Variety-4 (V_4) and rest of the variety were unable to germinate above 100 µ M/L concentration. Root growth was halted in V_3, whereas V_4 showed resistance. Most sensitivities regarding relative water content were expressed by V_1 and V_5 retained more turgidity in 50 µ M/L concentration. In case of the speed of germination, V_4 affected much less as compared to others. Root and shoot fresh and dry weight percent reduction showed maximum reduction for V_4T_4. All the growth parameters were negatively correlated with As concentration. The speed of germination was significantly positively correlated with germination percentage. From the results obtained in the present study, it can be concluded that indigenous rice varieties are sensitive to As concentration and among the varieties, V_4 is come out to be the most resistant to As stress.

Keywords: Arsenic, germination, speed of germination, hypocotyls, radicle, sensitive, tolerant

1. Introduction

Arsenic (As) is a toxic metalloid that is ubiquitous in the environment. It has raised serious concern for both environmental and human health perspectives. Contribution of As in soil gathered from both geogenic and anthropogenic sources, which include, metal mining and smelting, arsenic-containing pesticides, herbicides uses and irrigation with arsenic-contaminated water (Mitra *et al*. 2017 [24]). The groundwater is getting contaminated with As and drinking of the water cause great exposure of As to human kind. (Smith *et al*. 2000 [35]). Moreover, the agricultural soils are being contaminated by As at a rate of 1000 t As per year as for the irrigation with As laddened ground water. (Duxbury and Panaullah 2007 [8]). So, crops grown on the arsenic-contaminated soils serve as a secondary source of arsenic for human beings. Studies showed that As concentrations in rice plant depends firstly on the presence of As in soil and secondly on the As contaminated groundwater sources and other factors depend on plant-rhizopheric characters which do play role in As mobilization and stabilization. (Mandal *et al*. 1996 [20]; Ullah 1998 [37]; Rahman *et al*. 2007 [27]). In recent times, the south east Asian countries are facing serious threat to arsenic (As) toxicity due to the extensive cultivation of boro (winter) rice with As contaminated groundwater and that results in people of Bangladesh, India, China, Korea, Taiwan, and Thailand to arsenic intake from rice i.e. the staple food of these region. (Bakhat *et al*. 2017 [4]; Zhao *et al*. 2010 [38]). Occurrence of it in ground water beyond permissible limit has become a serious threat for these countries.

The transfer of arsenic from soil to plant systems is a serious issue that leads to considerable human exposure. Concentration of As in plants depends on plants root ability to uptake and transport it from soil to roots/shoots. Rice is the most severely affected staple food crop to arsenic contamination as compared with other crops like wheat, maize, and barley due to its (rice) cultivation in flooded conditions. 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 V) in the soil water and reduction of As V into more mobile arsenite (As III) (Bakhat *et al*. 2017 [4]; Punshon *et al*. 2017 [26]). In rice, As is taken up by plant roots using macro-nutrient transporters; As V via the phosphate while As III through Si transporters (Ma *et al*. 2008). Then As finds its way into grain (ICAR report 2001; Roychowdhury *et al*. 2002 [29]; Zhao *et al*. 2006 [39]).

In West Bengal (India) groundwater arsenic contamination is in alarming condition for long time. In rural areas the arsenic-contaminated groundwater used for drinking purpose as well as irrigation of various crops, especially rice. Many plant species including agricultural crops can accumulate higher amounts of heavy metals than those present in the soil (Seregin and Kozhevnikova 2008 [31]). Seedling and seed germination stage of plant life are sensitive to environmental factors such as heavy metals pollution (Abedin and Meharg 2002 [2]). Germination inhibition is among the best-known effects of toxic impact of heavy metals (Ernst 1998 [9]). Many plants at seed germination and seedling stages are sensitive to environmental factors. Therefore, the change of plant growth at the germination and seedling stage under heavy metal stress has become an important index to evaluate plant's heavy metals tolerance (Peralta-Videa *et al*. 2002 [25]). In West Bengal the cultivation of indigenous rice varieties are gaining popularity and are come under the threat of As pollution.

Most of the published reports focused mainly on the uptake and accumulation of arsenic in the rice plant irrigated with arsenic-contaminated water (Abedin *et al*. 2002 [1]; Rahman *et al*. 2004 [28]). Thus, in this study, the objective was to evaluate the germination performance and initial growth habit of indigenous rice varieties in response to different concentration of As in the growing solution.

