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Phytotoxicity of Arsenate and Salinity on Early Seedling Growth of Rice (*Oryza sativa* L.): A Threat to Sustainable Rice Cultivation in South and South-East Asia

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Abstract

Arsenic (As) contamination is an important environmental consequence in some parts of salinity-affected South (S) and South-East (SE) Asia. In this study, we investigated the individual and combined phytotoxicity of arsenic (As) [arsenate; As(V)] and salinity (NaCl) on early seedling growth (ESG) of saline-tolerant and non-tolerant rice varieties. Germination percentage (GP), germination speed (GS) and vigor index (VI) of both saline-tolerant and non-tolerant rice varieties decreased significantly (p[0.01) with increasing As(V) and NaCl concentrations. The highest GP (91%) was observed for saline non-tolerant BRRI dhan28 and BRRI dhan49, while the lowest (62%) was for saline tolerant BRRI dhan47. The ESG parameters, such as weights and relative lengths of plumule and radicle, also decreased significantly (p\0.01) with increasing As(V) and NaCl concentrations. Relative radicle length was more affected than plumule length by As(V) and NaCl. Although VI of saline-tolerant and non-tolerant rice seedlings showed significant variation (p\0.05), weights and lengths of plumule and radicle of different rice varieties did not show significant variation for As(V) and NaCl treatments. Results reveal that the combined phytotoxicity of As(V) and NaCl on rice seed germination and ESG are greater than their individual toxicities, and some saline-tolerant rice varieties are more resistant to the combined phytotoxicity of As(V) and NaCl than the saline non-tolerant varieties.

Keywords: Rice, Arsenic, Salinity, Phytotoxicity.

Introduction

Arsenic (As) contamination in groundwater has been reported from over 70 countries of which the situation is worst in Bangladesh (Brammer and Ravenscroft 2009). Beside the use of As-contaminated groundwater for drinking and household purposes, this water have also been extensively used for crop irrigation, particularly paddy rice (*Oryza sativa* L.), in Bangladesh and other South (S) and South-East (SE) Asian countries (Meharg and Rahman 2003). About 75% of the total cropped area and 83% of the irrigated area in Bangladesh is under rice cultivation (Meharg and Rahman 2003). Background levels of As in paddy soils in Bangladesh range from 4 to 8 mg kg^{-1} (Williams et al. 2006), and long-term use of As-contaminated groundwater for irrigation has resulted in significant increase of As concentration in the topsoil (about 150 mm) of paddy fields (Brammer and Ravenscroft 2009). About 83 mg kg^{-1} As was found in paddy soils that had been rrigated with As-contaminated groundwater (Williams et al. 2006). As contamination in underground irrigation water and paddy soils is also an environmental problem in West Bengal (India), China, Myanmar, Nepal, Thailand, Taiwan and Vietnam (Nordstrom 2002), and is important concern due the adverse effects of As on yield and food contamination (Brammer and Ravenscroft 2009; Rahman et al. 2008). Meharg (2004) reported that As in irrigation water and paddy soils poses a serious threat to sustainable rice cultivation in S and SE Asia. Soil salinity is another environmental problem in S and SE Asian countries. About 49 million hectare (m ha) of soils in humid regions of S and SE Asia are salinity-affected, and 27 m ha of these are coastal saline soils (Ponnamperuma and Bandyopadhya 1980). About 23.2, 2.5, 1.5, and 1.1 m ha of soils in India, Bangladesh, Thailand, and Vietnam, respectively, are salinity affected

(Ponnamperuma and Bandyopadhya 1980), which is a threat to sustainable rice cultivation in large areas of these countries.

There is great regional and temporal variability of the As concentration in ground water as well as in paddy soils in S and SE Asia (Fendorf et al. 2010), and a large part of the As-affected areas of this regions are also affected by soil salinity (Ponnamperuma and Bandyopadhya 1980). For example, high As-contamination in groundwater and soils has been found across the south-costal part of Bangladesh (Brammer and Ravenscroft 2009; Meharg and Rahman 2003). Groundwater and soils of the coastal areas of the country are also affected by salinity (Ponnamperuma and Bandyopadhya 1980; Rahman et al. 2011). To avoid As problems, peoples of As and salinity affected southcoastal part of Bangladesh are extensively using groundwater from deeper aquifers, which is increasing salinity in groundwater by seawater intrusion (Rahman et al. 2011). This pattern of concurrent occurrences of As and salinity problems has also been found in some parts of other S and SE Asian countries like Myanmar, Vietnam, and West Bengal of India (Fendorf et al. 2010; Ponnamperuma and Bandyopadhya 1980).

