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ORIGINAL ARTICLE

North East India Rice Genotypes: Screening of Arsenic Tolerant and Sensitive Rice at Germinating Stage

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Arsenic (As) accumulation in rice is hazardous to both plant and human because of its toxicity and carcinogenic properties. North-East (NE) rice genotypes (76) were screened at germination stage under As treatments (0, 50 and 100 μM) in order to discover As tolerant and sensitive NE rice genotypes. Results showed a differential response to As with different degree of impact that can be categorized as tolerant, moderately tolerant and sensitive rice genotypes. This study reveals a significant background for the selection of the highest tolerant and sensitive rice genotypes.

Key words: Arsenic, germination, north east rice, sensitive, tolerant

The toxic and carcinogen As is ubiquitous in the environment through natural sources and human activities, causing serious problems to the ecosystem. The two most common inorganic forms of As are arsenite (AsIII) and arsenate (AsV), dominant in submerged soil and aerobic soil respectively, where factors like pH, redox conditions, and microbial activities influence the interconversion of these two forms. As enters in plant from soil and water and then enters food chain affecting both plant and animal. Several studies revealed that As stress in plant results in the generation of reactive oxygen species (ROS) which induced oxidative stress to the biomolecules and finally resulted into cell death as reviewed by Abbas et al. (2018). Among the various crops, the major staple crop rice (*Oryza sativa L.*) causes a big impact on human health through consumption as rice can accumulate high concentration of As (Bakhat et al., 2017).

Rice (*Oryza sativa* L.), one of the most important cereal crops, being a paddy field crop particularly susceptible to As stress. Accumulation of As occurs higher in rice than other cereals, affecting the yield, moreover, becomes one of the major routes of As exposure in humans through food chain (Davis et al., 2017) and so, rice contribute a substantial amount to the total arsenic consumption in contaminated area (Roxane Guillod-Magnin et al., 2018). Availability of As in soil and physiological properties of plant, these two factors control the accumulation of As in plants (Santra et al., 2013). It has been found that As species and their concentration differ with soil condition, and rice cultivars (Awasthi et al., 2017).

In North East (NE) India, rice is the primary staple food crop but being an efficient accumulator of As, rice greatly imparts a big impact on human health on consumption. Moreover, rice quality and yield are also greatly affected by As contamination. In order to counteract these problems, there is an urgent need to produce locally adapted or tolerant rice which have low arsenic accumulation. Thus, the study was undertaken to screen NE rice genotypes in germination stage to discover As tolerant and sensitive genotype. This was studied by analysing germination percentage, length of plumule and radical, fresh mass and dry mass and arsenic tolerant index (%) of 76 NE rice under As treatment.

MATERIALS AND METHODS

Viable rice seeds (76 genotypes) were procured from Rice Research Station, Wangbal, Manipur, India; Regional Rainfed Lowland Rice Research Station (RRLRRS) Gerua, Guwahati, Assam, India, ICAR-National Bereau of Plant Genetic Resources (NBPGR) Regional Station, Umiam, Meghalaya, India and ICAR-Research complex North Eastern Hills region, Mizoram, Kolasib, Mizoram, India. The seeds were surface sterilized with 0.1% mercuric chloride ($HgCl_2$) solution and set for germination in petri dishes having filter paper moistened with 10 ml of 50 µM and 100 µM As at 28 ± 2ºC for 3 days. For control (0 µM) distilled water was used. On the fourth day of germination uniformly germinated seeds were selected and done the analysis.

Phenotype of the rice seeds

Seed colour and seed shape were determined on the basis of visual observation. Seed length and breadth were measured by using a scale of ten seeds and took mean value. Seed weight also measured.

Germination percentage (GP)

20 sterilized seeds of each variety were set for germination separately and using the following formula (Abdul et al., 2014), germination percentage was analysed

GP = (Number of total germinated seeds/ Total number of seeds tested) × 100

Growth of seedlings

Growth was determined by measuring the length of the radical and plumule and fresh and dry mass of the seedlings. Dry mass of the seedlings was determined after the seedlings were oven dried for 72 h at 80ºC. Reduction percentage of radical length, plumule length and fresh and dry mass of the seedlings were determined by the formulas (Vibhuti et al., 2015):

Radical length percentage reduction (RLPR) = 100 × [1 - (Radical length As stress/ Radical length control)]

Plumule length percentage reduction (PLPR) = 100 × [1 - (Plumule length As stress/ Plumule length control)]

Fresh weight percentage reduction (DWPR) = 100 ×

[1 - (Fresh weight As stress/ Fresh weight control)]

Dry weight percentage reduction (DWPR) = 100 × [1 - (Dry weight As stress/ Dry weight control)]

Arsenic tolerant index (ATI%)

Using the formula described by Wilkins (1978), ATI (%) was calculated.