2. Materials and Methods

2.1. Seeds

Seeds of five aromatic rice : Gobindabhog (V_1), Radhunipagal (V_2), Lal Badshabhog (V_3), Sugandha (V_4) and Kalonunia (V_5) were collected from field trial of 'RKVY (Rashtriya Krishi Vikas Yojana) Project on Bengal rice' in Bidhan Chandra Krishi Viswavidyalaya, Kalyani, West Bengal, India. Harvesting of rice grain were performed in peak maturity stage and stored in proper moisture content to retain its germinability. Organically cultivated species of these aromatic rice seeds were free from pesticides and other toxic substances

2.2. Seed surface sterilisation

A two steps process was used to sterilise seed surfaces prior to germination trials. First step involved soaking of seeds in 70% ethanol for a period of 2 min and seeds are placed onto Whatman No. 1 filter paper to remove excess fluid. Seeds were then treated with 10% w/v sodium hypochlorite solution. After that seeds were triple washed in sterile distilled water and then placed on Whatman No. 1 filter paper to remove excess fluid.

2.3. Germination assay

Effects of different concentrations of Arseinic (i.e. arsenate) on the seed germination of the different aromatic rice varieties were evaluated. The concentrations of arsenate were 0, 50, 100, 150 and 200 μMl^{-1} and were prepared freshly as Sodium arsenate dibasic heptahydrate ($Na_2HAsO_4.7H_2O$). Seed germination was tested on moist filter paper. A piece of filter paper was placed on a Petri plate and moistened it with 5.0 ml aqueous solution of arsenate. 5 ml distilled water was used for controls to moisten the filter paper. Twenty five seeds of each variety were placed in each plate, covered by lid, and incubated in dark at 25±1°C. Geminated seeds were counted each days after initiation. Each treatment was replicated three times following randomized complete design (CRD). The seeds were allowed to germinate and grow for 10 days. During this period the Petri dishes were moistened by respective solutions of As when needed

2.4. Germination percentage (GP)

Germination was daily recorded upto 10 days. Seeds were considered germinated when both the plumule and radicle were extended to more than 2 mm from their junction. Germination percentage was calculated by the following formula

$$GP(\%) = \frac{Ng}{Nt} \times 100 \qquad \text{(i)}$$

Where, Ng= number of germinated seeds and Nt= number of total seeds

2.5. Speed of germination

Speed of germination was calculated by the following formula

$$\text{Speed of germination} = \frac{\text{n1}}{\text{d1}} + \frac{\text{n2}}{\text{d2}} + \frac{\text{n3}}{\text{d3}} + \cdots \qquad \text{(ii)}$$

Where, n = number of germinated seeds, d= number of days.

2.6. Hypocotyl and radicle length

After 10 days growth, hypocotyl length was measured from culms base to the tip of the longest leaf and radicle length was measured from the root-shoot junction to the tip of the longest root.

2.7. Dry and fresh weight of root and shoot

The seedlings were separated in shoots, roots and were weighted separately and expressed in miligram (mg) as the shoot and root fresh weight. Dry weight of seedling shoots and roots were recorded and expressed in miligram (mg) after oven drying at 72 ºc for 48 h according to Ahmadvand *et al*. (2012) [3].

2.8. Weight reduction percentage

The shoots and roots were separated and the fresh weights were measured; after being dried at 72 °C in Pasteur oven during 48 hours, the dry weights were immediately taken. According to each treatment, the fresh and dry weights rate, referred to the control, were calculated in percent, by the following equations (Goumi *et al*. 2014 [12]):

Fresh weight (FW) percentage reduction:

$$FWPR\% = (1 - \frac{FWs}{FWc}) \times 100 \qquad \text{(iii)}$$

Where, FWs= fresh weight under stress and FWc= fresh weight in control

Dry weight (DW) percentage reduction:

$$DWPR\% = (1 - \frac{DWs}{DWc}) \times 100 \qquad \text{(iv)}$$

Where, DWs= dry weight under stress and DWc= dry weight in control

2.9. Relative water content (RWC)

The water content respective to the fresh weight was calculated as described by Barrs and Weatherly (1962) [5]

$$RWC\ \% = \frac{FW-DW}{FW} \times 100 \quad \text{(v)}$$

Where, FW= fresh weight and DW= dry weight

2.10. Statistical analysis

The experiment was made as a completely randomized design (CRD) with three replications. The variances from each Petri dish comfort the data to be reliable. The data were statistically treated by Fisher's analysis of variance (ANOVA) using IBM SPSS Statistics 23. Duncan's multiple range tests were performed to determine significant difference between means at a significance level of $P< 0.05$.