Rice is the staple food crop in S and SE Asia, and saline tolerant rice varieties have been cultivated in the salinity affected areas. Saline soils should keep flooded (waterlogged), and must not be allowed dry over the whole rice growing period to minimize the phytotoxicity of salinity (Gregorio et al. 1997). This flooded (reducing) condition increases As bioavailability to rice plant (Duxbury et al. 2003). Although As phytotoxicity on germination and early seedling growth (ESG) of rice (*O. sativa* L.) have been investigated (Abedin and Meharg 2002), the combined toxicities of As and salinity on seed germination and ESG of rice has not been investigated. Therefore, the phytotoxicity of As and salinity on seed germination and ESG of rice were investigated using saline-tolerant and non-tolerant Bangladeshi rice varieties. Since arsenate [As(V)] is the main species in underground irrigation water (Chatterjee et al. 1995), we used this species in this study. This is the first report on the combined phytotoxicity of As(V) and salinity (NaCl) on seed germination and ESG of rice, which will be relevant to the agronomy and sustainable rice cultivation in As- and salinity-affected areas in S and SE Asia.

Materials and Methods

Saline-tolerant (BRRI dhan47, BRRI dhan53 and BRRI dhan54) and non-tolerant (BRRI dhan28, BR 11 and BRRI dhan49) rice varieties were collected from the Bangladesh Rice Research Institute (BRRI). About 30 g seed of each varieties were surface sterilized using 50 mL of 1% NaOCl solution. The seeds were immersed into the solution for five min, and then washed with deionized (DI) water [using an E-pure system (Barnstead)] and dried using tissue paper.

Stock solutions of As(V) and salt were prepared by dissolving sodium arsenate ($Na_2HAsO_4.7H_2O$) and sodium chloride (NaCl), respectively, in DI water. NaCl was used to prepare the salt solution because it is easily soluble without causing any precipitation of different ions (Aslam et al. 1993). Rice seeds were exposed to four As(V) (3, 6, 9 and 12 mg L-1) and two salt (2 and 4 g L-1) concentrations in different (individual and combined) combinations. All chemical reagents used in this experiment were of analytical grade. The experiment was performed as a three factorial randomized block design with three replications of each treatment. For each treatment, 25 pre-sterilized rice seeds of each variety were germinated in 10 cm diameter Petri dishes. Two pieces of filter paper (Whatman No. 1) were placed in each Petri dish, and moistened with 10 mL aqueous solution of As(V) and NaCl (individual or combined) treatments. Petri dishes were coved by the lid, and were incubated in a plant growth chamber with conditions of 14:10 h light/dark schedule, 100–125 lE m^{-2} s^{-1} light intensity, and 30 ± 2°C.

Seeds were considered to be germinated when the length of both plumule (shoot) and radicle (root) were equal or more than 2 mm. The number of germinated seeds in each Petri dish was counted daily during the 9 days experiment. The lengths and weights of plumule and radicle of 10 randomly selected seedlings from each of the Petri dishes were measured at harvest (after 9 days). Moisture content of the ten seeds was also calculated from dry and weight weights after oven drying at 65°C for 72 h. Fresh weight (f. wt.) of radicle (root) and dry weight (d. wt.) of plumule (shoot) were used to determine the seedling growth. Germination speed (GS) and vigor index (VI) were used to measure the seedling vigor. The VI was calculated from radicle and plumule lengths and germination percentage (GP) of the seeds.

Moisture contents of rice seeds were determined after 50 h from the initiation of germination using the following formula (Kim et al. 2005)

$$Water\ content\ \ (\%) = \frac{\text{Fresh weight} - \text{dry weight}}{\text{Fresh weight}} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

GP (also termed as germination index GI) of rice seeds was calculated at 9th day following the formula recommended by International Rice Research Institute (IRRI) (Gummert 2011)

$$GP = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds}} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

The GS of the seeds was calculated using the formula proposed by the International Seed Testing Association (ISTA) (Hampton and Tekrony 1995)

$$SG = \sum \left(\frac{\text{Number of germinated seeds}}{\text{Days of first count}}\right) + \cdots + \left(\frac{\text{Number of germinated seeds}}{\text{Days of final count}}\right) \; (3)$$

The VI of rice seedlings was calculated using the following formula (Baki and Anderson 1972)

$$VI = (\text{Mean of root length} + \text{Mean of Shoot length}) \times \text{Seed GP} \ldots\ldots\ldots (4)$$

Data analysis was performed by SPSS 17.0 for windows. Comparison of means of the treatments was made by Duncan’s Multiple Range Test (DMRT).

