Characterization of Rice Germplasms for Sufficient Selenium and Low Arsenic Accumulation in Grains

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Abstract

Arsenic (As) contamination of soil is a serious problem limiting the rice grain nutrients production. Aim of this study was to identify the low grain As and sufficient selenium (Se) accumulating cultivars which may be suitable for human consumption and cultivation in As prone areas. Field trials at As contaminated sites of West Bengal, India were conducted to assess total As, inorganic arsenic (iAs) and Se content in rice grains by using HPLC-ICP-MS. Over a period of two years, 89 rice cultivars were cultivated at three different sites in West Bengal (India) having variable soil-As level. Selenium and As content in rice grains of various cultivars revealed that the number of safe cultivars decreased with increasing soil As level and also showed negative co-relation (R=-0.997**) between grain As and Se accumulation. However, total As content showed a positive correlation (R=0.903*) with grain iAs content, while Se content in rice grains was negatively affected by As uptake. Only ten cultivars viz., IET-4786, CN1646-5, CN1794-2, Dusmix-40, S. Sankar, IR-64, IET-19226, Nayanmoni, CN1643-3 and CN1646-2 accumulating low grain As had sufficient Se. Therefore, cultivation of these ten genotypes may reduce the risk of possible dietary human As exposure and thus may be recommended for the cultivation in As affected areas in India.

Key words: Arsenic, Maximum tolerable daily intake, Selenium, Recommended daily intake, Rice, West Bengal.

1. Introduction

Rice (*Oryza sativa* L.) is the major staple crop being the source of proteins and micronutrients including selenium (Se) to nearly half of world's population reliant upon it for sustenance. India is the second highest rice producer, producing more than one-fifth of rice worldwide (Williams *et al*., 2009a). Indian rice is known to be enriched in Se, but, rice cultivation in arsenic (As) contaminated areas is leading to high level of As in grains (Meharg, 2004; Dwivedi *et al*., 2010b, 2012; Norton *et al*., 2013), which has emerged as an issue of huge concern. West Bengal is the rice bowl of India and is highly As contaminated reaching upto 20.2 mg kg^{-1} in the surface horizon of pedon (Ghosh *et al*., 2006).

Zavala and Duxbury (2008) reported total As concentrations in rice grains of different countries to be 0.005 to 0.710 mg kg^{-1}. Norton *et al*. (2012) reported the 3-34 fold range in grain As accumulation in selected six field sites. Geographical variation in total and inorganic As (iAs) in rice grains has also been reported (Meharg *et al*., 2009). Daily intake of iAs loaded rice grains may constitute a potential As exposure pathway for humans (Meharg and Rahman, 2003) leading to serious health hazards such as bladder, lung, skin and prostate cancer (Naujokas *et al*., 2013; Sofuoglu *et al*., 2014; Joseph *et al*., 2015). According to a study carried out in West Bengal (India), rice was found to contribute about 44% of total average iAs intake (Mondal and Polya, 2008). Arsenic level (0.079 to 2.7 mg kg^{-1}) has been reported in rice from Indian field sites (Norton *et al*., 2012). Correspondingly, Se content in Indian rice has been shown to be within the 0.005-0.233 mg kg^{-1} range (Williams *et al*., 2009a).

Selenium is an essential micronutrient required by animals and human beings. Selenocysteins is the main component of selenoproteins or selenoenzymes (Rayman, 2002) in organisms requiring Se. Arsenic and Se compounds can be biologically antagonistic to each other, because selenite (SeIV) and arsenite (AsIII) have the same electronic structure (Rosen and Liu, 2009). Thus, Se acts as a natural antidote to As by accelerating As excretion, and acting as an antioxidant component of the enzyme glutathione peroxidase, that may counteract the cancer (Spallholz *et al*., 2004). In contrast to As, supplemented Se intake is effective in reducing mammary, prostate, lung, colon and liver cancer risk (Rayman, 2000; Duffield-Lillico *et al*., 2003). Selenium also prevents As-induced chromosomal damage, increases biliary and urinary As excretion and reduces body As load (Sweins, 1983). Plants, more importantly rice constitute the main source of Se especially for those consuming low protein diets (Williams *et al*., 2009b).

Selenate is the predominant form under well oxidized soils, however SeIV dominates under reduced soil conditions (Rosen and Liu, 2009). Plant uptake of selenate (SeVI) can be mediated by sulfate transporters while silicon influx transporter (Lsi1) is identified for undissociated selenite uptake at low pH in rice (Zhao *et al*., 2010). Arsenate (AsV) is transported through phosphate transporters (Tripathi *et al*., 2007), while AsIII through aquaporins (NIPs) in rice (Ma *et al*., 2008). Thus, As and Se chemistry and dynamics in paddy fields are complex. Zhang *et al*. (2006) reported the genetic difference in Se accumulation in grains of japonica rice. While, Williams *et al*. (2009b) concluded As accumulation has been found to limits grain Se in Bangladesh rice. Genetic variation and the effect of different environments on grain As accumulation has also been demonstrated by Norton *et al*. (2009a, b).