3. Results and Discussion

The analysis of variance on germination and initial plant growth parameters (Table 1) showed that variety and As treatments had significant effect on germination percentage as well as shoot length, root length, root and shoot fresh and dry weights and relative water content (RWC).The interactions among the variety and As treatment had also significant effect.

3.1. Germination percentage

Concentration of As showed inhibitory effects on seed germination. The degree of inhibition increased with increasing concentration of As in the solution (Fig1). Seed germination is energy (ATP) consuming process in which many enzymes that stored in seeds are broken down into simple forms and these are translocated to the growing region of seedlings (Meharg, 1994 [22]). According to Liu *et al*. (2005) [17] germination of seeds proved to be very sensitive to As contamination. Maximum GP was recorded for V_4 followed by V_2 at treatment T_1 (Table I). However except variety 4 (V_4) no germination was observed above As concentration 100 µM/L. Increasing concentration from 50 to 100 µM/L, GP decreased about 46% and it was curtailed more than 62% when the concentration increased from 100 to 150 µM/L. Energy for germination of seeds and for growth of roots and shoots is provided by sugars metabolism and for this purpose α-amylase converts endospermic stored starch into metabolizable sugars which is not adequate in arsenic stress condition(Kaneko *et al*. 2002 [15]). Results thus showed that V_4 and T_1 were the best for germination percentage. No interaction effects were observed between the variety and As concentration.

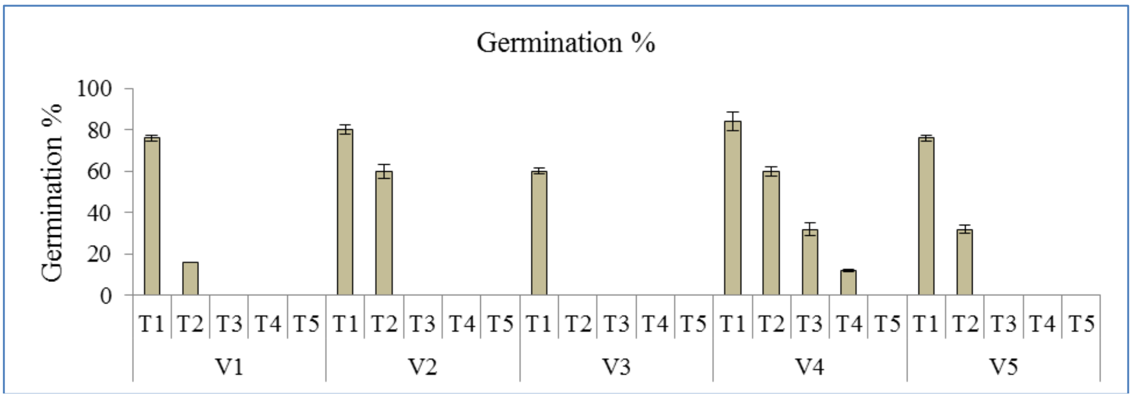

Fig 1: Effect of Arsenic concentration on the seed germination percentage of five different rice varieties (O. Sativa L.) with five Arsenic treatments()(error bars represent the standard error of mean

Table I: Effects of As stress on seed germination and seedling growth of rice genotypes at 10 DAS

		GP	Shoot length(Cm)	Root length (Cm)	Shoot fresh wt(mg)	Shoot dry wt(mg)	Root fresh wt(mg)	Root dry wt(mg)	RLWC
V_1	T1	76^{b}	4.25^{b}	2.55^{bc}	11.20^{bc}	1.41^{cd}	9.90^{a}	2.40^{a}	94.80^{a}
	T2	16^{e}	0.70^{f}	1.00^{f}	8.73^{de}	0.96^{e}	7.30^{b}	0.98^{bc}	82.94^{c}
	T3	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	T4	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	T5	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
V_2	T1	82^{a}	4.23^{b}	2.33^{cd}	12.43^{bc}	1.50^{bcd}	4.65^{c}	1.45^{c}	95.16^{a}
	T2	68^{c}	3.34^{cd}	1.88^{cde}	11.00^{bc}	1.35^{cd}	2.73^{cde}	1.00^{cd}	88.35^{b}
	T3	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	T4	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	T5	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
V_3	T1	60^{c}	3.40^{cd}	1.50^{de}	10.25^{cd}	1.20^{de}	4.43	0.66^{d}	94.07^{ab}
	T2	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	T3	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	T4	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	T5	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
V_4	T1	84^{a}	4.65^{a}	3.40^{a}	15.73^{a}	1.98^{a}	3.40^{cd}	1.87^{b}	96.00^{a}
	T2	60^{c}	3.43^{cd}	1.78^{de}	12.87^{b}	1.40^{cd}	3.37^{cd}	0.60^{d}	92.10^{ab}
	T3	32^{d}	3.20^{d}	1.30^{ef}	10.28^{cd}	1.35^{cd}	2.20^{de}	0.56^{e}	91.56^{ab}

