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Screening and selection of low grain arsenic (As) accumulating rice germplasms under West Bengal condition

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ABSTRACT

For developing (As) tolerant rice variety, screening and selection of As tolerant rice germplasms (low As containing grain) is an essential pre-requisite. Keeping this objective in mind, a collaborative research programme has been undertaken and three different As contaminated locations viz, Purbasthali-1, Birnagar and Beldanga-1(As content ranges from 10 µg /L to >50 µg /L) were chosen for field trials along with a control set at RRS, Chinsurah. During *Boro* 2007-2008, hundred (100) rice germplasms were grown at the said locations and all of the entries were evaluated. The result of the experimental trial revealed that out of the hundred entries, only seven entries are promising with respect to grain arsenic content. Arsenic content in grain was very high in three entries and low in four entries irrespective of locations. The result of the arsenic analysis of rice grains suggests that diversified rice germplasms have differential reactions towards uptake and translocation of arsenic into the grains and this differential reaction is mainly due to genotypic differences of the rice varieties.

Key words: Aresenic, germplasm and tolerance

Arsenic (As) hazard has become a global concern due to its ever increasing contamination in many regions in the world particularly in South-East Asia. It is potentially toxic to human beings causing severe diseases including cancer. Groundwater As contamination in the Bengal delta is known as the largest chemical disaster in history (Smith *et.al*., 2000). A large part of Ganga-Meghna-Brahmaputra plain with an area of 569,749 sq.km and population over 500 million is at risk (Chakraborty *et.al*., 2004). In West Bengal, 111 blocks of 12 districts are arsenic affected. Not only drinking water but also staple food grains like rice contain arsenic well in excess of permissible limit. Rice has been reported to accumulate As upto 1.8 mg kg^{-1} in grains and upto 92 mg kg^{-1} in straw (Abedin *et al.*, 2002). Compared to other cereals (wheat, barley, maize etc.) rice accumulates much higher levels of As in the roots, shoots and grains (Duxbury and Panaullah., 2007). The study further revealed that the roots of rice and wheat grown in contaminated fields had 169-178 mg kg^{-1} of arsenic, nearly 20 times higher than the 7.7 mg kg^{-1} in an uncontaminated location. Concentrations of arsenic in rice grain did not exceed the food hygiene concentration limit (1.0 mg of As kg^{-1} dry weight). The concentrations of arsenic in rice straw (up to 91.8 mg kg^{-1} for the highest As treatment) were of the same order of magnitude as root arsenic concentrations (up to 107.5 mg kg^{-1}), suggesting that arsenic can be readily translocated to the shoot (Abedin *et. al.*, 2001) Rice being the major food crop of As contaminated areas also, notable accumulation of As in grains of rice through As contaminated irrigation water is alarming. Arsenic uptake and accumulation in rice plant from irrigation water may differ depending on cultivars used (Xie and Huang, 1998). The high arsenic concentrations may have the potential for adverse health effects on the cattle and an increase of arsenic exposure in humans via the plant-animal-human pathway. Arsenic concentrations in rice plant parts except husk were not affected by application of phosphate (Abedin *et. al.*, 2001). Decontamination of arsenic from soil is not being a feasible approach. So to produce arsenic free grain without compromising projected demand, development of arsenic tolerant rice cultivars which should be genotypic in nature is the best way of solution. For developing As tolerant rice variety, screening and selection of As tolerant rice germplasms (low As containing grain) is an essential pre-requisite. Keeping this objective in mind, a collaborative research programme with NBRI, Lucknow, U.P. has been undertaken at Rice Research Station, Chinsurah, West Bengal.

MATERIALS AND METHODS

Field experiment was conducted during Boro season 2007 – 08 at three different arsenic contaminated locations at different Agro-climatic Zones viz, Purbasthali-I (Burdwan), Birnagar (Nadia) and RRS, Chinsurah (Hooghly) (As content ranges from 10 µg l^{-1} to >50µg l^{-1} in ground water). Birnagar is situated at district Nadia ($22^0 41^{/}\ 23^{//}$ N and $72^0 51^{/}\ 24^{//}$E) and As level in ground water is >10µg l^{-1}. Purbasthali-I is situated at district Burdwan ($23^o 53^{/}$N -$22^o 56^{/}$N and 83°25′E - 86°48′E) and As level in groundwater ranges from 10 µgl^{-1} to >50 µg l^{-1}. RRS, Chinsurah is situated at district Hooghly (22°52′N and 88°24′ E) and As level in ground water is >10µg l^{-1} in boro rice growing areas only. During *Boro* 2007-2008, one hundred (100) rice entries (56 Boro Rice Germplasms (BRG) and 44 rice varieties) were grown at the said locations for screening and evaluation. All of the hundred entries cultivated at RRS, Chinsurah, Purbosthali-I and Birnagar were evaluated for their grain arsenic content. 30-35 days old seedlings of each entry were planted at 20 cm x 20 cm spacing following other standard agronomic practices. Samples were collected from all locations separately for chemical analysis. Speciation of grain arsenic content was done in the laboratory of NBRI, Lucknow.

