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Research Article

SCREENING & EVALUATION ON GRAIN ARSENIC ACCUMULATION OF RICE (*Oryza sativa* L.) GENOTYPES UNDER ALLUVIAL ZONE OF WEST BENGAL

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Abstract- The present investigation was conducted to evaluate 14 rice genotypes for grain arsenic concentration in two seasons (2015 boro and 2016 boro) over three different locations of Nadia District of West Bengal, India with differential arsenic concentration in soil. Among 14 rice genotypes two genotypes Puspa and Satabdi showed low grain arsenic concentration, linear response and least deviation from linear regression and the genotypes PNR 546, Khitish and Nayanmoni had highest grain arsenic concentration with b_i values significantly higher than 1.0. There was less variation in mean grain arsenic concentration of genotypes between two seasons and also between locations. The range of accumulation of arsenic in boro season 2016 was lower than 2015 boro season in every site. The mean grain arsenic concentration of Puspa and Satabdi was lowest irrespective of location in both the seasons. Hence, Puspaans Satabdi were identified as suitable for growing over environments with high arsenic concentration under study and can be used for further.

Keywords- Arsenic, concentration, grain arsenic, rice cultivars, tolerance

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Introduction

Arsenic (As) is a well-known human carcinogen [1] which has influenced human history more than any other toxic element or compound [2] causing serious health hazards, including cancers of the skin, lung, bladder, liver, and kidney as well as many other cardiovascular, neurological, hematological, renal, and respiratory diseases [3-6]. Arsenic (As) hazard has become a global concern due to its increasing contamination of groundwater in many regions in the world including Argentina, Bangladesh, Bolivia, Brazil, Chile, China, Ghana, Greece, India, Japan, Korea, Mexico, Mongolia, Nepal, New Zealand, Poland, Taiwan, Vietnam, and the USA [7-13]. However, the situation is at critical level in Asian region particularly the Gangetic Delta of Bangladesh and West Bengal in India [14,15]. Drinking As-contaminated groundwater is likely the major pathway of human exposure. However, recent studies have revealed that not only drinking water but also staple food crops, like rice, can accumulate arsenic well in excessive amount and can be a potential route of human exposure [16-20]. Rice, the principle food crop of more than 3 billion people of the world, especially in Southeast Asia accumulates much higher levels of As in the roots, shoots and grains [21] compared to other cereal crops. Rice has been reported to accumulate as upto 1.8 mg kg-1 in grains and upto 92 mg kg-1 in straw [22]. Arsenic contamination occurs in paddy fields through various sources, including metal mining [23-25], pesticides, fertilizer application [26,27], and irrigation with As-rich groundwater [28,29]. Irrigation with As-rich groundwater is the most common source, for increased As concentration in the soil [30-32] and uptake by rice [33,29,16,34]. High accumulation of as in grains of rice through As contaminated irrigation water is alarming. Different cultivars have Variation in Arsenic uptake and accumulation in rice grain from irrigation water may differ depending on cultivars [35]. Some of this variability has been explained by differences in groundwater irrigation levels of as and soil as concentrations. Decontamination of arsenic from soil is not a feasible approach. So to produce arsenic free rice grain without compromising demand of grain production, development of arsenic tolerant rice cultivars is the best way of solution. The objective of the present study was to reveal the extent of variation in total arsenic accumulation in grains of the experimental genotypes and to screen and identify genotypes with relatively low grain arsenic suitable for cultivation in boro and kharif season.

