SHORT COMMUNICATION Oryza Vol. 48 No. 3, 2011 (270-273)

Genetic analysis of arsenic accumulation in grain and straw of rice using recombinant inbred lines

M Chatterjee, S Sarkar, S Debnath, S Ghosh, S Bhattacharyya*, Sanyal S K

Crop Research Unit, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal - 741252

ABSTRACT

Recombinant Inbred Lines (RILs), developed from a cross between a low arsenic accumulating rice genotype (field-mutant of Swarna) and a high As accumulating rice genotype, IR 36, were grown in farmer's field during wet season 2008 and 2009 to understand the inheritance of As accumulation in rice. As accumulation in both grain and straw was controlled by number of genes as concluded from continuous frequency distribution pattern of As accumulation data in RILs. A separate set of genes was responsible for arsenic accumulation in straw and grain of rice as no correlation was observed between arsenic accumulation in grain and that of straw. It was concluded that genes from low accumulating parent were playing a major role in grain As accumulation but genes from both parents are contributing equally for straw As accumulation. A significant amount of transgressive segregation towards high accumulating parent, had also been observed in straw As accumulation data of RILs.

Key words: *rice, arsenic, RILs, grain, straw*

Arsenic (As), a metalloid released from natural, geogenic and anthropogenic sources, ranks as the number one toxic environmental contaminant, as there is no threshold value below which it does not causes cancer (Smith *et al.,* 2008). Arsenic contamination in groundwater is a problem especially along the coastal belt of Bangladesh (Dhar *et al.,* 1997), Indo-Gangetic region of India (Mandal *et al.,* 1996) and part of China (Liangfang and Jianghong, 1994). In these regions, level of arsenic content in drinking water exceeds the safe limit of 10µg/l set by WHO (WHO 2001) and even 50µg/l specified in India and Bangladesh. Other than drinking water it is also became a threat by entering in the food chain through As contaminated groundwater irrigation (Sanyal *et al.,* 2002). Rice which is cultivated in flooded condition accumulates higher amount of As in grain and straw than other cereals, principally in inorganic form (Williams *et al.,* 2005). This poses a potential health risk to high rice consuming areas (Zhao *et al.,* 2010). Rice generally exceeds 50 µg kg^{-1} inorganic form of arsenic and reaches levels as high as 900µg kg^{-1}. Inorganic forms, either arsenate or arsenite are the predominant species of As in rice grain (Torres-Escribano *et al.,* 2008). Rice is efficient in arsenic accumulation as arsenite transport in rice roots shares the same pathways of silicon transport (Ma *et al.,* 2008). Transporters belong to the Nod 26 like Intrinsic Protein subfamily of aquaporins in rice are permeable to arsenite but not to arsenate. Although mechanism of As uptake by different plant species (Meharg and Macnair, 1992; Meharg, 1994) is known, but little work (Zhang *et al.,* 2007) has been done on inheritance of As accumulation in rice, particularly in field condition. In this study, 101 Recombinant Inbred Population (RILs), developed from a cross between a low arsenic accumulating rice genotype, (natural mutant of Swarna) and a high arsenic accumulating rice genotype, IR 36 were grown in two consecutive wet season (2008 and 2009) and few photoinsensitive lines were selected. Purpose of the study is to unveil the inheritance of As accumulation phenomenon to aid breeding programme in low arsenic accumulating rice suitable for human and ruminant consumption.