The nature of As speciation in rice grains was determined using the trifluoroacetic acid (TFA) extraction technique of Abedin et al. (2002).After grinding of rice in a stainless steel grinder (Breville, Sydney, New South Wales, Australia), a portion (0.25 g) of rice material was weighed into 100-mL glass digestion tubes to which 2 mL 2 M TFA was added. The digestion tubes were placed on a heating block, and the temperature was increased to 100°C for 6 hr. The digest was evaporated to dryness and the residue dissolved in deionized water, filtered (0.22-µm filters), and made up to 20 mL with deionized water. The extracts were stored at –20°C before analysis by high performance liquid chromatography inductively coupled plasma mass spectrometer (HPLC)-ICP-MS (Agilent Technologies, Tokyo, Japan, Model No. Agilent 7500ce).The nature and concentration of As species in extract solutions was determined by HPLC-ICP-MS (Agilent Technologies).Samples were injected onto a PRP-X100 anion-exchange column (25.041 mm internal diameter, 10 µm; Hamilton, Reno, NV, USA) using a fixed 50-µL sample loop. The column temperature was maintained at 40°C and the mobile phase (20 mM NH_4H_2PO4 adjusted to pH 5.6 with aqueous NH_3) flow rate was 1.5 mL/min. Arsenic compounds were quantified by external calibration with standard solutions of arsenite (AsIII), AsV, dimethylarsinic acid (DMA), and monomethylarsonic acid (MMA).

RESULTS AND DISCUSSION

Out of hundred entries, seven (7) entries viz., BRG-12, BRG-15, BRG-20, CN-1646-2, Nayanmoni, Gotrabhog and CN-1646-5 have shown promising result with respect to grain arsenic content. On the basis of arsenic accumulation in the grain two distinct categories are developed viz., Category-I and Category-II. Varieties which are **unsafe** for subsistence diet fall under Category-I and varieties which are **safe** for subsistence diet fall under Category-II (WHO, 1993). From the result it is clearly evident that As content in grain was very high and it ranges from 1034±182.01 µg/kg to 2650±590.99 µg/kg in all the three entries viz., BRG-12, BRG-15 and BRG-20 irrespective of locations (Table-1). So these three entries are placed under category-I. Whereas As content in grain was found low and that ranges from 78.65±1.202 µg/kg to 256.05±77.56 µg/kg in another four entries viz., CN-1646-2, Nayanmoni, Gotrabhog and CN-1646-5 irrespective of locations. So these four entries are placed under category – II. Remaining 93 entries have As content in between 256.05±77.56 µg/kg to 1034±182.01 µg/kg. The three entries containing high As in grain under category-I have been shown to have different level of As in grain at three different trial locations. In all of the three test locations, As content in soil ranges from 10.59±1.82 mg kg^{-1} to 15.54±1.32 mg kg^{-1} soil. Data shown in Table-1 revealed that BRG-12 has very high As content in two test locations namely Purbosthali-I and Birnagar and comparatively low As content in grain at Chinsurah location. But contrasting result was found in BRG-15, it has low As content in grain at Purbosthali-I and Birnagar whereas very high As content at Chinsurah. Similarly, BRG-20 has low As content in Purbosthali-I and Chinsurah locations whereas very high As content at Birnagar location. Fig. 1 shows the grain arsenic content of different rice germplasms grown at Purbasthali – I. The result of the arsenic analysis of rice grains of different test entries grown in different arsenic contaminated locations suggest that diversified rice germplasms have differential reactions towards uptake and translocation of arsenic into the grains and this differential reaction is mainly due to the genotypic differences of the rice germplasms under trial. Out of the hundred test entries, four entries are found to have low level of grain-arsenic (safe varieties for subsistence diet) in all of the three locations. In future, these low-grain arsenic varieties (arsenic tolerant varieties) may be utilized as donors in breeding programme for the development of arsenic tolerant high yielding popular rice varieties, either in conventional ways or by transgenic methods.

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Table 1: Grain Arsenic level (μg kg^{-1}) in some contrasting rice germplasms showing common performance at three sites in West Bengal during boro season.