Results and Discussion

Irrespective of the rice variety and NaCl treatments, GP of rice seed decreased significantly ($p < 0.01$) with increasing of As(V) concentration (Table 1). Seed germination was reduced by 5%, 11%, 19% and 43% for 3, 6, 9 and 12 mg L^{-1} As(V) treatments, respectively. The highest germination (97%) was found in the As-control treatment, while the lowest (54%) was found in the 12 mg L^{-1} As(V) treatment. Irrespective of rice variety and As(V) treatment, GP also decreased significantly ($p < 0.01$) with increasing NaCl levels (Table 1). Combined treatments of 3, 6, 9 and 12 mg L^{-1} As(V) and 2 g L^{-1} NaCl decreased the seed germination by 5%, 12%, 19% and 44% for, respectively. In addition, combined treatments of 3, 6, 9 and 12 mg L^{-1} As(V) and 4 g L^{-1}

NaCl decreased the seed germination by 12%, 20%, 35% and 69% for, respectively (Table 2). Although previous studies have reported the toxic effects of As on rice seed germination (Abedin and Meharg 2002; Asaduzzaman et al. 2009), this study for the first time investigated the combined toxicities of As(V) and NaCl on germination and ESG rice seeds, thus is of great relevance to the S and SE Asian perspective. Rice seed germination decreased significantly with increasing As(V), which is consistent with the results of previous study (Abedin and Meharg 2002). However, the combined toxic effect of As(V) and NaCl on rice seed germination was observed to be greater than the individual phytotoxicity of As(V) and NaCl (Tables 1, 2).

The phytotoxicity of As(V) and NaCl on rice seed germination differed with rice variety. The combined toxicities of As(V) and NaCl on seed germination of different rice varieties are shown in Fig. 1. Among the saline-tolerant varieties, BRRI dhan54 showed the highest tolerance to As(V) and NaCl phytotoxicity whereas BRRI dhan47 was found to be highly susceptible to their combined phytotoxicity. Germination of BRRI dhan47 seeds failed completely in the 12 mg L^{-1} As(V) and 4 g L^{-1} NaCl treatments (Fig. 1f, d). Saline-tolerant BRRI dhan53 showed moderate resistance to As(V) and NaCl phytotoxicity up to 9 mg L^{-1} As(V) treatments, but the phytotoxicity was significantly higher in the 12 mg L^{-1} As treatment (Fig. 1e). Among saline non-tolerant varieties, BRRI dhan28 showed greater tolerance to the combined phytotoxicity of As(V) and NaCl than BR 11 and BRRI dhan49 (Fig. 1a–c). The results reveal that the individual toxicities of As(V) and NaCl on seed germination of both saline tolerant and non-tolerant varieties were not significant, while the combined toxicities of As(V) and NaCl on seed germination were highly significant. Moreover, the GP of saline non-tolerant varieties was significantly ($p < 0.05$) higher than the saline-tolerant varieties (Table 1) indicating that saline-tolerant varieties were more susceptible to As(V) phytotoxicity than the saline non-tolerant varieties.

The effect of As(V) and NaCl on early seedling vigor was evaluated by seed GS and VI. Both GS and VI decreased significantly ($p < 0.01$) with increasing As(V) concentration (Table 1), and the combined toxicities of As(V) and NaCl on GS and VI were severe than their individual phytotoxicity (Table 2). The GS was the highest (6.1) in control treatment, while the lowest (2.9) was in 12 mg L^{-1} As(V) treatment indicating that As(V) phytotoxicity delays seed germination. The highest VI (1,027) was observed in control treatments, while the lowest (44.75) was found in 12 mg L^{-1} As(V) and 4 g L^{-1} NaCl treatments (Table 2). These results show that increasing concentrations of As(V) and NaCl and their combined treatments decrease seedling vigor and post germination seedling growth. The combined toxic effects of As(V) and NaCl on seedling vigor differed with rice varieties (Table 1). The highest VI was found for BRRI dhan28, while the lowest was for BRRI dhan47, indicating that saline non-tolerant varieties have higher seedling vigor than the saline-tolerant varieties.

Plumule length (PL) decreased significantly ($p < 0.01$) with increasing As(V) and NaCl concentrations (Table 1). The relative plumule lengths (RPL) at different As(V) and NaCl concentrations are presented in Fig. 2. Irrespective of NaCl concentrations and rice varieties, PL decreased by 16%, 28%, 40% and 54% for 3, 6, 9 and 12 mg L^{-1} As(V) treatments, respectively. The highest PL (3.9 cm) was found in the As-control treatment, while the lowest (1.8 cm) was observed in the 12 mg L^{-1} As(V) treatment. The combined effect of As(V) and NaCl on RPL was greater than their individual effect, and the RPL differed between saline-tolerant and non-tolerant varieties. Among saline non-tolerant varieties, the PLs of BRRI dhan28 and BRRI dhan49 were higher (Fig. 2a, c) than BR11, and the PL of BR11 was significantly reduced in the 12 mg L^{-1} As(V) treatment (Fig. 2b). On the other hand, PL of salinetolerant BRRI dhan53 (Fig. 2e) was less affected by the combined phytotoxicity of As(V) and NaCl than BRRI dhan47 and BRRI dhan54 (Fig. 2d, f). Irrespective of rice varieties, plumule d. wt. decreased significantly ($p < 0.01$) with the individual increase of As(V) and NaCl concentrations (Table 1). Compared to control treatment, plumule d. wt. was reduced by 16%, 24%, 35% and 49% at 3, 6, 9 and 12 mg L^{-1} As(V) treatments, respectively.