ATI % = [(Dry weight of treated plants)/(Dry weight of control plants)] x 100.

Statistical analysis

All data obtained were subjected to one-way analyses of variance (ANOVA) and LSD test was used for comparison between mean of treatments and between rice genotypes. All the experiments were repeated three times and each bar represents mean ± standard error of the three experiments. Asterisk and letters indicate a significant mean difference at $P = 0.05$.

RESULTS AND DISCUSSION

Phenotype of the 76 rice genotypes were studied which is given in Table 1. Most of the rice genotypes bear straw yellow colour, only few of them have different colours like blackish brown of R43, R49 and R56, reddish yellow of R21, R28, R35, R46, R55 and brownish yellow of R24, R50, R51, R52 and R73. Long bold is the common shaped of the rice seeds but unusually, slight spindle shaped in R16 and oval shaped in R32 are also observed.

From the analysis, ten highest sensitive and ten highest tolerant rice cultivars to As were selected (Fig 1). Germination percentage of the 20 selected rice genotypes under As treatment is given in Fig 2A which showed differential responses of the rice to As. RT3 and RT6 rice genotypes germinated at the rate of 100% with respect to control in both 50 and 100 µM, revealing 100% germination tolerant percentage (GT%) whereas in genotypes like RS11, RS12 and RS16, germination was inhibited almost by 50% in 100 µM, of which the GT % are 54.21%, 51.66 % and 55.55% respectively. The inhibition of germination might be due to the toxic effect of As on seed metabolic activities that resulted into inhibition of certain enzymes require for seed germination and growth. Previous studies also reported inhibition of seed germination under heavy metal stress by lowering of carbohydrate-metabolizing enzymes thereby affecting digestion and mobilization of reserved carbohydrate of seeds (Singh et al., 2011, Pena et al 2011). The study of Adrees et al. (2015) also demonstrated inhibition of seed germination by heavy metal stress including As. The genotype that germinated 100% with respect to control may be due to proper activation of internal As detoxification mechanisms or restriction of As uptake inside the plant thus less toxic effect. Plant operates different mechanisms against stress, different plant response differently. The response to As differs with the different genotypes.

The growth of the rice seedlings, i.e., the length and biomass are also affected by As (Fig 3 & 4). It is found that radical length of the rice genotypes namely RS11, RS13, RS16 and RS19 is greatly affected by As in dose dependent manner where maximum reduction was recorded at 100 µM As, having RLPR of 95.55%, 98.76%, 99.74% and 95.63% respectively with respect to control (Fig. 3A), revealing high sensitivity to As. Whereas in rice genotypes like RT6, RT8, RT9 and RT10, it is found that the growth of the radical is less affected by As treatments, showing only 14.19%, 18.75%, 18.84% and 10.31% reduction comparing with control suggesting resistivity to As. In comparison with the impact of As on the radical length, the length of plumule is less affected (Fig. 3B). In RT8, RT9 and RS16 genotypes, plumule length is not distress under both 50 and 100 µM As treatments comparing with control, showing only 3.92%, 6.19% and 6.64% reduction at 100 µM with respect to control. This may be due to restriction of As translocation to plumule or well developed defence mechanism against As in plumule. Tiwari and Lata (2018) reviewed that survivability of plant against heavy metal stress depends on the balance between the uptake and accumulation of metal ions and the activation of detoxification mechanisms in plant. However, in rice genotypes RS13 and RS20 plumule length are greatly affected by both 50 and 100 µM As, having 45.83% and 77.96% reduction at 100 µM respectively with respect to control. The difference responses to As may be due to the differential responses of defence mechanisms operating inside the different rice genotypes which resulted into resistivity or sensitivity to As.