	T4	12^{e}	1.95^{e}	1.20^{ef}	7.60^{e}	1.30^{d}	1.00^{ef}	0.50^{e}	66.30^{d}
	T5	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
V_6	T1	78^{b}	4.10^{b}	3.13^{ab}	15.30^{a}	1.83^{ab}	2.80^{cde}	1.17^{cd}	81.67^{c}
	T2	32^{d}	3.83^{bc}	1.78^{de}	10.40^{cd}	1.68^{abc}	1.75^{def}	0.83^{d}	79.68^{c}
	T3	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	T4	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	T5	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00

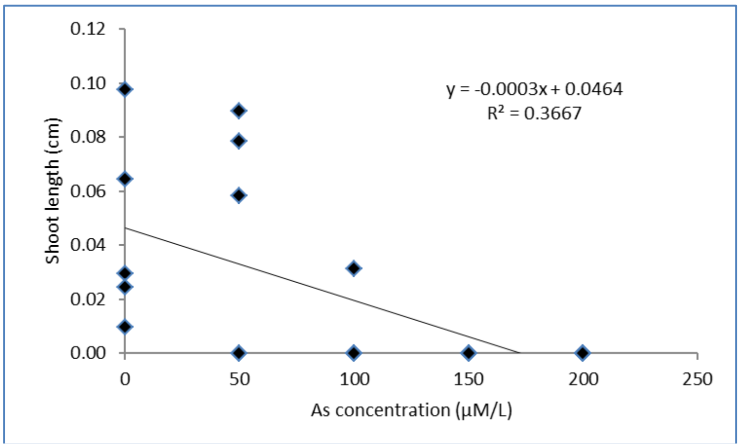

Fig 2: Relationship between arsenic concentration and shoot length for aromatic rice varieties.

3.2. Speed of germination

Speed of germination was recorded statistically maximum in case of V_4 control (14.84). With the increase in the concentration of As in solution, speed of germination decreased continuously. Generally controlled experienced the higher speed of germination than the As treatments ones. According to Shankar (2006) [32] seed germination is very sensitive physiological process affected by heavy metals. There were 35 %, 6.4 %, 2.6% and 42.5 % decrease in speed of germination when the As concentration increased from 0 to 50 µM/L in V_1, V_2, V_4 and V_5, respectively. In case of V_4 from 50 to 100 µM/L and 100 to 150 µM/L As concentration the speed of germination decreased 48 % and 56 % respectively. Decreased germination rate in response to As exposure in rice has been reported by Shri *et al*. (2009)[34]. In addition, Sharma (2012) [33] concluded that poor seed germination rate due to As was attributed to poor cell wall metabolism and hormonal signaling.

3.3. Hypocotyl length

For all the cultivars, the hypocotyls lengths decreased as As concentrations were increased in solution (Fig2): moreover, for both shoots and roots the maximum lengths value were expressed in the control conditions (Fig3 & Table I). Under the As treatments, the highest mean hypocotyls length (3.83 cm) were showed by V_5 at 50 µM/L As treatment (T_2). The highest negative effect on shoot length of aromatic rice was obtained in V_3 and V_1. The shoot length of control were statistically at par for V_1, V_2 and V_5. There was 83 % decrease in hypocotyls length from 0 to 50 µM/L As concentration in V_1 which was 20 % and 6.5 % for V_2 and V_5, respectively. However, in V_4, the decrease of hypocotyls length from 0 to 50 µM/L, 50 to 100 µM/L and 100 to 150 µM/L were at 26 %, 6.7 % and 39 % respectively. Geng and his co-workers (2005) [11] observed that the inhibition was stronger in the root than in the shoot when treated with As. The interaction among variety and As concentration was significant($p<0.01$). Plant cells are very much sensitive to toxic elements and at the cellular level arsenic toxicity cause electrolyte leakage due to membrane damages with increased production of malondialdehyde, a by-product of lipid peroxidation (Finnegan *et al*. 2012 [10]).