Considering the role of Se in efflux of As from human body along with being antagonistic it seems worthwhile to screen out rice cultivars which accumulate least As and sufficient Se irrespective of soil As content, which could eventually be useful for cultivation in As prone areas, safer for human consumption. For this purpose in the present study three field sites were selected in As affected areas of West Bengal, for cultivation of 89 local rice genotypes during *Boro* season. After maturation, plants were analyzed for Se and As accumulation in grains and were categorized in terms of Se and As levels, taking mean of the two *Boro* seasons. Further, grain As speciation of contrasting cultivars was also performed.

2. Materials and Methods

2.1. Experimental sites and growth conditions

Field trials were conducted over a period of two *Boro* seasons at three randomly selected sites each in three districts of As contaminated areas of West Bengal. District Hoogly, Chinsurah (latitude 220 53' N, longitude 880 24' E), district Bardhaman (latitude 230 15' 0 N, longitude 870 45' 0 E) is situated in the Gangetic alluvial zone in which, the block Purbasthali-I is severely As affected. While Birnagar of district Nadia (latitude 220 53’-240 12’ N, longitude 880 01’-880 48’ E) was highly As contaminated. During the present study, As level in groundwater of Chinsurah, Purbosthali and Birnagar site was around 17, 27 and 53 µg l^{-1}, however, soil As of these selected sites was 10.43, 12.59 and 15.54 mg kg^{-1}, respectively. Besides, conducting rice field trials in these selected sites, the As accumulation in native plants growing naturally in these paddy fields were also analyzed and results indicated that some medicinal plants, accumulate more As than its permissible limit and data published elsewhere along with soil physico-chemical properties (Tripathi *et al*., 2012).

The seeds of 89 rice germplasms were obtained and cultivated with the cooperation of Rice Research Station, Chinsurah, West Bengal in a randomized block design by following standard agronomic practices. Among the 89 rice germplasms, 60 were traditional type and called *Boro* Rice Germplasm (BRG), 12 were promising aromatic lines (PAL), 4 were Iron rich lines (FeRL), 11 were high yielding varieties (HYV) and 3 were segregating aromatic rice varieties (SAV). Among these rice germplasms most of them are widely cultivated in As affected areas of West Bengal and consumed by local population, these are also supplied to nearby markets for further transportation to National and International markets.

Seedlings (25 days old) of these 89 cultivars were transplanted in a prepared plot at a spacing of 20x15 cm between rows and plants. All the field sites were fertilized with chemical fertilizers like N, P and K supplied in the form of urea, single super phosphate (P_2O_5) and muriate of potash (K_2O) @ 100, 50 and 50 kg ha^{-1} respectively. Half of N fertilizer and full dose of P_2O_5 and K_2O were applied as basal dose, remaining half N fertilizer was applied as top dressing in two equal doses, first at the time of maximum tillering stage and second during panicle initiation stage. The paddy field was irrigated continuously with groundwater and shallow level of submergence (6±2 cm) was maintained throughout the growth period.

2.2. Panicle harvesting and preparation of samples

Mature panicles of each cultivar were harvested and kept in polythene bag with proper code number and were brought to the laboratory for Se and As analysis in the grain. In the laboratory, seeds were processed to white rice by the help of rice milling machine (Satake Corporation Hiroshima, Japan). Grains of each cultivar were powdered with mortar and pestle, oven dried at 80ºC for 2 d and weighed for further analysis. Grain weight of each cultivar (1000 seeds) was taken by balance for the grain yield at different sites.

2.3. Sample digestion and quantification of Se and As

Estimation of Se and As in rice grains, grain powder (0.5 g) was taken and digested in 3 ml of HNO_3 (Dwivedi *et al*., 2010a). These metalloids (Se and As) were quantified with the help of inductively coupled plasma mass spectrometer (ICPMS, Agilent 7500cx).

2.4. Determination of As species

For As species, samples were powdered; 0.2 g was weighed in a centrifuge tube, and extracted with 1 ml of 1.52 mM NaH_2PO_4 buffer containing 0.198 mM Na_2EDTA, 3 mM $NaNO_3$, 10 mM CH_3COONa and 1% C_2H_5OH (pH 6.0) (Zheng *et al.*, 2011). The extraction solutions were centrifuged and passed through a 0.45 µm nylon syringe filter. Samples were kept on ice and in the dark and analyzed within a few hours after extraction to minimize potential further transformation of As species. Arsenic species in the extracts were assayed by HPLC-ICP-MS (Agilent 7500cx ICPMS, HPLC Agilent Technologies 1200 series). Chromatographic columns consisted of a Column 150x4.6 mm, (Anion exchange resin hydrophilic polyacrylete as basic resin, PEEK1, Agilent Technologies, Inc., Tokyo, Japan). The mobile phase consisting of 1.52 mM NaH_2PO_4 buffer containing 0.198 mM Na_2EDTA, 3 mM $NaNO_3$, 10 mM CH_3COONa and 1% C_2H_5OH (pH 6.0) was run isocratically at 1 ml min^{-1}. Standard compounds of AsV and AsIII were used to obtain retention times.

2.5. Quality control and quality assurance

The standard reference materials of metals (E-Merck, Germany) were used for the calibration and quality assurance for each analytical batch. Analytical data quality of metalloids was ensured with repeated analysis (n=6) of quality control samples and the results were found within (±2.82 mg kg^{-1} dw) the certified values. Recovery of Se from grains was found to be more than 93.5% as determined by spiking samples with a known amount of Se while, for As, rice flour NIST 1568a was used as a reference material with known spiked samples and recovery of total As were 95% (±2.82; n=5) and 96% (±3.1; n=5), respectively. The quantification limit for As and Se was 1 µg l^{-1}.