Table 1. Phenotype of the 76 NE rice genotypes

Code No.	Name	Seed colour	Seed shape	Seed length in mm	Seed breath in mm	Seed weight in g
R1.	Anjali	Straw yellow	Medium bold	8	3	0.025
R2.	Bahadur	Straw yellow	Medium bold	8	3	0.023
R3.	Bias muthi	Straw yellow	Medium bold	8	3	0.021
R4.	Bio 25	Straw yellow	Medium bold	8.3	2.8	0.025
R5.	Chandan	Straw yellow	Long bold	9.3	3	0.024
R6.	CR 310	Straw yellow	Medium bold	7.6	3	0.022
R7.	CR 311	Straw yellow	Medium bold	8.6	3	0.024
R8.	CR 601	Straw yellow	Long bold	9	3	0.025
R9.	CR 909	Straw yellow	Long bold	9.6	3	0.025
R10.	Dhusari	Straw yellow	Long bold	10	3	0.028
R11.	Hawai	Straw yellow	Medium bold	8	3	0.027
R12.	Ikora	Reddish brown	Medium bold	8	3.1	0.028
R13.	Kanchan	Straw yellow	Medium bold	7.3	2.1	0.016
R14.	Mia	Straw yellow	Medium bold	8	3.8	0.028
R15.	Naveen	Straw yellow	Medium bold	8.3	3	0.022
R16.	Padmini	Straw yellow	Slender (Slightly spindle)	8.3	2	0.016
R17.	Panchnum	Yellow brown	Slender	8	2.1	0.018
R18.	Pankaj	Straw yellow	Medium bold	7.6	3	0.022
R19.	Parijat	Straw yellow	Long bold	9	2.8	0.023
R20.	Prasad	Straw yellow (dark)	Medium bold	8	3.3	0.023
R21.	Sahibhagi dhan	Reddish yellow	Slender	9	2.3	0.023
R22.	Satyabhama	Straw yellow	Medium bold	8.3	3	0.026
R23.	Subnum	Straw yellow (light)	Slender	8.3	2	0.014
R24.	Tarkekoo	Brownish yellow	Medium bold	8.6	3.6	0.023
R25.	TN1	Straw yellow	Medium bold	7.6	3	0.019
R26.	Chakhao	Brown	Long bold	9.6	3	0.025
R27.	Chakhao Amubi	Brown	Long bold	9.6	3	0.031
R28.	Chakhao Anangbi	Reddish yellow	Long bold	10	3.3	0.028
R29.	Chakhao Angaobi	Straw yellow	Medium bold	8.6	3	0.033
R30.	Changlei	Straw yellow	Long bold	9.7	3	0.25
R31.	Changmen chakhao	Straw yellow	Medium bold	7.6	2.8	0.019
R32.	Heimang	Straw yellow (dark)	Oval	5.6	3.6	0.021
R33.	Heitup	Straw yellow	Long bold & slender	10	4 & 2.5	0.029
R34.	Huikap	Straw yellow	Medium bold	7.3	3.1	0.025
R35.	Kabok	Reddish yellow	Long bold	9	3	0.024
R36.	Katan chakhao	Straw yellow (blacken at tips)	Long bold	9.6	4	0.032
R37.	Keibi	Straw yellow	Medium bold	8.3	3.8	0.026
R38.	Kono aro	Straw yellow	Long bold	10.3	3	0.029
R39.	Kumbi	Straw yellow	Long bold	10	3	0.027
R40.	Moirang phaongaoba	Straw yellow	Medium bold	8.6	3	0.022
R41.	Moirang phou	Straw yellow (dark)	Long bold	9.6	3.8	0.030
R42.	Pat	Straw yellow	Long bold	9.6	3.1	0.028
R43.	Phaugak	Blackish brown	Medium bold	7.4	3	0.23
R44.	Phauren amubi	Blackish yellow	Long bold	10	3	0.28
R45.	Phoudum	Straw yellow	Medium bold	8.3	3.8	0.028
R46.	Phoungang	Reddish yellow	Long bold	9.3	3.1	0.031
R47.	Phouren	Straw yellow	Long bold	9.6	3.1	0.028
R48.	Phouren khongNgangbi	Straw yellow	Long bold	9.6	3	0.027
R49.	Poireiton chakhao	Blackish brown	Long bold	9.3	3	0.023
R50.	Sangsangba	Brownish yellow	Long bold	9.3	3.7	0.29
R51.	Taothabi	Brownish yellow	Medium bold	8.6	3.1	0.023
R52.	Tungoo	Brownish yellow	Long bold	9	3.3	0.029
R53.	Wangoo	Straw yellow	Long bold	9.8	3	0.029
R54.	Yenthik	Straw yellow (light)	Long bold	10	3	0.031
R55.	137492	Reddish yellow	Medium bold	8.3	3	0.021
R56.	140000	Blackish brown	Long bold	10	3.1	0.024
R57.	146079	Straw yellow	Medium bold	8	3	0.020
R58.	200507	Straw yellow	Medium bold	8.3	3	0.020
R59.	200536	Straw yellow	Medium bold	8.3	2.7	0.025
R60.	207937	Straw yellow	Medium bold	8	2.65	0.021
R61.	207949	Straw yellow	Medium bold	7.3	3	0.024
R62.	264486	Straw yellow	Medium bold	7.3	2.9	0.021
R63.	324307	Straw yellow	Long bold	9.1	2.35	0.025
R64.	330250	Straw yellow (dark)	Medium bold	7.3	3	0.020
R65.	350076	Straw yellow	Medium bold	8.3	3	0.022
R66.	350818	Straw yellow	Medium bold	7.7	2.9	0.017
R67.	380488	Straw yellow	Long bold	10	3	0.02
R68.	463756	Straw yellow (light)	Medium bold	7.3	3.85	0.031
R69.	466632	Straw yellow	Medium bold	7.3	2.7	0.019
R70.	466717	Straw yellow	Long bold	10	4	0.035
R71.	Jwain	Straw yellow	Long bold	9.3	3	0.029
R72.	Myari	Straw yellow	Long bold	9.3	3	0.030
R73.	Pnet	Brownish yellow	Long bold	10	3.65	0.035
R74.	Saw	Straw yellow	Medium bold	8.7	3	0.032
R75.	Hawangsen	Brownish yellow	Long bold	9.3	3	0.026
R76.	Rokamlova	Straw yellow	Long bold	10	3	0.027