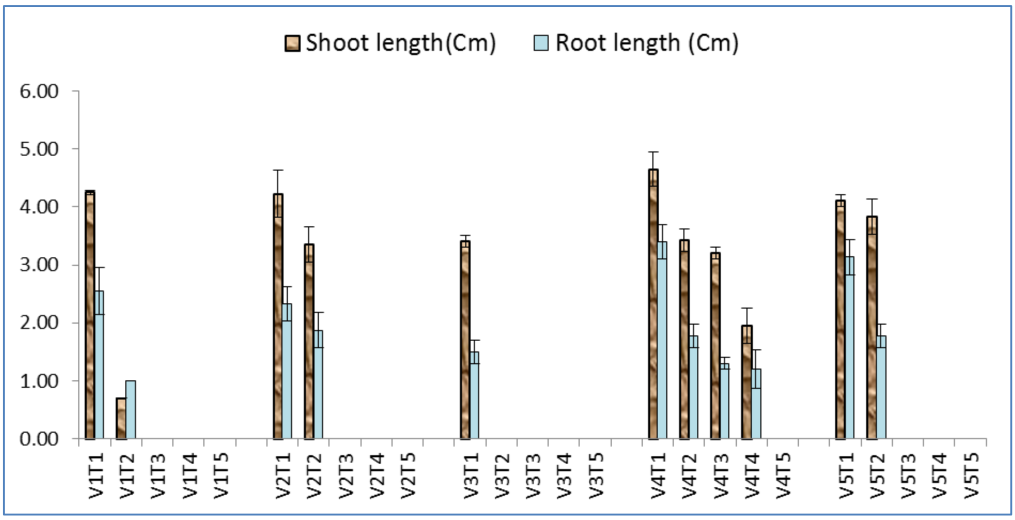

Fig 3: Effect of Arsenic stress on shoot and root length of 10 DAS old seedling of different rice varieties (error bars represent the standard error of mean).

3.4. Radicle length

Radicle length was relatively more sensitive to As concentration in different varieties than that was for hypocotyls length(Fig3). As concentration in solution caused a marked reduction in radical length of aromatic rice seedlings (Fig4). Besides inhibitory elongation, other morphological abnormalities occurred as many of the varieties caused twisted radical growth. Control treatment (T_1) experienced highest radical length where V_4 showed significantly the highest (3.40 cm) value followed by V_1 (2.55 cm). Root growth was almost halted by As treatments in V_3 (Table I) however, V_1 showed higher sensitivity at T_2 treatment than V_2, V_4 and V_5, respectively. Cultivator V_4 showed the resistancy towards As concentration. A decrement of 47 %, 27 % and 8 % in root length of the treatments from 0 to 50 µM/L, 50 to 100 µM/L and 100 to 150 µM/L, respectively was observed. Reduced root growth in response to arsenic exposure has been reported by a number of investigators in other plants (Hartley-Whitaker *et al*. 2001[13]). Under T_2 treatment V_1, V_2 and V_5 experienced a reduction of 60 %, 19 % and 43 % in radical length respectively. The variety and As concentrations showed significant interaction (Table III) among them. Usually, due to translocation in plants, the accumulation of arsenic decreased from root to above ground parts. It might be due to induction of phytotoxicity by As resulting in restricted root growth.