2.6. Characterization of rice genotypes

Recommended daily intake of Se is 55 µg d^{-1} while, provisional maximum tolerable daily intake of As (MTDI) is 2 µg kg^{-1} body weight d^{-1} (WHO, 1989). Hence, assuming a person consumes minimum of about 300 g of rice per day as staple diet (Williams *et al*., 2009a), the rice grains should have at least 0.183 µg g^{-1} Se and less than 0.4 µg g^{-1} iAs (for 60 Kg body weight) to be considered safe for human consumption. In Bengal Delta region (Bangladesh and West Bengal, India) the Se intake has been estimated as 26 µg d^{-1}, this is based on >80% calories and protein from rice, 16% calories from fruits and vegetables and the balance of calories provided by fish (Spallholz *et al*., 2008). Therefore, rice is the major source of proteins and micronutrients including Se in this region. Thus, the cultivated rice germplasms are divided into four categories on the basis of earlier described limits (µg kg^{-1}) of Se and As, as follows:

Type A: Sufficient-Se and low-As accumulating cultivars (>0.183 Se; <0.25 As)

Type B: Sufficient-Se and high-As accumulating cultivars (>0.183 Se; >0.25 As)

Type C: Insufficient-Se and low-As accumulating cultivars (<0.183 Se; <0.25 As)

Type D: Insufficient-Se and high-As accumulating cultivars (<0.183 Se; >0.25 As)

2.7. Statistical analysis

The field experiment was conducted following a randomized block design. Two-way analysis of variance (ANOVA) and Duncan's multiple range test (DMRT) was performed with all the data. Correlation analysis was performed which has been given within text at relevant places ($p<0.001$***; $p<0.01$**; $p<0.1$*; ^{NS} non significant) (Gomez and Gomez, 1984). Regression co-efficient was done using SPSS 17.0 for windows statistical software.

3. Results

3.1. Grain Se and As accumulation

The median Se concentration (mg kg^{-1} dw) at the three sites differed significantly being 0.153, 0.132 and 0.060 for Chinsurah, Purbosthali and Birnagar sites respectively (Table 1), also a significant negative correlation (R= -0.997**) was found between median grain As and Se content in the rice grains. Similarly, negative correlation (R=-0.978**) was also found between soil As level and median grain Se content. However, a positive correlation (R=0.978**) was established between soil As and total grain As content. Arsenic accumulation and grain yield showed a significant negative correlation (R=-0.958**). Chinsurah site, the safest category A showed prolific response, while at Birnagar site except for 10 cultivars all were found grouped as category D; the most unsafe category (Table 2).

Table 1: Evaluation of rice cultivars for grain As accumulation cultivated in As contaminated area of West Bengal

Locality	Grain Se accumulation (mg kg^{-1} dw)			Grain As accumulation (mg kg^{-1} dw)		
	Median	Mean	Min.-Max.	Median	Mean	Min.-Max.
Chinsurah site	0.153	0.154	0.020-0.306	0.174	0.205	0.089-0.666
Purbosthali site	0.132	0.136	0.017-0.232	0.303	0.287	0.130-0.833
Birnagar site	0.060	0.073	0.010-0.197	0.721	0.610	0.181-1.196

Table 2: Characterization of rice genotypes as per recommended daily intake (RDI) for selenium and maximum tolerable daily intake (MTDI) of arsenic

Categories	Locality		
	Chinsurah	Purbosthali	Birnagar
Type A	21	18	10
Type B	7	12	0
Type C	45	16	0
Type D	16	43	79

Type A: Sufficient-Se and low-As accumulating cultivars (>0.183 Se; <0.25 As)

Type B: Sufficient-Se and high-As accumulating cultivars (>0.183 Se; >0.25 As)

Type C: Insufficient-Se and low-As accumulating cultivars (<0.183 Se; <0.25 As)

Type D: Insufficient-Se and high-As accumulating cultivars (<0.183 Se; >0.25 As)

Data obtained from Chinsurah field showed a significant genotypic difference in grain Se (mg kg^{-1} dw) accumulation ranging between 0.020 to 0.306. At this site mean value of Se was 0.154 while median range was 0.153 mg kg^{-1} dw however, grain As accumulation ranged between 0.089-0.666 mg kg^{-1} dw with a mean and median grain As content of 0.205 and 0.174 mg kg^{-1} dw respectively (Table 1). Rice genotypes viz. BRG-39 (0.020), BRG-14 (0.022), BRG-38 (0.028), BRG-7 (0.031) and BRG-11 (0.031) showed less (<0.183) Se level. However, cultivars CN1646-2 (0.307), CN1643-3 (0.306), BRG-31 (0.305), BRG-30 (0.298) and IR-64 (0.296) accumulated high amount of Se. At this site highest grain As was measured in BRG-16>BRG-3>BRG-18>BRG-15>BRG-12 while it was lowest for CN1794-2 <IET-19226<CN1646-2<Nayanamoni < IET-4 786. At this site, 66 genotypes showed less grain As content, while 21 cultivars accumulating high As content in their grains. However, 21 cultivars accumulating sufficient amount of Se with low grain As, in contrast to 16 cultivars having insufficient Se with high As level.