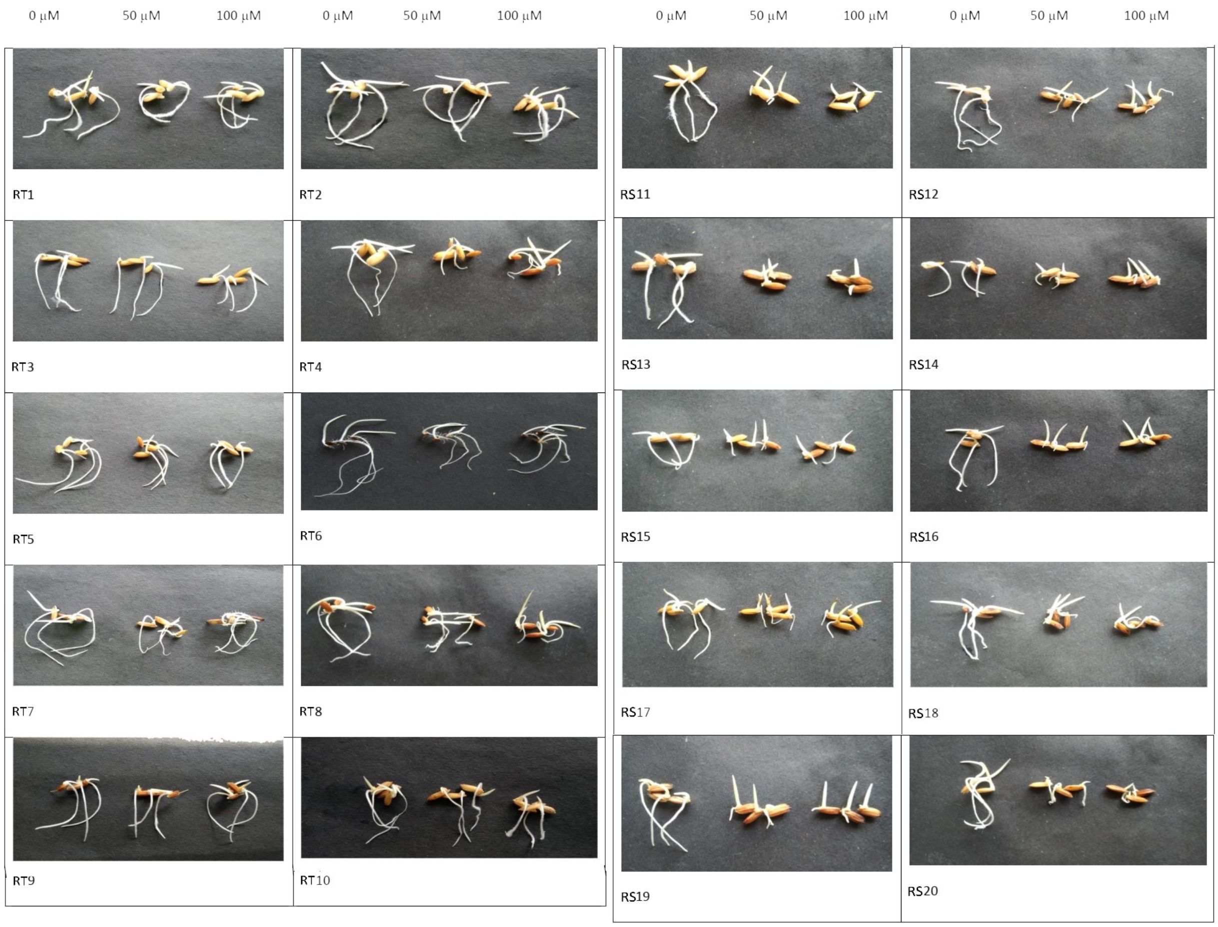

Figure 1. Effect of As (0, 50 and 100 µM) in germinating seedlings of the 20 NE rice genotypes.

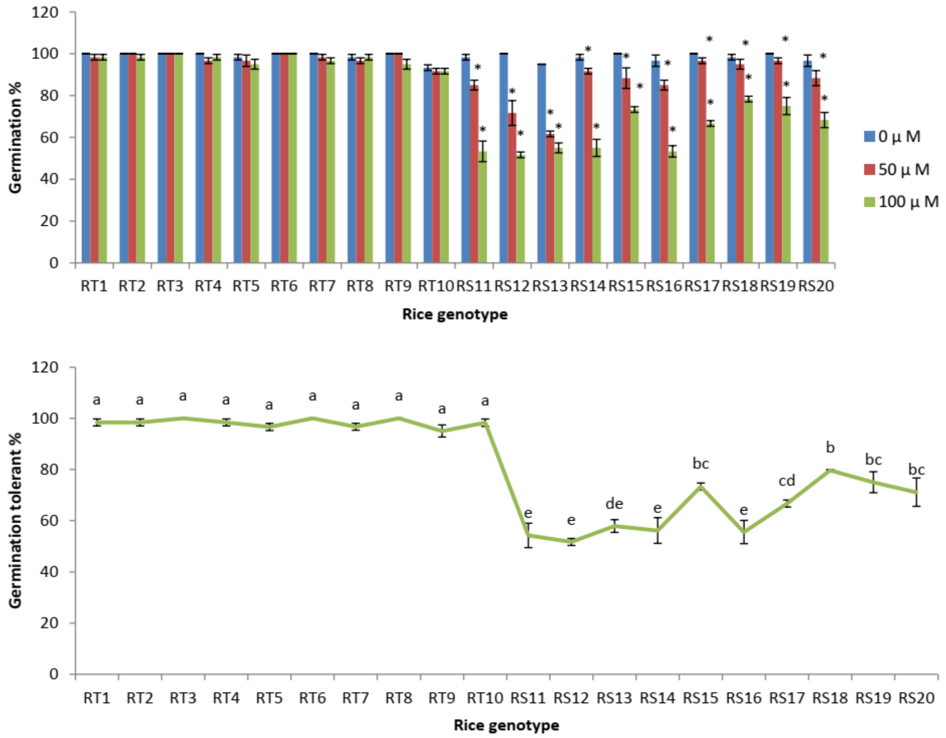

Figure 2. Changes in germination under As treatments (0, 50 and 100 µM) and germination tolerant % at 100 µM As of the rice genotypes. The data presented are mean ± SE (n = 3). * and letters superscripted indicate significant mean difference from control and between the genotypes respectively at 5% level of significance in multiple comparison by LSD test