Irrespective of rice varieties, radicle length (RL) decreased significantly ($p < 0.01$) with increasing As(V) and NaCl concentrations (Table 1). The combined toxic effects of As(V) and NaCl on relative radicle length (RRL) are shown in Fig. 3.The RL was reduced by 43%, 73%, 84%and 91% for 3, 6, 9 and 12 mg L^{-1} As(V) treatments, respectively. In most cases, the radicle do not emerge in the 12 mg L^{-1} As(V) and 4 g L^{-1} NaCl treatments. The highest RL (5.8 cm) was observed in the As-control treatment where as the lowest (2 mm) was observed in the 12 mg L^{-1} As(V) and 4 g L^{-1} NaCl treatments (Table 2). Radicle f. wt. was severely affected and reduced by 40%, 70%, 80% and 89% for 3, 6, 9 and 12 mg L^{-1} As(V) treatments, respectively. The combined effect of As(V) and NaCl on RRL was greater than their individual effect (Fig. 2). Saline-tolerant and non-tolerant varieties did not show significant variation in RL for either individual or combined effects of As(V) and NaCl.

Seed germination in this study was not found to be significantly affected in the 3 mg L^{-1} As(V) treatment, whereas Abedin and Meharg (2002) reported significant germination reduction in the 4 mg L^{-1} of the same As species. Seed germination was significantly reduced at high As(V) concentrations (6 mg L^{-1}), which is consistent with the results of Asaduzzaman et al. (2009). However, Abedin and Meharg (2002) found that out of eight rice varieties tested, two showed tolerance in germination at high As(V) concentration. The results reveal that the individual phytotoxicity of As(V) in seed germination depends on rice varieties. Varietal differences of As phytotoxicity to rice seed germination have been reported in other studies (Abedin and Meharg 2002; Marin et al. 1992). The present study also shows that saline-tolerant rice varieties are more susceptible to As(V) phytotoxicity than the saline non-tolerant varieties. The susceptibility of the saline-tolerant varieties to As(V) might be related to the genetic constituents of these varieties since several studies reported the involvement of some genes in As(V) tolerance (Dasgupta et al. 2004; Norton et al. 2008). Seed germination involves several physical and biochemical processes such as imbibition, enzyme activation, and post-germination growth stages. In the imbibition stage, seeds take up substantial amount of water. In the present study, the moisture content of rice seeds was found to be decreased significantly ($p < 0.01$) with increasing As(V) concentrations (Table 1). Mishra and Dubey (2006) also reported that rice seeds were substantially water deficient at high As concentration. Thus, water deficiency might reduces seed germination by reducing the activities of enzymes that are needed for the degradation of stored materials in endosperm (Liu et al. 2005a), and for normal metabolic processess and development of seedlings at early stage (Mishra and Dubey 2006).

NaCl also decreased rice seed germination ESG significantly (Table 1). Significant reduction of rice seed germination at high NaCl concentration can be explained by the osmotic and toxic effects of NaCl (Lutts et al. 1996). Reduction of ESG parameters, such as plumule and radicle growths, by NaCl has been reported previously (Lutts et al. 1996; Rahman et al. 2001). At high saline condition, Na^+ and Cl^- uptake creates ionic imbalance in the tissues of rice seedlings (Lutts et al. 1996), and Cl^- produces phytotoxicity that inhibits the growth of plumule and radicle. In addition, the epidermal, cortical, root cap, meristem and stellar cells appeared to be damaged at high NaCl condition (Rahman et al. 2001) resulting radicle growth inhibition. The combined phytotoxicity of As(V) and NaCl causes poor seedling establishment, and reduces seedling quality and production, decreases seed germination speed and seedling vigor (Table 1 and 2). Other ESG parameters, such as plumule length and dry weight, radicle length and weight, were also reduced significantly by As(V), which agree with the previous reports of Liu et al. (2005b) and Abedin and Meharg (2002). In the present study, it was also observed that the radicle growth was more affected than the plumule growth, which is also in agreement with the previous studies (Abedin and Meharg 2002; Liu et al. 2005a). Inhibition of plumule and radicle growth of rice seedlings is a typical response to As (Abedin and Meharg 2002) because of complexation or extra chelation of As(V) with the functional groups of radicle-producing and root-growing enzymes or hormones (Kumar and Banerji 1992; Sneller et al. 1999). In very high As(V) concentration (12 mg L^{-1}), radicle emergence in germinating seeds was stopped though the plumule emerged. Abedin and Meharg (2002) also observed the same event at high As(V) concentration.