At Purbasthali, grain Se level ranged as 0.017-0.232 mg kg^{-1} dw with the mean and median Se content as 0.136 and 0.132 mg kg^{-1} dw respectively. Five rice cultivars with high Se accumulation were in the order as CN1646-2<IR-64<BRG-2<IR-36<BRG-1 while, BRG-51>BRG-46>BRG-44>BRG-19>BRG-3 showed least Se in their grains. At this site grain As content ranged between 0.130-0.833 mg kg^{-1} dw with a mean and median As level 0.287 and 0.303 respectively (Table 1). The five genotypes with the lowest grain As concentration were CN1794-2<IET-4786<CN1646-2< Nayanmoni, while those containing maximum grain As included BRG-12>BRG-3<CN1642-1>>BRG-23>BRG-18. As per our categorization, a total of 18 cultivars showed low As accumulation with sufficient Se level and thus came under the safest category (type A). In contrast 43 cultivars were found highly unsafe (type D). Other varieties were either belonged to type B or C, i.e. they either had sufficient Se alongwith high As (12) or insufficient Se with low As (16).

At Birnagar field, Se accumulation showed significant genotypic variability ranging between 0.010-0.197 mg kg^{-1} dw. Grain As ranged between 0.181-1.196 mg kg^{-1} dw with mean and median As being 0.721 and 0.610, respectively (Table 1). At this site, the least As accumulating genotypes were CN1794-2<Nayanamoni<IET-4786<CN1643-3<IET-19226<CN1646-2, while BRG-3>BRG-15>BRG-12>BRG-18>BRG-24>BRG-10 represented high grain As accumulating genotypes. Similarly, evaluation of these cultivars according to our categorization showed that number of genotypes from the safest category (type A) fell from 18 at Purbasthali site to just 10 at Birnagar site, whereas the number of genotypes in the least safe category (type D) increased from 43 to 79. Therefore, with an increase in As level in soil, only 10 genotypes were able to retain their characteristics of accumulating low As and high Se, while most of the other varieties fell under medium or least safe categories i.e. containing high As and high / low Se.

3.2. Grain As speciation

Various iAs species extracted from the rice grain indicated significant genotypic differences in the level of total grain iAs content at the selected sites (Table 3). Results revealed that iAs species (AsIII and AsV) were dominant in all common cultivars. The mean iAs ranged between 45-87% at all three sites. There was an 18-fold difference in grain iAs accumulation at Chinsurah however, 11-fold at Birnagar. Total As had positive correlation (R=0.903*) with grain iAs content.

3.3. Grain yield

The seed weight of different cultivars declined significantly with increasing As concentrations in paddy fields, being maximum at Birnagar site i.e., 70% for cultivar BRG-48. However, percentage yield reduction at Purbasthali site ranged as 0.78% to 27% of the tested cultivars. The moderate decrease (13-23%) in seed weight was observed in the common low As accumulating eight cultivars (Table 4).

Table 3: Inorganic As accumulation of different rice genotypes cultivated at three As affected sites of West Bengal during Boro season

Cultivars code	Chinsurah site	Purbosthali site	Birnagar site
BRG-1	0.144±0.002	0.296±0.036	0.525±0.096
BRG-2	0.146±0.020	0.252±0.029	0.570±0.114
BRG-3	0.486±0.036	0.543±0.039	0.572±0.067
BRG-4	0.110±0.006	0.228±0.030	0.269±0.068
BRG-5	0.139±0.014	0.324±0.020	0.558±0.025
BRG-6	0.126±0.013	0.262±0.029	0.565±0.029
BRG-7	0.102±0.009	0.113±0.020	0.332±0.041
BRG-8	0.138±0.030	0.134±0.005	0.902±0.021
BRG-9	0.070±0.024	0.192±0.018	0.421±0.044
BRG-10	0.085±0.027	0.191±0.014	0.613±0.045
BRG-11	0.085±0.005	0.220±0.009	0.272±0.007
BRG-12	0.317±0.018	0.567±0.018	0.615±0.066
BRG-13	0.072±0.011	0.170±0.016	0.516±0.025
BRG-14	0.146±0.013	0.326±0.021	0.267±0.024
BRG-15	0.341±0.030	0.407±0.040	0.481±0.065
BRG-16	0.369±0.016	0.416±0.036	0.483±0.029
BRG-17	0.078±0.005	0.520±0.387	0.433±0.033
BRG-18	0.392±0.021	0.479±0.044	0.524±0.062
BRG-19	0.193±0.012	0.572±0.064	0.636±0.012
BRG-20	0.108±0.005	0.456±0.030	0.943±0.050
BRG-21	0.093±0.005	0.379±0.031	0.358±0.030
BRG-22	0.082±0.016	0.349±0.029	0.265±0.005
BRG-23	0.071±0.006	0.355±0.027	0.625±0.081
BRG-24	0.050±0.006	0.185±0.012	0.831±0.074
BRG-25	0.115±0.015	0.278±0.021	0.632±0.006
BRG-26	0.107±0.021	0.223±0.020	0.635±0.063
BRG-27	0.104±0.027	0.318±0.007	0.416±0.045
BRG-28	0.173±0.038	0.178±0.006	0.799±0.066
BRG-30	0.148±0.021	0.119±0.015	0.457±0.054
BRG-31	0.300±0.016	0.242±0.021	0.611±0.035
BRG-32	0.157±0.007	0.220±0.288	0.282±0.027
BRG-33	0.099±0.020	0.194±0.030	0.396±0.020
BRG-34	0.171±0.024	0.313±0.020	0.320±0.016
BRG-35	0.116±0.009	0.244±0.011	0.864±0.077
BRG-36	0.198±0.020	0.192±0.018	0.754±0.052
BRG-37	0.089±0.011	0.221±0.015	0.485±0.041
BRG-38	0.088±0.014	0.263±0.020	0.597±0.045
BRG-39	0.186±0.038	0.167±0.022	0.738±0.030
BRG-40	0.094±0.015	0.291±0.039	0.385±0.042
BRG-41	0.092±0.005	0.206±0.020	0.262±0.015
BRG-42	0.192±0.029	0.234±0.024	0.812±0.041
BRG-43	0.146±0.024	0.221±0.007	0.487±0.032
BRG-44	0.074±0.006	0.213±0.024	0.368±0.021
BRG-45	0.061±0.008	0.151±0.024	0.336±0.015
BRG-46	0.147±0.009	0.243±0.029	0.683±0.021
BRG-47	0.112±0.010	0.213±0.029	0.238±0.016
BRG-48	0.082±0.008	0.126±0.029	0.292±0.020
BRG-49	0.113±0.006	0.288±0.023	0.810±0.048
BRG-50	0.141±0.012	0.122±0.008	0.205±0.009
BRG-51	0.070±0.006	0.215±0.012	0.357±0.029
BRG-52	0.123±0.030	0.270±0.020	0.606±0.025
BRG-53	0.118±0.024	0.189±0.024	0.329±0.006
BRG-54	0.043±0.007	0.077±0.007	0.193±0.030
BRG-55	0.239±0.016	0.071±0.005	0.676±0.005
BRG-56	0.140±0.027	0.245±0.042	0.518±0.040