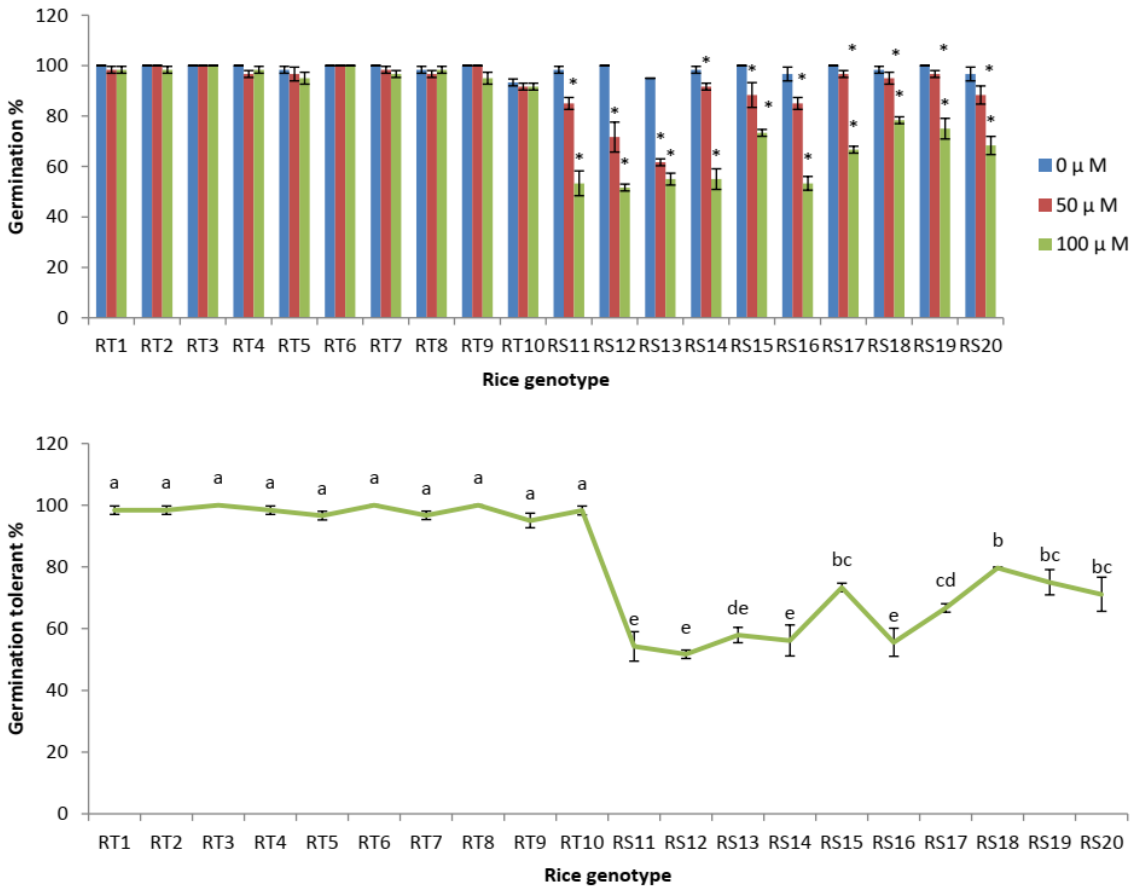

Figure 3. Changes in length of radical and plumule under As treatments (0, 50 and 100 µM) and radical reduction % and plumule reduction % at 100 µM As of rice genotypes. The data presented are mean ± SE (n = 3). * and letters superscripted indicate significant mean difference from control and between the genotypes respectively at 5% level of significance in multiple comparison by LSD test