The experiment was laid out in a randomized complete block design with three replications and 3x3 sq.m plots. Fertilizers were applied at elemental rates of 70-30-30 kg ha^{-1} of N-P-K using urea, triple superphosphate and muriate of potash. Nitrogen was

PDF created with pdfFactory Pro trial version www.pdffactory.com

applied in three equal splits, a basal dose before transplanting, at active tillering and at panicle initiation stage. Rice seedlings of 101 Recombinant inbred lines (RILs) along with their two parents, 30-40 days old, were transplanted into puddle soil of farmer's field in Nonaghata mouza of the Haringhata block of Nadia district in West Bengal at the rate of one seedling per hill with a hill to hill spacing of 20 x 20 cm. The arsenic loading of the groundwater which is used as irrigation source in farmer's field varied from 0.53 to 0.66 mg L^{-1}. and Olsen extractable arsenic in soil is 3.2 to 4.2 mg kg^{-1}. Control site was selected at regional research substation of Bidhan Chandra Krishi Viswavidyalaya situated at Sekhampur, Birbhum, West Bengal. The samples were appropriately labeled, dried in an air-oven at 105^0C for 24 hours. Then a portion (1 gm) of the ground samples were digested on a sand bath with tri-acid mixture (HNO_3 : H_2SO_4 : $HClO_4$: : 10 : 1 : 4 , by volume) to obtain a clear digest. The arsenic content of the digested sample was measured using AAS (Analyst 200) coupled with FIAS 400, a hydride generator (Perkin Elmer) as described by Mukhopadhyay *et al.,* 2004. The frequency distribution pattern of all the lines was graphically plotted against their As content in different plant parts. Correlations between grain, straw and root arsenic were calculated following Microsoft Excel. Broad sense heritability was calculated following ANOVA table for As accumulation data of RILs.

Arsenic content in root, straw and grain was measured in µg kg^{-1} of dry weight for each year at the mature stage. Estimation was made from the field grown plants in wet season as one of the parents used for developing RILs was strictly photosensitive. There was a significant ($P < 0.01$) difference in arsenic accumulation of grain and straw within 101 RILs and also among the two parents (Table 1). Arsenic content in grain of RILs showed a range of 0.028 mg kg^{-1} to 0.170 mg kg^{-1}, where as in straw in between 0.392 mg kg^{-1} and 2.602 mg kg^{-1} (Table 2). As accumulation in grain and straw of IR36 was 0.181mg kg^{-1} and 0.883mg kg^{-1}, respectively and in other parent it was 0.058mg kg^{-1} and 0.392mg kg^{-1} respectively. The frequency distribution of As accumulation both in grain and straw showed a continuous variation. So, arsenic accumulation in grain and straw were supposed to control by a number of genes rather by a single gene. A small transgressive segregation was evident only towards the direction of low accumulating parent for grain- As (only four RILs accumulated As at the range of 0.025 to 0.045 mg kg^{-1}). On the other hand, twenty six RILs accumulated As in straw above the mean value of IR36, 0.883mg kg^{-1}. Arsenic concentrations in soil were reported often high enough to cause of concern even in wet season rice, because rice appears to be particularly efficient in As assimilation compared with other cereal crops (Williams *et al.,* 2007). Therefore, arsenic already deposited in the study site (4.2mg kg^{-1}) could be sufficient for accumulation of significant amount of As in grain and straw that may cause risks for human and ruminant. So, suitable genotypes and management are required not only for *boro* rice but also for wet season rice. Abedin *et al.,* (2002) reported that accumulation of As decreased gradually from root to straw and then to grain. Pot experimentation using DH population derived from Japonica and Indica cross also showed continuous distribution pattern (Zhang *et*

Table 1. ANOVA for grain and straw arsenic accumulation of 101 RILs grown on arsenic contaminated field

Sources of variation	df	Grain As accumulation		Straw As accumulation	
		F-value	Probability	F -value	Probability
RIL	100	233.5601	0.01 **	342.34	0.01**
Replication	2	0.1658		1.0970	
Year	1	0.1992	NS	0.0014	NS
RIL X Year	100	0.0236	NS	0.0005	NS

Table 2. Mean, SD and range of As accumulation data for 101 RILs and their parents