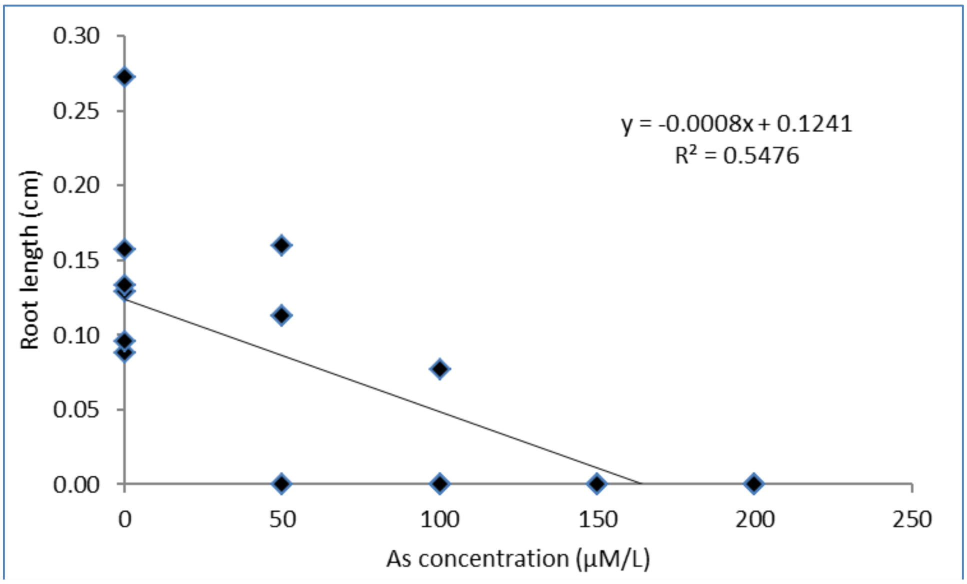

Fig 4: Relationship between arsenic concentration and root length for aromatic rice varieties

Table II. Results of the two-way ANOVA and Tukey multiple range tests for the effects of As on the seed germination and seedling growth parameters of different rice genotypes.

Source of variance	df	GP	Shoot length(Cm)	Root length (Cm)	Shoot fresh wt(mg)	Shoot dry wt(mg)	Root fresh wt(mg)	Root dry wt(mg)	RLWC
Variety	4	2.73*	84.42**	16.39**	70.54**	14.50**	50.29**	20.17**	472.42**
As treament	4	12.97**	388.92**	89.69**	290.29**	59.85**	162.59**	16.63**	2116.32**
Var * As	16		37.33**	4.40**	18.02**	8.14**	13.98**	20.14**	174.36**
Error	72		0.076	0.108	2.54	0.322	6.25	0.075	19.25
CoV(%)			0.056	0.113	0.049	0.124	0.076	0.115	0.023

DAS: Days after sowing.[a] F-values. ns: not significant F ratio (p < 0.05); *, ** and *** indicate significant at P < 0.05, 0.01 and 0.001, respectively.

Table III. Results of Matrix correlation for the effects of As on the seed germination and seedling growth parameters of different rice genotypes.

Correlations								
	As	Shoot length(Cm)	Root length (Cm)	Shoot fresh wt(mg)	Shoot dry wt(mg)	Root fresh wt(mg)	Root dry wt(mg)	RLWC
As	1							
Shoot length(Cm)	-0.851**	1						
Root length (Cm)	-0.813*	0.919**	1					
Shoot fresh wt(mg)	-0.85*	0.960**	0.943**	1				
Shoot dry wt(mg)	-0.86**	0.966**	0.917**	0.988**	1			
Root fresh wt(mg)	-0.811*	0.698**	0.721**	0.740**	0.699**	1		
Root dry wt(mg)	-0.543*	0.425*	0.317*	0.394*	0.406*	0.259	1	.
RLWC	-0.865*	0.918**	.896**	0.967**	0.957**	0.792**	0.444*	1

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

3.5. Shoot dry weight

Dry weight of shoot differed with varieties and different As treatments. In control condition, dry weights of shoot were significantly higher than those under treatments. The experimental results for shoot weight indicated that V_3 was most sensitive and V_4 came out to be the most resistant variety against As stress. Again, increases in As concentration decreased dry shoot materials in all the varieties. Shoot growth was clearly restricted in all concentration above 50 μM/L (T_2) in all varieties except V_4. Reduced shoot growth in rice due to application of arsenate had been reported by Abedin *et al*. (2002) [1]. Significantly maximum shoot growth was observed in V_4 control (1.98 mg) followed by V5 control (1.83 mg). There were 32%, 10 % and 8% reduction in shoot dry weight under the varieties V_1, V_2 and V_5 respectively from 0 to 50 μM/L As treatment. The reason is that plant roots were the first point of contact for these toxic arsenic species in the nutrient media (Abedin and Meharg 2002 [2]). Cultivar V4 showed shoot growth in all the As treatments except T5 (300 μM As/L). The shoot growth decreased significantly at the rate of 29 %, 4% and 4under V4 variety from increasing As concentration 0 to 50 μM/L, 50 to 100 μM/L and 100 to 150 μM As/L, respectively. The interaction effect against variety and As concentration was also noted significant (Table 2).