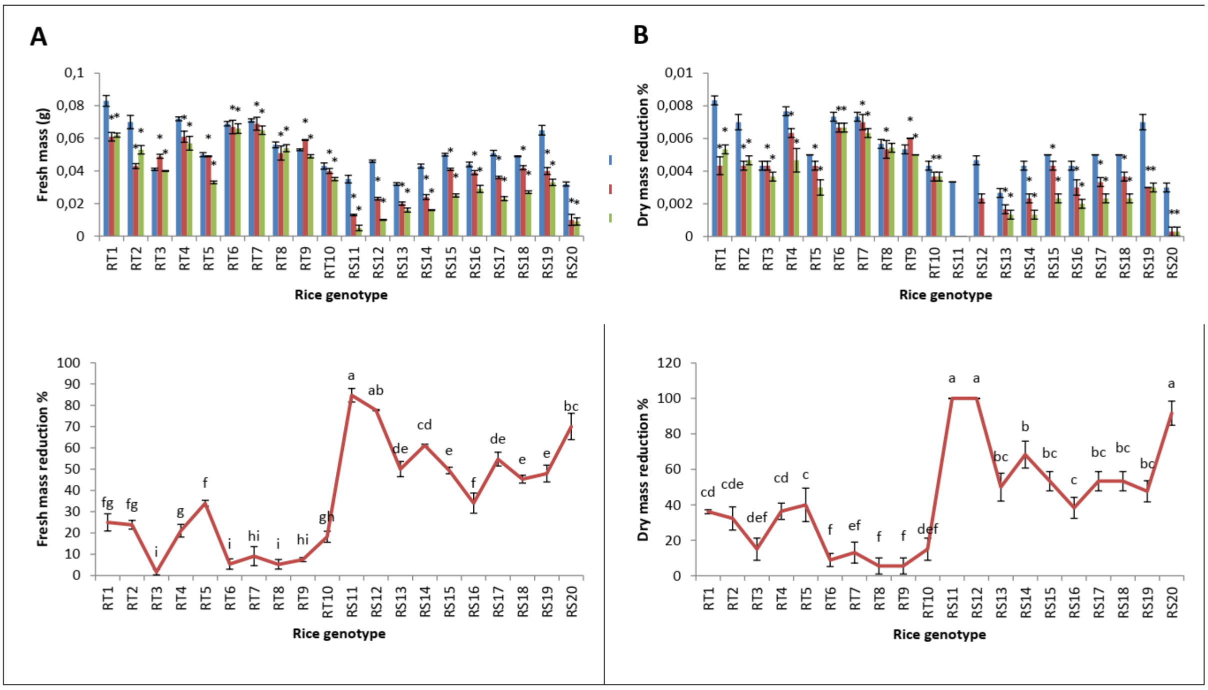

Figure 4. Changes in fresh and dry mass of rice seedlings under As treatments (0, 50 and 100 µM) and reduction % of fresh and dry mass at 100 µM As of rice genotypes. The data presented are mean ± SE (n = 3). * and letters superscripted indicate significant mean difference from control and between the genotypes respectively at 5% level of significance in multiple comparison by LSD test.