BRG-57	0.236±0.029	0.045±0.003	0.221±0.007
BRG-58	0.087±0.005	0.073±0.004	0.097±0.015
BRG-59	0.124±0.009	0.114±0.020	0.249±0.010
BRG-60	0.111±0.007	0.114±0.029	0.682±0.029
CN1641-1	0.123±0.021	0.270±0.024	0.397±0.033
CN1641-2	0.374±0.029	0.380±0.027	0.362±0.006
CN1642-1	0.316±0.009	0.386±0.045	0.56±0.050
CN1643-1	0.240±0.030	0.191±0.007	0.561±0.014
CN1643-2	0.163±0.028	0.114±0.018	0.393±0.014
CN1644-1	0.040±0.003	0.130±0.029	0.399±0.029
CN1646-1	0.109±0.005	0.270±0.018	0.433±0.035
CN1646-3	0.126±0.029	0.095±0.009	0.150±0.016
CN1646-4	0.057±0.006	0.096±0.007	0.306±0.022
CN1646-5	0.041±0.002	0.052±0.005	0.108±0.009
CN1794-1	0.322±0.023	0.371±0.033	0.580±0.074
CN1794-2	0.041±0.003	0.055±0.007	0.083±0.020
IR72406-BR-3221	0.227±0.029	0.378±0.015	0.670±0.007
IR68144-2B-2231127	0.206±0.020	0.136±0.009	0.575±0.030
IR68144-2B-2231120	0.262±0.021	0.177±0.010	0.446±0.019
IR75494-1122312	0.093±0.025	0.088±0.005	0.174±0.016
Satya	0.090±0.007	0.180±0.029	0.260±0.014
Khitish	0.081±0.006	0.148±0.024	0.283±0.029
Dusmix-40	0.074±0.023	0.077±0.005	0.139±0.010
PNR-546	0.086±0.006	0.086±0.014	0.204±0.014
S. Sankar	0.036±0.006	0.078±0.004	0.107±0.012
Erima	0.166±0.010	0.145±0.006	0.279±0.016
Pusa Bas-I	0.071±0.007	0.231±0.023	0.268±0.024
CR-126-42-1	0.085±0.005	0.097±0.014	0.282±0.007
IR-36	0.093±0.006	0.490±0.035	0.706±0.015
IR-64	0.047±0.004	0.091±0.014	0.113±0.030
IET-19226	0.035±0.002	0.066±0.003	0.075±0.016
Nayanmoni	0.039±0.002	0.058±0.006	0.068±0.006
CN1643-3	0.046±0.003	0.047±0.004	0.093±0.010
CN1646-2	0.071±0.002	0.072±0.005	0.084±0.016