The present study shows that seed germination and ESG of rice decreased significantly with increasing As(V) and NaCl concentrations. The combined effect of As(V) and NaCl on seed germination and ESG of rice was greater than their individual effect. Therefore, irrigation of As-contaminated water in salinity-affected paddy fields will pose a greater risk to seed germination and ESG than in non-saline paddy fields. Long-term irrigation of As-contaminated water would lead to a steady build up of As in soil, and the combined effect of As and salinity will be a potential threat to sustainable rice cultivation in the As- and salinity-affected areas, especially in the southern part of Bangladesh and West Bengal (India). Field scale studies are necessary to understand the real threat of As and salinity to rice seed germination and seedling growth. However, the results of the present study provides useful preliminary information about the possible consequences of As and salinity phytotoxicity to rice seed germination and ESG of rice. Since some saline-tolerant rice varieties were found to be more resistant to the combined phytotoxicity of As(V) and NaCl than the saline non-tolerant varieties, breeding of saline-tolerant and As-resistant rice varieties would be a good solution to this problem.

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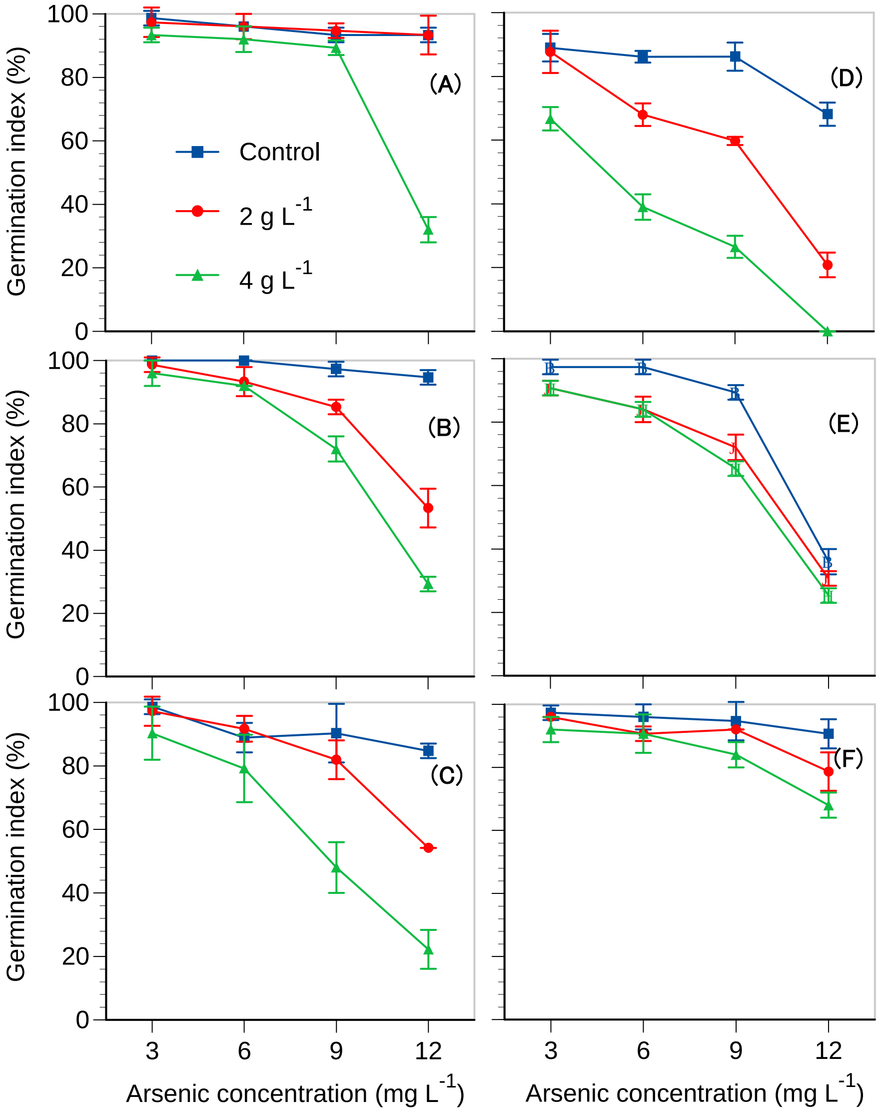

Fig. 1: Effect of arsenic concentrations and salinity levels on seed germination of saline non-tolerant (BRRI dhan28 **(A)**; BR11 **(B)**; BRRI dhan49 **(C)**) and tolerant (BRRI dhan47 **(D)**; BRRI dhan53 **(E)**; BRRI dhan54 **(F)**) rice varieties. Data represent mean ±SD (*n* = 10).