Materials & Methods

Field experiment was conducted with fourteen rice genotypes where IR 36 and IET 21845 were taken as high accumulating and low accumulating check respectively, at three locations all in farmers field Mitrapur, Dakshin Panchpota and Nonaghata villages of Nadia district in West Bengal in Boro season in 2015 and Boro season in 2016. The Mitrapur field site had an average soil as content of 28.18 mg Kg^{-1}in *boro* and 26.89 mg Kg^{-1} in kharif season respectively. The field site at Nonaghata had an average 21.90 mg Kg-1 and 20.84 mg Kg^{-1} of total soil arsenic in boro and kharif season respectively while the Dakshin Panchpota field site contained 16.12 mg Kg^{-1} soil arsenic in *boro* and 15.01 mg kg soil arsenic in boro and kharif seasons respectively. The fourteen rice germplasms were planted in randomized block design with three replications with spacing of 6m x 15cm

The grain samples were collected from 5 randomly selected plants from each plot excluding those at the border and appropriately and dried in an air-oven at 105°C for 24 hours. The dried grain samples were ground and made ready for digestion. Grain samples were digested with a mixture of acids e.g. $HN0_3$, $HClO_4$ and H_2SO_4 in a proportion of 10:4:1 (v/v) [36] and filtered by Whatman No. 42 filter

paper, the filtered solution was ready for analysis and estimations were carried out as follows: The total arsenic of grain samples was estimated from the above filtered solution. The filtrate was diluted to 50 ml. 5 ml of the aliquot was taken in 50 ml volumetric flask, 5 ml of concentrated HCl and 1 ml. of mixed reagent [5% KI (w/v) + 5% Ascorbic acid (w/v)] were added to it, kept for 45 minutes to ensure complete reaction and the volume was made up to 5O ml. The resultant solution was analyzed in a Perkin-Elmer Atomic Absorption Spectrophotometer with Flow Injection Analysis System (FIAS 400) @ Xmax=193.7 nm where the carrier solution was 10% v/v HCl, the reducing agent (to ensure all As species be reduced to AsH3 and to be measured against a calibration with standard As^{+3} solution) was 0.2% $NaBH_4$, in 0.05% NaOH. Blanks were included for quality control. The data were subjected to stability analysis of genotypes in total grain As concentration [37], mean performance of genotypes for grain As concentration in overall environment for three locations.

Results & Discussion

The analysis for variance for total grain arsenic yield revealed significant differences among the genotypes and environments [Table-1]. Partitioning of mean sum of squares in to that of genotypes, environments + (genotypes x environments) and pooled error revealed that the genotypes were highly significant for mean squares due to genotype x environment (GXE) interaction which revealed that the genotypes interacted considerably with environmental conditions. It indicated that it might be taken into account while breeding for low accumulating genotype. E (L), G x E (L) and PD also tested significant.

Table-1 *ANOVA (pooled) for stability of total grain arsenic concentration as per Eberhart and Russel (1996) model***.**

Source of variation	Degrees of freedom	Mean sum of square
G	13	4.16**
E	2	1.66
G x E	26	6.87**
E + G x E	28	7.57
E(L)	1	3.32**
G x E (L)	13	1.13**
P D	14	2.25**
P E	78	5.86

** significant at 0.01% level

G: Genotype, **E:** Environment, **G x E:** Genotype x Environment, **E (L):** Environment (Linear), **G x E (L):** Genotype x Environment (Linear), **PD:** Pooled Deviation, **PE:** Pooled Error