Year	Tissue-sample		Mean	(±)SD	Range
2007	Grain	RILs	0.112	0.035	0.028-0.170
		Parent	0.054	0.009	0.048-0.061
		IR36	0.179	0.014	0.147-0.182
	Straw	RILs	0.957	0.314	0.398-1.988
		Parent	0.392	0.042	0.368-0.402
		IR36	0.889	0.062	0.880-0.932
2008	Grain	RILs	0.099	0.037	0.031-0.168
		Parent	0.58	0.012	0.048-0.063
		IR36	0.167	0.014	0.141-0.176
	Straw	RILs	0.877	0.328	0.392-2.062
		Parent	0.388	0.035	0.359-0.397
		IR36	0.912	0.051	0.883-1.07

PDF created with pdfFactory Pro trial version www.pdffactory.com

al., 2007). In our present study continuous frequency distribution of arsenic accumulation in straw and grain implied involvement of numbers of genes. It is the first report of inheritance of As accumulation using RILs developed from Indica x Indica cross grown in the contaminated field. The frequency distribution of As accumulation in grain skewed towards low accumulating parent suggest that genes responsible for low accumulation in grain might have dominant role over genes responsible for higher accumulation. Transgressive segregation for both grain and straw arsenic accumulation in uni-parental direction in present study instead of bi-parental direction in earlier studies (Zhang *et al.,* 2007) might be due to different parental combinations used for developing RILs. There was no correlation between arsenic content in root with straw (r = 0.29) or with grain (r = 0.03). Insignificant correlation was also observed between straw arsenic with grain (r = 0.34). High broad sense heritability (h^2 >0.9) and also good correlations (r > 0.80) among two years for straw and grain As accumulation suggested that arsenic accumulation in grain and straw is stably heritable trait (Table 3). No correlation was observed among root arsenic accumulation across the years so it is difficult to assess the inheritance of root arsenic accumulation in rice. When a few photoinsensitive RILs were grown both in *boro* and wet season, it was observed that lines accumulating high As at wet season were also maintained the same trend at *boro* season. Root arsenic accumulation was not consistent across the year as iron plaque on root surface had a strong effect on arsenic uptake in rice (Chen *et al.,* 2005). The high correlations in straw and grain arsenic in successive two years in 2008 and 2009 (grain=0.83, straw=0.92), showed the robustness of sampling and arsenic estimation method. An interesting result of this study was insignificant correlation of straw arsenic with that of grain. So, different sets of transporter genes were responsible for accumulation of arsenic in straw and grain. It had also been corroborated by identification of QTLs on chromosome 2, responsible for straw arsenic at the seedling stage but QTLs on chromosome 6 and 8 are controlling grain arsenic at the maturity (Zhang *et al.,* 2007). It was difficult to get an idea from wet season data that how much quantity of arsenic will be accumulated in grain or straw if same line was grown in *boro* season as there was no linear correlation exists. But As accumulation was higher in *boro* rice, as expected, where groundwater was used as a main source of irrigation.

Table 3. Estimation of GCV, PCV, and heritability of the grain and straw arsenic accumulation for RILs (wet season)

Arsenic accumulation in different parts of rice	GCV	PCV	ECV	Heritability
Grain	73.23	74.07	11.14	0.98
Stem	48.95	50.40	11.98	0.94

These RILs were currently in use for identification of QTLs responsible for straw and grain arsenic accumulation at maturity in field condition. So for developing low As accumulating rice genotypes, arsenic accumulation in grain and straw of the parents should be considered separately and evaluation should be made both at wet and *boro* seasons.

ACKNOWLEDGEMENT

This study is supported by NAIP comp-4 project entitled "Arsenic in food-chain: cause, effect and mitigation" and ICAR-Niche area of Excellence programme entitled 'Arsenic management options including organic agricultural system in West Bengal' and one of the author Sandip Debnath acknowledges the INSPIRE fellowship programme of DST, GOI for providing financial assistance.

REFERENCES

Abedin JM, Feldmann J and Meharg AA 2002. Uptake kinetics of arsenic species in Rice plants. Plant Physiol. (128):1120-1128.