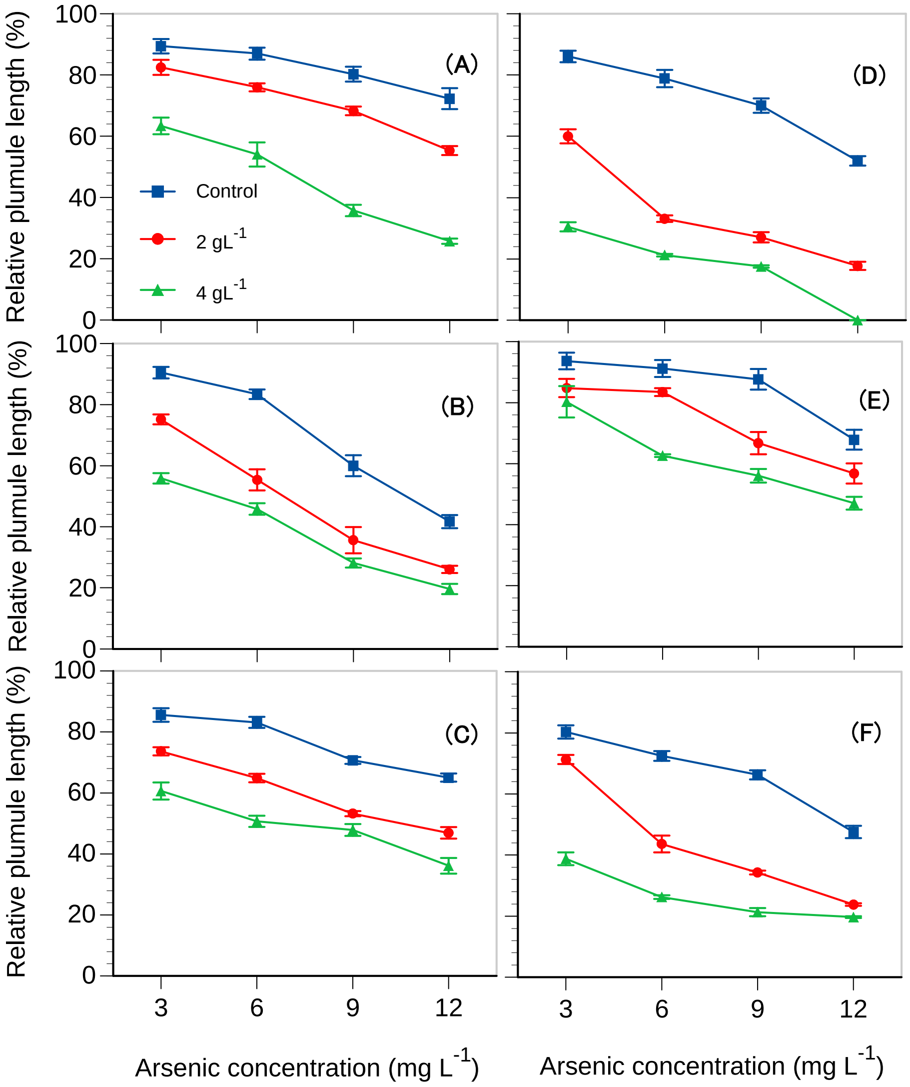

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Fig. 2: Effect of arsenic concentrations and salinity levels on relative plumule length of saline non-tolerant (BRRI dhan28 **(A)**; BR11 **(B)**; BRRI dhan49 **(C)**) and tolerant (BRRI dhan47 **(D)**; BRRI dhan53 **(E)**; BRRI dhan54 **(F)**) rice varieties. Data represent mean ± SD (n = 10).