4. Discussion

Nutritional factors are known to influence As metabolism in humans, Se being an essential trace element along with being antagonistic to As, contributes significantly towards amelioration of arsenicosis. Many factors limiting grain Se levels are geochemical in origin and subject to natural variation in mineral and soil composition (Williams *et al*., 2009a). Thus, the level of Se in rice grains is of utmost importance especially in several Asian countries where rice is the staple crop and is grown on soil contaminated due to irrigation with As laden ground water (Takahashi *et al*., 2004; Mirza *et al*., 2014). Though, out of 89 rice genotypes; 16 contrasting (low and high) As accumulating genotypes were selected to study the impact of grain As accumulation on amino acid profiling (Dwivedi *et al*., 2012). Here we compared the response of 89 rice genotypes at three selected sites based on MTDI and RDI for As and Se in grains. Williams *et al*. (2009a) reported 0.002-1.370 mg kg^{-1} Se concentration in rice grains after market basket survey of white rice produced in different countries; however they also reported 0.035-0.371 mg kg^{-1} Se in rice from India. During present study, it was observed that Se concentration at the selected sites declined significantly (6% at Purbosthali site and 57% at Birnagar site) with increasing soil As concentrations (Fig. 1). Williams *et al*. (2009b) have also reported a similar observation that As limits Se uptake in Bangladesh rice grains. Further, rice cultivars were categorized on the basis of set limits as per the RDI of Se and MTDI of As for rice based diet. Selenium and As concentrations in grains showed significant differences among the rice genotypes grown at the three sites. A negative correlation (R= -0.997**) was found between grain Se level and grain As level, which was probably due to increased soil As content, and subsequently limits the grain Se level (Williams *et al*., 2009b). Hence, the number of genotypes having sufficient Se decreased from 28 (21+7) at Chinsurah site to 10 at Birnagar site. However, at the same time, the number of varieties having high As (>0.25 mg Kg^{-1}) increased from 23 at Chinsurah, 55 at Purbasthali to 79 at Birnagar site, which may be due to increased soil As concentrations. The possible reason for inhibition of Se accumulation in rice grain by soil As may be due to competition between AsIII and SeIV, as both these species share the same transport pathway, i.e., aquaporins (NIPs; Lsi1) in rice (Ma *et al*., 2008; Zhao *et al*., 2010). In essence, the number of safe cultivars decreased significantly from 21 at Chinsurah, 18 at Purbasthali and 10 at Birnagar. These variations substantiate the observations of Lu *et al*. (2009) suggesting that baseline soil variation is a major factor in grain As accumulation in Bengal Delta. Despite the impact of soil As, ten genotypes (CN1646-5, CN1794-2, IET-4786, Dusmix-40, IR-64, S. Sankar, IET-19226, Nayanmoni, CN1643-3 and CN1646-2) could maintain their low As and sufficient Se accumulating behavior giving common performance at all the three sites and thus indicating genetic control for As and Se accumulation. Norton *et al*. (2009a) reported stable genetic response across the two environments regarding grain As level (Bangladesh and Indian field sites). These findings indicated that factors affecting grain As included both field As level and genotype in concern and hence, a significant site by genotype interaction was observed (Norton *et al*., 2009b). According to Rai *et al*. (2011) rice genotypes responded differentially under AsV and AsIII stress in terms of gene expression, amino acids and antioxidant defenses for As tolerance which is controlled by a set of genes (Kumar *et al*., 2014a, b; Dixit *et al*., 2015; Singh *et al*., 2015).

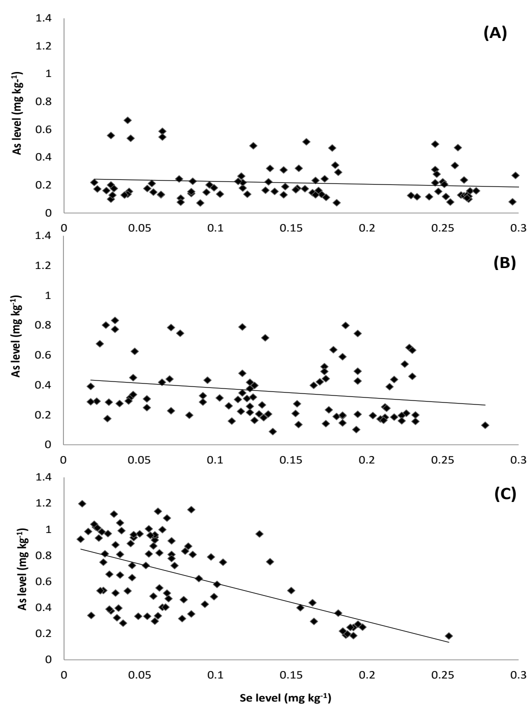

Fig. 1: Correlation analysis of grain As and Se level at (a) Chinsurah, (b) Purbosthali and (c) Birnagar sites

Arsenic speciation plays an important role in contributing to toxicity caused by its accumulation. Speciation analysis of grains indicated that iAs species were dominant in the rice grains collected from Asian countries (Norton *et al*., 2009a, 2012; Dwivedi *et al*., 2010b; Wu *et al*., 2011). The concentration of total iAs (mg kg^{-1} dw) in grains varied significantly indicating genotypic characteristics of the rice cultivars. In general, genotypes like BRG-3, BRG-12 and BRG-15 accumulated high amounts of iAs, whereas CN1794-2, CN1646-2 and Nayanmoni accumulated less iAs at all the three sites further indicating the genetic variability for As accumulation in rice (Norton *et al*., 2009a, 2012). The high concentration of iAs in Bengal rice poses threat to the regional human population in Bengal delta not only because it is non threshold class-I carcinogen but also rice is the staple diet in this region and will enhance the risk for arsenicosis through prohibiting Se uptake (Spallholz *et al*., 2004).