In the present study S^2di values for all genotypes were zero or did not significantly deviate from zero which indicates that there is genetic stability. The genotypes Khitish, CNRH 102 and CNRH 103 were found to be suitable for a general adaptation, i.e. suitable for all environmental conditions as their b_i (linear response) was around 1.0 with least deviation from linearity and above or around average mean [Table-2]. The genotypes PNR 546, KhitishIR-36 and Nayanmoni had high grain arsenic concentration compared to the high arsenic accumulating check variety IR-36, with b_i values significantly higher than 1.0 were found to be suitable for environments with low As levels. The mean of genotypes with total grain arsenic content ranged from 0.37 mg/kg to 1.87 mg/kg with a population mean of 0.72 mg/kg. The mean grain arsenic content of genotypes CNRH 103, IR 64 ,IET 21845, Satabdi, Ajit, GS 3, IET 21261 and Puspa was lower than that of population mean which is desirable but only Ajit, Satabdi and Puspa had grain arsenic concentration lower than that of the low accumulating check IET 21845. Ajit contained 0.52 mg/kg of grain As while Satabdi and Puspa both had 0.37 mg/kg of As in their grains which is much lower than that of the low accumulating check IET 21845. Thus Satabdi and Puspa were the best performing varieties in this study. These two varieties had bi value less than 1 and s^2di value zero or not significantly deviating from zero. Thus they are stable varieties adapted to soils with high As levels. Therefore, these genotypes reflect negligible response to the environmental changes i.e. remain steady under poor conditions but cannot exploit the positive improvement in the environment. In the present study only two genotypes Satabdi and Puspa fulfilled the conditions for an ideal variety with low grain arsenic concentration, linear response and least deviation from linear regression. Hence, these genotypes were identified as suitable for general adaptation i.e. suitable for growing over environments with high arsenic concentration under study. The mean of total grain arsenic concentration in all the rice genotypes per location for *boro* season in 2015 and 2016 are presented in [Table-3]. There was less variation in mean grain arsenic concentration of genotypes between two seasons and also between locations. In the Mitrapur site mean grain arsenic concentration ranged from 0.35-2.77 mg/kg in 2015 while in 2016 it ranged from 0.34-1.46 mg/kg. In Dakshin Panchpota site it ranged from 0.33-1.09 mg/kg in 2015 and from 0.32-1.50 mg/kg in 2016 and in the Nonaghata site the range was from 0.14-1.75 mg/kg in 2015 and from 0.32-1.50 mg/kg in 2016. As concentration in rice collected from the Murshidabad District of West Bengal was reported to be varied from 0.09–0.66 mg/kg in 2002 and 0.08–0.55 mg/kg in 2003 [38,39]. As concentrations in rice from Kolkata, West Bengal, was noted to be ranged between 0.02 and 0.40 mg/kg [40]. Though in this study the minimum and the maximum range of grain arsenic content is much more higher than the studies which have been done earlier in West Bengal. In 2015 highest accumulation of grain arsenic was observed in Mitrapur (0.85 mg/kg) and between sites there was large variation. The results of this study were much more similar to that of the studies in Bangladesh for grain arsenic concentration in rice genotypes. The highest level of As, up to 2.05 mg/kg (range, 0.05–2.05 mg/kg), was reported in the southern part (Gopalganj, Rajbari and Faridpur) of Bangladesh [41], whereas it was up to 1.84 mg/kg (range, 0.03–1.84 mg/kg) in western Bangladesh (Nawabgong and Naogoan) [28]. In boro season 2016 very little variation in mean grain arsenic accumulation of genotypes between locations are observed. Accumulation of arsenic in boro season 2016 was lower than 2015 boro season in every site. The range of mean grain arsenic concentration of different genotypes in boro 2016 was 0.36-1.79 mg/kg. In boro 2016 the highest grain arsenic concentration of different genotypes was observed in Mitrapur site followed by Dakshin Panchpota and Nonaghata. The genotypes IR-36, PNR 546 and Khitish were observed to have the highest grain arsenic content in three different trial locations in both the seasons. The genotypes Satabdi and Puspa were observed to be the least arsenic accumulators as their mean grain arsenic concentration was lowest irrespective of location in both the seasons. The results of the arsenic analysis of rice grains of different genotypes grown in different arsenic contaminated sites suggest that different genotypes have differential reactions towards uptake and translocation of arsenic into the grains and this differential reaction is mainly due to the genotypic differences of genotypes under trial. Out of the fourteen genotypes only two are found to have low level of grain arsenic in all of the three locations in both the seasons. In future, these low-grain arsenic varieties (arsenic tolerant varieties) may be utilized as parents in breeding program for the development of arsenic tolerant high yielding popular rice varieties, The ranking of genotypes for low grain As (mg/kg) has been presented in [Table-4]. Among the fourteen rice genotypes two genotypes Puspa and Satabdi showed promising result with respect to grain arsenic content.