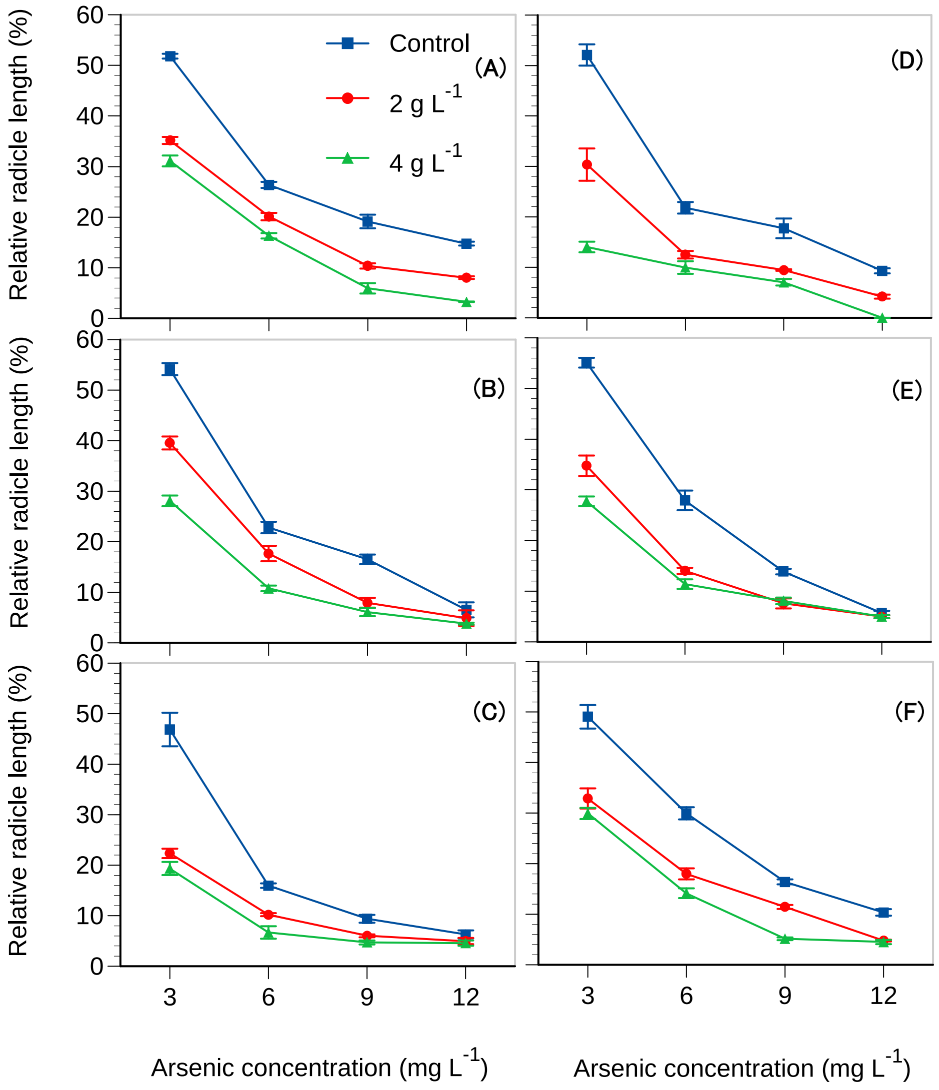

Fig. 3: Effect of arsenic concentrations and salinity levels on radicle length of saline non-tolerant (BRRI dhan28 **(A)**; BR11 **(B)**; BRRI dhan49 **(C)**) and tolerant (BRRI dhan47 **(D)**; BRRI dhan53 **(E)**; BRRI dhan54 **(F)**) rice varieties. Data represent mean ± SD (n = 10).

Table 1 Effect of As(V) and NaCl concentrations on germination and ESG of saline-tolerant (BRRI dhan47, BRRI dhan53, BRRI dhan54) and non-tolerant (BRRI dhan28, BR11, BRRI dhan49) rice varieties

Treatment means from ANOVA test								
	Germination percentage	Speed of Germination (SG)	Vigor Index (VI)	Plumule length (cm)	Plumule weight (mg)	Radicle length (cm)	Radicle weight (mg)	Seed Moisture content
Variety								
V1	91.2 a	5.6 a	547.6 a	3.7 a	2.0 b	2.0 a	4.8 ab	30.3 b
V2	87.3 ab	4.6 b	387.5 bc	2.3 c	2.0 b	1.7 ab	4.5 abc	27.8 d
V3	78.5 bc	5.2 ab	375.4 bc	3.1 b	2.3 ab	1.2 b	3.3 bc	29.9 bc
V4	62.5 d	3.2 c	246.7 d	1.9 d	1.6 c	1.2 b	4.4 abc	31.1 a
V5	77.3 c	4.9 ab	339.3 cd	2.7 bc	2.4 a	1.2 b	2.9 c	29.6 c
V6	91.2 a	5.2 ab	475.0 ab	3.0 b	2.1 ab	1.9 a	5.6 a	27.7 d
	**	**	**	**	**	*	*	**
Arsenic concentrations								
Control	97.0 a	6.1 a	744.0 a	3.9 a	2.7 a	3.7 a	9.7 a	30.3 a
3 mg L^{-1}	92.0 ab	5.6 a	508.1 b	3.2 b	2.3 b	2.1 b	5.8 b	29.6 b
6 mg L^{-1}	85.9 b	5.0 b	340.6 c	2.7 c	2.1 b	1.0 c	2.9 c	29.4 b
9 mg L^{-1}	78.2 c	4.3 c	243.7 d	2.3 d	1.8 c	0.6 d	1.9 d	29.0 bc
12 mg L^{-1}	53.6 d	2.8 d	139.8 e	1.8 e	1.4 d	0.3 d	1.0 d	28.7 c
	**	**	**	**	**	**	**	**
Salinity levels								
control	90.9 a	6.1 a	562.3 a	3.6 a	2.7 a	2.3 a	5.6 a	29.9 a
2 g L^{-1}	82.3 b	4.6 b	376.5 b	2.8 b	2.0 b	1.4 b	4.3 b	29.4 b
4 g L^{-1}	70.8 c	3.6 c	246.9 c	2.0 c	1.5 c	0.9 c	2.8 c	28.9 c
	**	**	**	**	**	**	**	**
Salinity tolerant and non-tolerant varieties								
Non-tolerant	85.7	5.1	436.8	3.0	2.1	1.6	4.2	29.5
Tolerant	77.0	4.4	353.6	2.5	2.0	1.4	4.3	29.4
	**	**	*	NS	NS	NS	NS	NS