Grain yield component of the selected genotypes were also analyzed to select the most suitable cultivars screened at As contaminated fields. Though increasing soil As level decreased the grain yield but some cultivars like CN1646-5, CN1794-2, IET-4786, Dusmix-40, S. Sankar, IET-19226, Nayanmoni, CN1643-3, CN1646-2 and IR-64 were moderately affected by the increasing soil As level, this may be due to less accumulation of As at all the sites. In most of the cultivars grain yield is significantly reduced being maximum (upto 69%) at Birnagar site followed by 27% at Purbosthali site (Table 4). Dwivedi *et al*. (2010a) reported 12 mg l^{-1} As application retarded the growth and yield of rice cultivars. Similarly, Abedin *et al*. (2002) also reported that application of AsV reduced root biomass and rice yield significantly, however, yield attributing characters were not affected considerably.

In conclusion, our study on rice cultivars in As contaminated field trials demonstrated genotypic as well as site wise variability regarding Se and As, providing holistic information on relative Se and As accumulation in grains. Se content in grains was greatly affected as As uptake increased, implicating that Se uptake pathways is hindered by As accumulation in rice. Se, a micronutrient also plays an antagonistic effect to As in humans leading to its efflux. Consumption of low Se - high As rice as staple diet would lead to disastrous effects on health. Out of eighty nine cultivars only ten rice cultivars were found safe having sufficient Se content and low grain As level at selected sites, which can be consumed by a person of 60 kg body weight in his subsistence diet of 300 g of rice per day also fulfilling Se requirement. Results also indicated that high accumulation of As significantly affected the grain yield. Cultivating rice genotypes that produce grains with safe levels of As and adequate source of Se nutrition is likely to be economic and safer solution for the rice consuming populations.

Besides, these can be also used as genetic resource material for breeding programs leading to development of productive rice lines with sufficient Se and low grain As. For validation of these safe genotypes, multi-locational field trials having varying soil profile and As contamination are needed without compromising farmer's interests in terms of yield.

Table 4: Seed weight of different rice genotypes cultivated at three As affected sites of W.B. during *Boro* season

Rice cultivars	Seed weight (g)			% yield reduction	
	Chinsurah site	Purbosthali site	Birnagar site	Chinsurah vs Purbosthali	Chinsurah vs Birnagar
BRG-1	23.64±0.95	21.66±051	20.20±1.80	8.38	14.55
BRG-2	28.76±0.68	27.96±0.03	21.68±0.45	2.78	24.62
BRG-3	20.62±0.29	19.75±0.36	14.52±0.91	4.22	29.58
BRG-4	23.16±0.47	21.56±0.71	16.34±0.92	6.91	29.45
BRG-5	22.44±0.39	21.32±0.42	17.58±0.37	4.99	21.66
BRG-6	26.91±0.87	24.22±1.63	15.37±2.46	10.00	42.88
BRG-7	24.86±0.14	21.48±1.12	14.24±1.23	13.60	42.72
BRG-8	27.48±1.12	24.91±0.94	14.79±0.66	9.35	46.18
BRG-9	24.74±1.6	21.60±0.36	19.62±1.12	12.69	20.70
BRG-10	22.64±0.94	21.57±0.72	20.54±1.34	4.73	9.28
BRG-11	24.08±1.90	22.96±0.82	12.65±0.41	4.65	47.47
BRG-12	18.94±0.64	17.26±0.34	16.86±0.29	8.87	10.98
BRG-13	22.68±1.02	21.7±0.42	13.77±4.79	4.32	39.29
BRG-14	21.92±0.80	21.01±0.90	17.37±1.03	4.15	20.76
BRG-15	22.26±0.87	21.69±0.35	19.39±0.54	2.56	12.89
BRG-16	27.92±1.42	25.24±1.08	23.56±0.37	9.60	15.62
BRG-17	33.49±0.75	32.08±2.00	23.84±1.62	4.21	28.81
BRG-18	29.08±0.72	27.58±0.67	24.72±0.38	5.16	14.99
BRG-19	20.81±0.95	20.91±1.11	15.40±0.54	4.32	26.00
BRG-20	23.08±0.95	22.90±1.12	20.39±0.47	0.78	11.66
BRG-21	28.67±1.20	27.34±1.04	21.85±1.80	4.64	23.79
BRG-22	26.14±0.89	23.13±0.80	21.60±0.29	11.51	17.37
BRG-23	26.8±1.89	25.14±0.84	23±0.45	6.19	14.18
BRG-24	25.88±0.33	25.72±0.42	23.16±0.77	0.62	10.51
BRG-25	20.91±0.88	19.08±095	16.90±1.12	8.75	19.18
BRG-26	25.05±0.47	23.99±0.11	22.26±0.89	4.23	11.14
BRG-27	26.57±0.37	25.14±1.34	19.60±0.88	5.38	26.23
BRG-28	29.54±0.74	28.65±0.64	24.83±0.40	3.01	15.94
BRG-30	26.96±1.28	25.44±0.85	22.99±0.87	5.64	14.73
BRG-31	24.22±0.75	23.25±0.55	22.7±0.91	4.00	6.28
BRG-32	28.50±0.33	27.45±1.24	22.70±1.48	3.68	20.35
BRG-33	27.08±0.85	25.18±0.87	22.68±2.28	7.02	16.25
BRG-34	16.36±0.88	15.71±0.39	10.90±1.59	3.97	33.37
BRG-35	19.52±0.78	19.26±0.77	15.85±0.81	1.33	18.80
BRG-36	29.94±1.71	26.84±0.94	14.78±1.28	10.35	50.63
BRG-37	26.72±0.97	25.41±0.55	23.28±0.84	4.90	12.87
BRG-38	23.64±0.91	21.12±0.77	17.52±0.41	10.66	25.89
BRG-39	25.25±0.76	18.63±0.60	18.60±0.66	26.22	26.34
BRG-40	29.67±0.67	29.08±0.16	21.31±0.07	1.99	28.18
BRG-41	22.67±0.23	21.42±0.53	14.99±0.80	5.51	33.88
BRG-42	24.5±0.44	22.24±0.98	18.96±0.46	9.22	22.61
BRG-43	26.95±0.69	26.30±0.65	19.32±0.59	2.41	28.31
BRG-44	26.16±1.03	24.12±0.76	15.68±0.83	7.80	40.06
BRG-45	29.02±1.27	28.09±1.94	11.45±0.47	3.20	60.54
BRG-46	21.94±0.31	19.54±0.39	13.42±0.46	10.94	38.83
BRG-47	22.45±0.15	20.40±1.43	17.10±0.75	9.13	23.83