Table-2 *Stability parameters of total grain arsenic concentration*

Sl. No	Variety	$\bar{X}$	bi	s^2di
1	Khitish	0.80	1.39	0.01
2	CNRH 103	0.69	1.39	0
3	IR 36	1.87	7.33	0.16
4	IR 64	0.56	0.27	0
5	IET 21845	0.56	0.13	0
6	Satabdi	0.37	0.52	0
7	Ajit	0.52	-0.92	0
8	CNRH 102	0.80	1.94	0
9	PNR 546	1.03	2.33	0
10	GS 3	0.65	-0.18	0
11	IET 21261	0.62	-1.12	0.02
12	Puspa	0.37	-0.13	0.01
13	GS1	0.63	-1.25	0.11
14	Nayanmoni	0.74	2.29	0
	Mean	0.72		

b_i: Regression Coefficient **S^2di:** Deviation from Regression
SE (m): Standard Error of Mean **SE (b):** Standard Error of b_i

Table-3 *Mean performance of genotypes for total grain arsenic concentration (mg/kg) over three locations in two season*

Genotypes	*Boro* 2015				*Boro* 2016			
	MP	DP	NG	Mean	MP	DP	NG	Mean
Khitish	0.97	0.63	0.81	**0.80**	0.85	0.61	0.74	**0.74**
CNRH 103	0.86	0.60	0.59	0.69	0.77	0.61	0.64	0.67
IR 36	2.78	1.09	1.75	**1.87**	2.41	1.46	1.50	**1.79**
IR 64	0.59	0.54	0.54	0.56	0.58	0.57	0.81	0.65
IET 21845	0.57	0.53	0.56	0.56	0.50	0.48	0.49	0.49
Satabdi	0.43	0.33	0.34	0.37	0.41	0.34	0.38	0.38
Ajit	0.41	0.59	0.57	0.53	0.47	0.51	0.54	0.51
CNRH 102	1.04	0.67	0.68	0.80	0.90	0.76	0.63	0.77
PNR 546	1.32	0.82	0.94	**1.03**	1.12	0.97	0.84	**0.98**
GS 3	0.62	0.67	0.64	0.65	0.73	0.71	0.62	0.69
IET 21261	0.49	0.79	0.59	0.62	0.55	0.57	0.61	0.58
Puspa	0.35	0.43	0.32	0.37	0.34	0.42	0.32	0.36
GS1	0.49	0.94	0.46	0.63	0.53	0.87	0.79	0.73
Nayanmoni	1.03	0.59	0.60	0.74	0.57	0.62	0.53	0.58
Range	0.35-2.77	0.33-1.09	0.32-1.75	0.36-1.87	0.34-2.41	0.34-1.46	0.32-1.50	0.36-1.79
Mean	0.85	0.66	0.67	-	0.77	0.68	0.68	-
CD (5%)	0.01	0.07	0.00	-	0.00	0.01	0.03	0.01

MP: Mitrapur, **DP:** Dakshin Panchpota, **NG:** Nonaghata

Table-4 *Ranking of genotypes for low grain arsenic concentration (mg/kg)*

Boro (2015)			*Boro* (2016)		
Genotype	As Conc. (mg/kg)	Rank	Genotype	As Conc. (mg/kg)	Rank
Puspa	0.36	1	Puspa	0.36	1
Satabdi	0.37	2	Satabdi	0.38	2
Ajit	0.53	3	IET 21845	0.49	3

Conclusion

The present study provided an evaluation of grain arsenic concentration of 14 rice genotypes over three locations with different range of soil arsenic concentration for two seasons. Significant differences among the genotypes and environment for grain arsenic content suggested the presence of wide variability. Both, components of genotype x environment interaction were significant, indicating considerable interaction of genotypes with the environment. From the present investigation it is concluded that among the 14 genotypes Puspa and Satabdi fulfilled the condition of a stable variety and can be suitable for adaptation over environments with high arsenic concentration with significantly low level grain arsenic content irrespective of locations and seasons.

Application of research: These genotypes can be used as donors in future breeding program for development of arsenic tolerant rice genotypes.

Research Category: Genetics & Plant Breeding

Abbreviations:
GxE: Genotype x Environment

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