3.6. Root dry weight

It was found that As concentration in the solution showed statistically significant inhibition on root elongation of the five varieties. Control showed significantly higher ($p<0.05$) root growth that the As stress treatments. Treatment T_2 (50 μM As/L) experienced minimum toxicity on test plants as it generally showed root growth in almost all the varieties. The 50 μM/L As had significantly retarded root growth 59 %, 31 %, 68 % and 29 % under V_1, V_2, V_4 and V_5 respectively than control. Carbonell *et al*. (1998) [7] showed that the plant biomass with low concentrations of As treatment was slightly higher than that of the control in the conditions of nutrition cultivating. The concentration of As above 100 μM/L restricted root growth in all the varieties excluding V4, however, the root dry materials reduced significantly 7 % and 11 % from 50 to 100 μM/L and 100 to 150 μM/L As concentration, respectively. As for example, in the Guandu wetland of Taiwan, arsenic concentration in plant parts of Kandelia obovata was decreased from the roots (19.74 mg kg^{-1}) to the stems (1.76 mg kg^{-1}), leaves (1.71 mg kg^{-1}), and seedlings (0.48 mg kg^{-1}) (Liu, C.W *et al*. 2014[16]; Liu, W.J *et al*. 2014[14]). A similar observation was also found in the Ratna variety of Oryza sativa (Aman rice), where, bioaccumulation of arsenic was found in decreasing order: root > basal stem > median stem > apical stem > leaves > grains (Bhattacharya *et al*. 2014 [6]; Liu *et al*. 2004 [19]).

3.7. Relative water content (RWC)

Arsenic (As) concentration significantly reduced RWC of shoots compared to control. Depending on decrease in shoot growth, RWC gradually declined with the increasing concentration of As (Fig5). Low RWC was observed under V_5, while, the maximum was for V_4. Te 50 μM/L As caused reduction of RWC than control having reduction of 12 %, 7 %, 4% and 2% under V_1, V_2, V_4 and V_5 respectively. Plant transpiration intensity was found to be reduced after arsenic exposure (Stoeva *et al*. 2003 [36]). Additionally, there were 1 % and 27 % decrease in RWC from 50 to 100 μM/L and 100 to 150 μM/L As concentration, respectively (Fig6). At a higher concentration, arsenic interferes with various metabolic processes, adversely affects the plant metabolism, and consequences in death. Abedin and Meharg (2002) [2] reported that early seedling growth of rice decreased significantly with increasing concentrations of As.

3.8. Shoot and root fresh and dry weight percentage reduction (FWPR/ DWPR)

Figure depicts that all the cultivars were affected in FWPR and DWPR by the As concentration in the solution. With the increase in As concentration both shoot FWPR and DWPR were increased and were made a significant relationship with them. FWPR was least for V_2T_2 and V_5T_2 treatments (statistically not significant) and least DWPR was noticed for V_5T_2 treatment. Roots fresh weights were significantly reduced under stress. Reduction was maximum for V_4T_4 in case of both in FWPR and DWPR. Rahman *et al*. (2007) [27] reported that regardless of rice varieties, accumulation of arsenic were 28 folds higher in the root than that of the shoot. That is the reason for higher values of FWPR. The V_1T_2 and V_5T_2 showed minimum FWPR and DWPR as indicated earlier in shoot. High resistance to arsenic could be achieved by (1) complexation of arsenic by such as peptides with SH-groups (SchmoEger *et al*. 2000 [30]), (2) reduction of arsenic influx by suppressing phosphate/arsenate uptake systems (Meharg and Macnair 1992 [21]; Meharg 1994 [22]), and (3) enhanced production of antioxidants that detoxify free reactive oxygen species (ROS) produced in response to arsenite and arsenate (Hartley-Whitaker *et al*. 2001 [13]). However, fresh and dry weight percentage reduction was lower in shoots than in roots. Plant physiological parameters i.e. plant height and biomass reduction with increasing concentration of As in both cultivation systems indicates As toxicity in rice plants as reported in many other studies (Geng *et al*. 2005 [11]).

3.9. Correlation of plant growth parameters

Results showed that positive and negative correlations were prevailed between the growth parameters. As concentration was in significantly negative correlation with shoot and root length; shoot and root fresh and dry weights and RWC. Root growth showed somewhat less negative correlation ($r=-0.543$, $p<0.05$). The shoot length with root length($r=0.919$, $p<0.01$), shoot fresh weight with root fresh weight ($r=0.740$, $p<0.01$), shoot dry weight and root dry weight ($r=0.406$, $p<0.01$), shoot dry weight with RWC ($r=0.957$, $p<0.01$) and root dry weight with RWC ($r=0.444$, $p<0.01$) were showed strong positive correlations. According to Mitchell and Barr(1995) [23] when uptake of nutrition was inhibited in roots, the growth of the whole plant was constrained, and the plant biomass decreased finally. Table indicated that RWC was significantly made strong correlation with shoot parameters than root parameters.