BRG-48	18.94±0.64	15.64±0.62	5.72±0.38	17.42	69.80
BRG-49	21.84±0.41	18.94±0.23	15.28±0.53	13.28	30.04
BRG-50	24.94±0.63	23.44±0.50	18.60±0.17	6.01	25.42
BRG-51	25.93±2.94	24.02±1.32	13.60±0.64	7.37	47.55
BRG-52	22.85±0.20	20.99±1.07	13.1±0.81	8.14	42.67
BRG-53	28.91±0.95	21.08±0.87	9.01±0.67	27.08	68.83
BRG-54	29.64±0.94	27.39±2.21	22.88±1	7.59	22.81
BRG-55	29.42±1.35	26.44±1.58	17.43±0.66	10.13	40.75
BRG-56	19±1.94	18.12±0.88	17.96±0.90	4.63	5.47
BRG-57	17.6±2.32	17.30±0.68	14±2	1.70	20.45
BRG-58	21.83±1.26	21.18±0.86	17.78±0.27	2.98	18.55
BRG-59	17.61±1.77	17.4±1.05	7.75±0.32	1.19	55.99
BRG-60	20.66±1.25	19.12±0.79	8.66±1.10	7.45	58.08
CN1641-1	16.42±1.94	15.86±0.49	5.23±0.84	3.41	68.15
CN1641-2	19.08±1.25	18.7±0.27	15.92±1.81	1.99	16.56
CN1642-1	12.18±1.94	11.60±0.36	10.79±1.66	4.76	11.41
CN1643-1	16.39±0.65	15.43±0.55	5.59±0.29	5.86	65.89
CN1643-2	15.58±1.47	14.68±1.70	8.76±1.61	5.78	43.77
CN1644-1	17.31±1.22	16.62±0.55	7.78±0.28	3.99	55.05
CN1646-1	19.40±1.31	18.11±0.25	12.61±0.46	6.65	35.00
CN1646-3	23.98±2.48	22.3±0.65	13.50±0.46	7.01	43.70
CN1646-4	20.64±1.2	19.84±1.26	13.54±0.32	3.88	34.40
CN1646-5	21.54±0.73	19.22±0.83	13.11±0.58	10.77	39.14
CN1794-1	22.95±1.19	21.64±0.51	14.95±0.17	5.71	34.86
CN1794-2	24.62±1.18	22.13±1.03	15.41±0.27	10.11	37.41
IR72406-BR-3221	23.64±0.96	21.34±0.77	19.40±0.51	9.73	17.94
IR68144-2B-2231127	15.9±0.74	15.26±0.78	11.78±0.34	4.03	25.91
IR68144-2B-2231120	19.42±0.41	18.45±0.60	13.63±0.58	4.99	29.81
IR75494-1122312	26.33±0.65	25.80±0.28	17.77±0.33	2.01	32.51
Satya	22.98±0.21	21.37±0.78	12.64±0.94	7.01	45.00
Khitish	23.30±0.60	21.18±0.86	19.91±2.02	9.10	14.55
Dusmix-40	18.09±1.00	17.52±0.42	10.68±0.49	3.15	22.96
PNR-546	21.30±0.68	20±2	14.90±0.48	6.10	30.05
S. Sankar	18.94±0.34	17.48±0.54	15.38±0.50	7.71	18.80
Erima	29.34±0.66	26.41±0.94	23.64±0.32	9.99	19.43
Pusa Bas-I	24.94±0.41	22.58±0.62	18.96±0.51	9.46	23.98
CR-126-42-1	26.75±3.07	25.51±0.43	18.91±0.39	4.64	29.31
IR-36	21.70±0.25	19.46±0.54	15.62±0.32	10.32	28.02
IR-64	28.29±0.58	27.1±0.98	17.04±0.39	4.21	23.77
IET-19226	11.71±0.47	10.22±0.81	9.44±1.25	12.72	19.39
Nayanmoni	22.07±0.69	21.37±1.03	19.13±0.47	3.17	13.32
CN1643-3	19.71±0.25	18.73±1.11	15.42±0.51	4.97	21.77
CN1646-2	16.39±0.99	15.60±0.58	13.94±0.41	4.82	14.95

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