Category	Germplasm No. /Name	Purbosthali – 1, Burdwan	Chinsurah, Hooghly	Birnagar, Nadia
Category – I (High As)	BRG-12	2196.72±106	1471.33±271	2309±539.32
	BRG-15	1050.65±197.07	2094±89.10	1034±182.01
	BRG-20	1177.66±92.63	1221.36±217.73	2650±590.99
Category – II (Low As)	CN-1646-2	78.65±1.202	118.38±21.62	Work in progress
	Nayanmoni	108.05±7.707	155.76±18.26	-do-
	Gotrabhog	112.26±22.721	256.05±77.56	-do-
	CN-1646-5	113.2±28.659	-	-do-
	Soil As level (mg/Kg)	10.59±1.82	12.43±1.53	15.54±1.32

N.B.: Category of germplasm vis-à-vis grain arsenic levels

Category - I	**:**	UNSAFE VARIETIES for subsistence diet [according to MTDI for 420 g of rice consumed by an average person of 60 Kg body weight
Category - II	**:**	SAFE VARIETIES for subsistence diet [according to MTDI for 420 g of rice consumed by an average person of 60 Kg body weight
MTDI	**:**	Provisional maximum tolerable daily intake of arsenic of 2 μg / Kg body weight
Ref	**:**	WHO, 1993

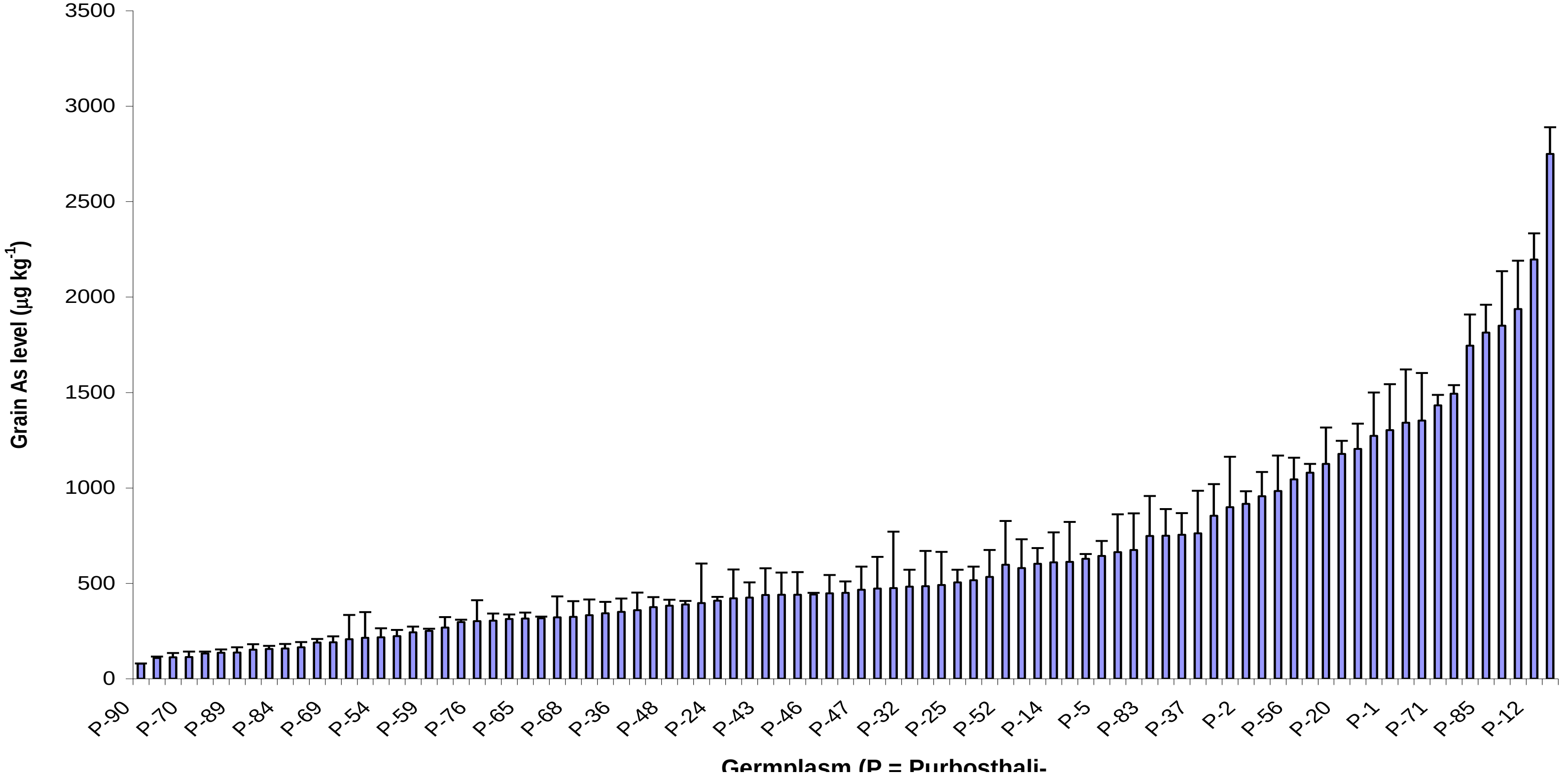

Fig. 1 : Grain arsenic level in different rice germplasms cultivated at Purbosthali – I.