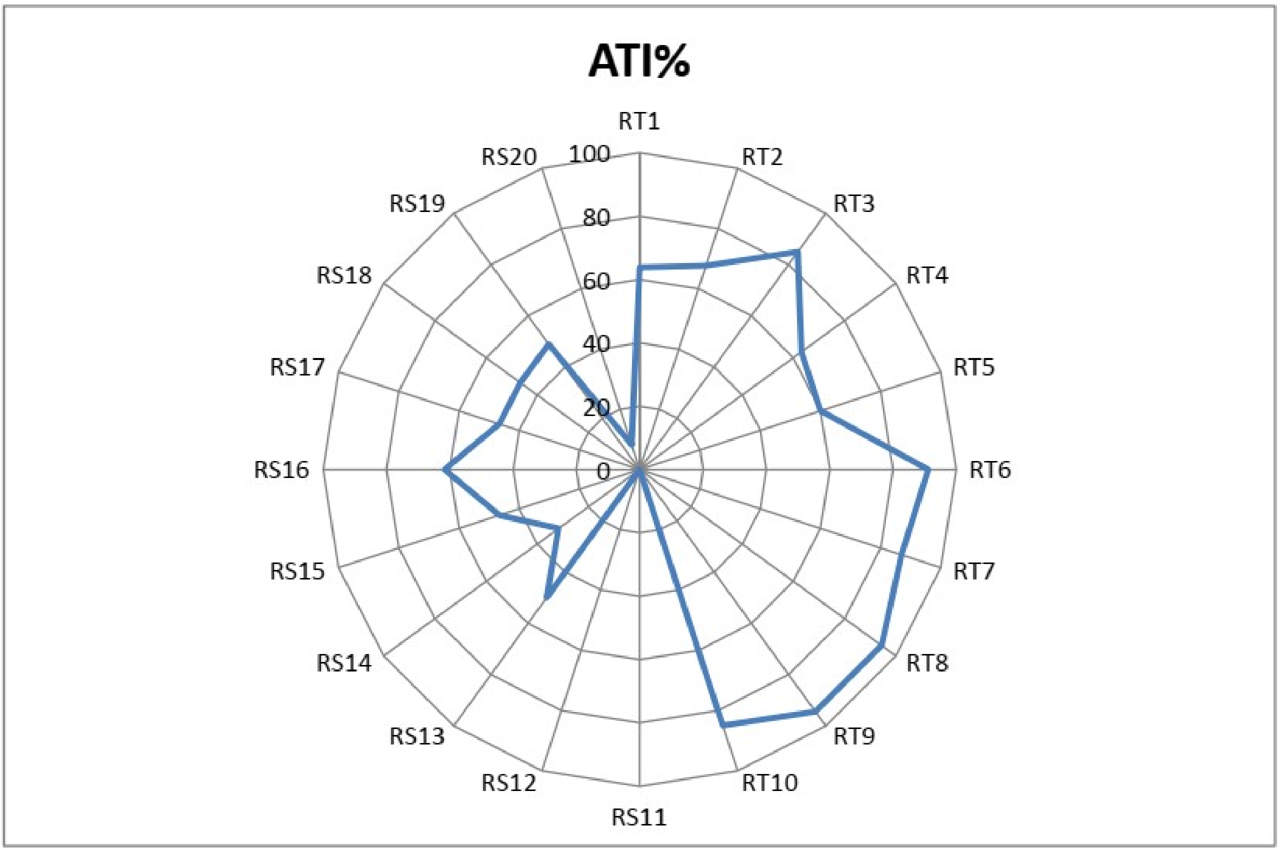

Figure 5. Arsenic tolerant index % (ATI%) of the 20 NE rice seedlings under 100 µM As. The data presented are mean ± SE (n = 3). Different letters superscripted indicates significant mean difference between the genotypes at 5% level of significance in multiple comparison by LSD test.

Fresh mass of the 20 rice genotypes is given in Fig. 4A, revealing differential response of the rice genotypes under As treatments. A gradual decrease in fresh mass is observed with the increased of As concentration where highest reduction is observed in the genotype RS11 with fresh mass reduction of 84.68% in comparison with control, which is followed by RS12 (77.70%), and RS20 (70.03%). Reduction may be due to the induction of oxidative stress to rice the seedlings by the As induced reactive oxygen species (ROS) production which lead to disturbances in various metabolisms (Liang 2018).

The rice genotype with lowest fresh mass reduction % with respect to control is RT3 (1.58%) followed by RT8 (5.26%), RT6 (5.39%), RT9 (7.47%) and RT7 (9.12%). The result of the dry mass analysis also revealed the alteration of rice plant growth under As treatment (Fig. 4B). Highest reduction of dry mass was observed in rice genotypes RS11 and RS12 at 100 µM As with respect to the control. However, it is seen that As showed less effect in the rice genotypes RT8 and RT9, with only 5.5 DWPR with respect to control. The result of the ATI% at 100 µM As also clearly revealed the variation in response to As stress in the NE rice genotypes. In the study, lowest ATI% is observed in RS11 and RS12 rice genotypes with 0 tolerant with As whereas, RT8 and RT9 showed highest tolerant index with 94.44 ATI% in each genotype, followed by RT6 (91.07%), RT7 (86.90%) and RT10 (85%) with respect to control. The variation in response to As of the rice genotypes may be due to genetic differences which resulted into difference protective mechanisms.

CONCLUSION

Germinating seedlings is the precise stage for screening against As stress, however the effect of As toxicity in germinating NE rice seedlings is not yet documented. This study provides a useful information of NE rice genotypes to As stress in germinating stage. It is found that NE rice genotypes response differently to As stress which can be categorized into tolerant, moderately tolerant/sensitive and sensitive rice. The genotypes RT8, RT9 and RT6 are found to be the highest tolerant which are lowland rice of Manipur (RT6 & RT8) and Assam (RT9) suggesting well developed defence mechanism against As is functioning inside the plants which might be due to prior experienced of As exposure. The upland Meghalaya rice genotypes RS11 and RS12 are found to be the highest sensitive in this study, reflecting no well developed protective mechanisms against As.

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