Different small letters in the columns explain significant differences among the rice varieties and different As(V) and NaCl treatments

** means significant at 1% level of significance

* means significant at 5% level of significance

NS means non-significant

Table 2 Combined effect of As(V) and NaCl on germination and ESG of saline-tolerant and non-tolerant rice varieties

Individual treatment means (arsenic concentrations and salinity levels)									
Arsenic conc.	Salinity levels	Germination percentage	Speed of germination	Vigor index	Plumule length (cm)	Plumule weight (mg)	Radicle length (cm)	Radicle weight (mg)	Seed moisture content
0 mg L^{-1}	0 g L^{-1}	98.7 a	7.0 a	1027.0 a	4.5 a	3.2 a	5.8 a	12.6 a	31.1 a
0 mg L^{-1}	2 g L^{-1}	97.3 ab	5.9 d	715.5 b	4.0 b	2.9 b	3.3 b	10.6 b	30.2 b
0 mg L^{-1}	4 g L^{-1}	95.1 bc	5.3 f	489.5 de	3.0 g	2.1 e	2.0 d	5.8 d	29.7 cd
3 mg L^{-1}	0 g L^{-1}	95.5 bc	6.5 b	668.2 c	3.9 c	2.8 b	3.0 c	7.8 c	30.3 b
3 mg L^{-1}	2 g L^{-1}	93.3 c	5.5 e	499.8 d	3.3 e	2.4 d	1.9 e	5.6 d	29.3 de
3 mg L^{-1}	4 g L^{-1}	87.1 e	4.7 g	356.3 g	2.4 i	1.7 g	1.4 f	4.0 e	29.1 defg
6 mg L^{-1}	0 g L^{-1}	92.8 cd	6.3 c	482.8 e	3.7 d	2.8 d	1.4 g	4.0 e	29.9 bc
6 mg L^{-1}	2 g L^{-1}	86.2 e	4.8 g	319.8 h	2.6 h	2.0 f	0.9 h	2.6 f	29.4 d
6 mg L^{-1}	4 g L^{-1}	78.6 fg	3.9 i	219.0 j	1.9 k	1.4 h	0.6 i	2.2 g	28.7 efgh
9 mg L^{-1}	0 g L^{-1}	90.6 d	5.9 d	383.0 f	3.2 f	2.5 c	0.9 h	2.5 f	29.2 def
9 mg L^{-1}	2 g L^{-1}	80.0 f	4.1 h	223.1 j	2.1 j	1.6 g	0.5 j	1.8 h	29.2 def
9 mg L^{-1}	4 g L^{-1}	64.2 h	2.7 j	125.2 k	1.5 k	1.1 i	0.3 k	1.3 i	28.5 gh
12 mg L^{-1}	0 g L^{-1}	76.8 g	4.7 g	250.4 i	2.6 l	2.0 f	0.5 j	1.3 i	29.1 efg
12 mg L^{-1}	2 g L^{-1}	54.6 i	2.6 j	124.4 k	1.7 m	1.4 h	0.3 k	1.2 i	28.6 fgh
12 mg L^{-1}	4 g L^{-1}	29.3 j	1.1 k	44.7 l	1.1 n	0.8 j	0.2 l	0.6 j	28.2 h
		**	**	**	**	**	**	**	**

Different small letters in the columns explain significant differences among the As(V) and NaCl treatments

** means significant at 1% level of significance