Chen Z, Zhu YG, Liu WJ and Meharg AA 2005. Direct evidence showing the effect of root surface iron plaque on arsenite and arsenate uptake into rice (*Oryza sativa*) roots. New Phytol. (165):91–97.

Dhar RK, Biswas BK, Samanta G, Mandal BK, Chakraborti D, Roy S, Jafar A, Islam A, Ara G, Kabir S, Khan AW, Ahamed SA and Hadi SA 1997. Groundwater arsenic calamity in Bangladesh. Curr. Sci. (73):48-59.

Liangfang W and Jianghong H 1994. Chronic arsenism from drinking water in some areas of Xinijang, China. In Jo Nriagu, ed, Arsenic in the environment, part II: Human Health & ecosystem effects. John Wiley & sons Inc, New York NY, pp 159-172.

PDF created with pdfFactory Pro trial version www.pdffactory.com

Ma JF, Yamaji N, Mitani N, Xu XY, Su YH, McGrath SP and Zhao FJ 2008. Transporters of arsenite in rice and their role in arsenic accumulation in rice grain. Proc Natl Acad Sci. USA. (105):9931–9935.

Meharg AA 1994. Integrated tolerance mechanisms – constitutive and adaptive plant – responses to elevated metal concentrations in the environment. Plant Cell Environ. (17):989-993.

Meharg AA and Macnair MR 1992. Suppression of the high affinity phosphate uptake system: a mechanism of arsenate tolerance in Holcus lanatus L. J Exp Bot. (43):519–524.

Mondal BK, Roy Chowdhury T, Samanta G, Basu GK, Chowdhury PP, Chanda CR, Lodh D, Karan NK, Dhar RK, Tamili DK, Das D, Saha KC and Chakraborti D 1996. Arsenic in groundwater in seven districts of West Bengal, India: the biggest arsenic calamity in the world. Curr sci. (70):976-986.

Mukhopadhyay D and Sanyal SK 2004. Complexation and release isotherm of arsenic in arsenichumic/fulvic equilibrium study. Australian Journal of Soils Research (42):815–824.

Sanyal SK and Nasar SKT 2002. Arsenic contamination of groundwater in West Bengal (India):Build-up in soil-crop systems. In: Analysis and Practice in Water Resources Engineering for Disaster Mitigation. New Age Internl (P) Ltd, Pub, New Delhi, pp 216-222.

Smith E, Juhasz AL, Weber J, Naidu R (2008) Arsenic uptake and speciation in rice plants grow under green house conditions with arsenic contaminated irrigation water. Sci Total Environ. (392):277–283.

Torres-Escribano S, Leal M, Vélez D and Montoro R 2008. Total and inorganic arsenic concentrations in rice sold in Spain, effect of cooking, and risk assessments. Environ Sci Technol. (42):3867–3872.

Tuli R, Chakrabarty D, Trivedi KP, Tripathi DR 2010. Recent advances in arsenic accumulation and metabolism in rice. Mol Breeding (26):307-323.

Williams PN, Price AH, Raab A, Hossain SA, Feldmann J and Meharg AA 2005. Variation in arsenic speciation and concentration in paddy rice related to dietary exposure. Environ. Sci. Technol. (39):5531–40.

Williams PN, Raab A, Feldmann J and Meharg AA 2007. Market basket survey shows elevated levels of as in South Central U.S. processed rice compared to California: consequences for human dietary exposure. Environ Sci Technol. (41):2178–2183.

WHO (2001) Arsenic and arsenic compounds. Environmental health criteria, pp 224.

Zhang J, Zhu YG, Zeng DL, Cheng WD, Qian Q, Duan GL 2007. Mapping quantitative trait loci associated with arsenic accumulation in rice (Oryza sativa). New Phytol. (177):350–356.

Zhao JF, McGrath PS and Meharg AA 2010. Arsenic as a food chain contaminant: Mechanisms of plant uptake and metabolism and mitigation strategies. The Annl. Rev. of Plant Bio. (61):535-559.

PDF created with pdfFactory Pro trial version www.pdffactory.com