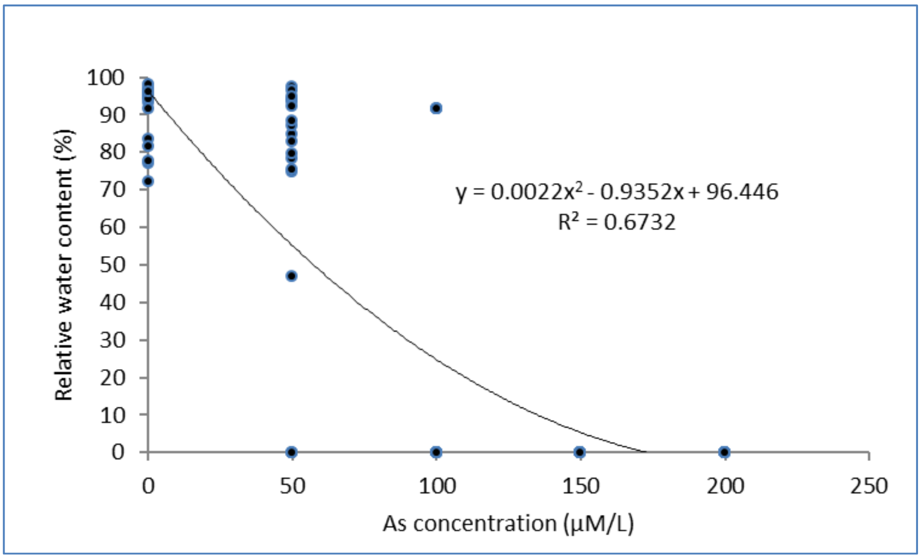

Fig 5: Relationship between arsenic concentration and relative water content for aromatic rice varieties.

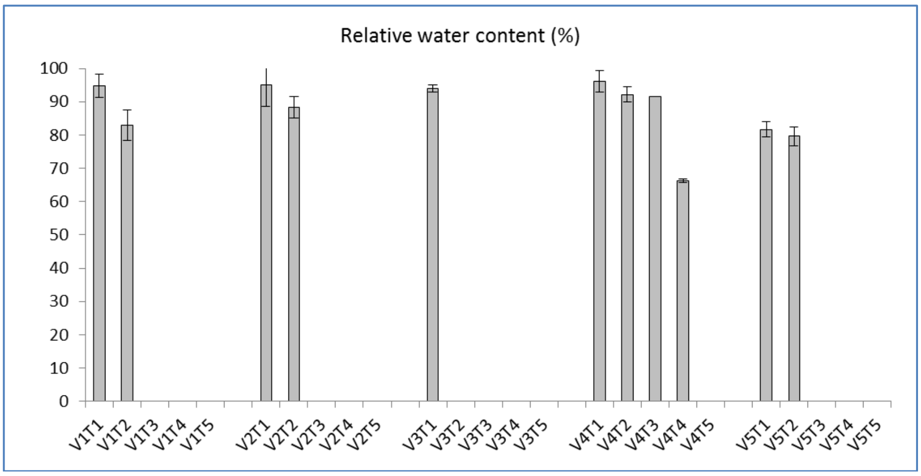

Fig 6: Relative water content of five indigenous rice varieties under different Arsenic treatment (error bars represent the standard error of mean)

3.10. Correlation of germination indices

Matrix correlation of the parameters clearly indicated that As was significantly negatively correlated with the germination indices (germination percentage and speed of germination). However, GP showed most strong negative correlation with rest of the indices. The speed of germination was significantly positive correlated ($r=0.986$, $p<0.01$) with GP.

4. Conclusion

The overall results showed that seeds of indigenous rice varieties had negative effect on As treatments. Accelerated As concentration decreases significantly the germination percentage, speed of germination and other growth parameters. The study also showed that Gobindabhog (V_1), Radhunipagol (V_2), Lal badshabhog (V_3) and Kalonunia (V_5) were unable to perform well above 50 µM/L As concentration. Roots were much less affected than shoot by As stress. The performance of the experiment concluded that Sugandha (V_4) is the best among all five varieties and Gobindabhog (V_1) is the most sensitive variety with respect to As stress.

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