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Assessment of potential dietary toxicity and arsenic accumulation in two contrasting rice genotypes: Effect of soil amendments

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Abstract

High concentration of arsenic (As) in rice is a serious problem worldwide. Pot experiments were conducted to assess the potential dietary toxicity of arsenic and effect of various soil amendments on arsenic accumulation in rice grains. Two basmati rice genotypes were used to conduct pot experiments using various levels of arsenic (10, 25, 50 and 100 mg kg^{-1} soil). In addition, plants were exposed to soil collected from a well documented arsenic contaminated site. Contrasting results for growth, yield and grain arsenic concentration were obtained for basmati-385 (Bas-385), exhibiting tolerance (56% yield improvement at 10 mg As kg^{-1}), while genotype BR-1 showed 18% yield decline under same conditions. Furthermore, application of soil amendments such as iron (Fe), phosphate (PO_4) and farmyard manure (FYM) at 50 mg kg^{-1}, 80 kg ha^{-1} and 10 t ha^{-1}, respectively improved the plant height and biomass in both genotypes. Accumulation of arsenic in rice grain followed a linear trend in BR-1 whereas a parabolic relationship was observed in Bas-385. Both genotypes exhibited a positive response to iron sulfate amendment with significant reduction in grain arsenic concentrations. Regression analysis gave soil arsenic threshold values of 12 mg kg^{-1} in Bas-385 and 10 mg kg^{-1} in BR-1 for potential dietary toxicity. This study suggests that genotype Bas-385 can be used for safe rice production in areas with soil arsenic contamination up to 12 mg kg^{-1} and that appropriate dose of iron sulfate for soil amendment can be used effectively to reduce translocation of arsenic to rice grain.

Keywords: Arsenic; iron sulfate; potential dietary toxicity; rice; soil amendments; soil arsenic thresholds.

1. Introduction

Arsenic (As) is a naturally occurring metalloid in the Earth's crust and predominantly occurs bound to iron oxides. However depending on geology, pH, redox status and microbial processes, it can exist in two oxidation states as arsenate (AsV) and arsenite (AsIII) (Li et al., 2017; Beiyuan et al., 2017a, Kumarathilaka et al., 2018a). Besides its natural occurrence in soil and water, arsenic contamination is increasing due to its use in pesticides and various industries, for example the production of precious trace elements. Extensive use of arsenic based pesticides caused accumulation of over 120 mg kg^{-1} in topsoil of cotton cultivation areas where arsenic was used as a defoliant (Smith et al., 1998; Niazi et al., 2011).

The presence of arsenic in soil and irrigation water can affect the growth and yield of crops, posing threats to human health as well as global food security. Soils of various regions have substantially high concentrations of arsenic in the form of minerals that may become available due to alkaline and redox conditions, contaminating water and crops thus leading to a serious environmental hazard (Beiyuan et al., 2017b). Arsenic is a known Class-1 human carcinogen, and exposure to it can result in skin and various other types of cancers and health disorders (Kumarathilaka et al., 2018a).

In the Sindh province of Pakistan, groundwater arsenic concentration has reached 1100 µg L^{-1} against the World Health Organization (WHO) permissible limit of 10 µg L^{-1} for drinking water. Moreover, about 36% of the population in the Punjab province of Pakistan and 20% of the population in the Sindh province is exposed to arsenic contamination above the prescribed limits of WHO (Shahid et al., 2018). In many cases the same water is used for irrigation purposes, causing elevated levels of arsenic in the surface soils and crops.

Human exposure to arsenic occurs through contaminated water and food supply, the later is particularly problematic in Asia where rice is used as major food since this plant species is known to accumulate relatively high arsenic due to the reducing conditions in paddy soils (Briat 2010, Kumarathilaka et al., 2018b). Contaminated food ingestion can promote the prevalence of diabetes (Li et al., 2007, Navas-Acien et al., 2008) while higher concentrations of arsenic can cause death by obstructing vital metabolic processes.

Arsenic can also negatively impact on germination, plant growth and plant development and thus poses a great threat to food production (Waseem et al., 2014; Abbas et al., 2018). In plants, most of the arsenic is retained in root cells and although translocation to shoots and grains is relatively low, it varies substantially both between and within species (Finnegan and Chen 2012). Arsenate acts as analogue of phosphate due to chemical similarity of phosphate and arsenate, thus it enters the cell using phosphate transporters (Tripathi et al., 2012).

Inside the cells, phosphate is an important element of different cellular processes and being its analogue, arsenate can cause the disruption of phosphate-dependent processes and metabolism (Finnegan and Chen 2012; Niazi et al., 2017). This similarity also means that a higher P/As ratio in the environment reduces arsenic accumulation in plants (Gomes et al., 2014). Application of iron to the soil has likewise been reported to play a key role in the reduction of arsenic accumulation in rice grain by increasing the iron percentage and by forming more iron plaque in the paddy field (Liu et al., 2015; Yu et al., 2016a, 2017). Addition of organic fertilizers can affect the bioavailability and mobilization of arsenic in a positive as well as a negative manner depending on soil conditions. In anaerobic conditions, organic matter content of soil affect the pH that cause the modification of iron redox cycle, mobilization of phosphate and

also the microbial community in the rhizosphere of paddy field, affecting the mobilization of metal (Yu et al., 2016b).

Rice is a major staple food crop and contributes1.3-1.6% to GDP in Pakistan. Beside its use as a staple food, rice is a major ingredient in a number of products especially baby formulas. Concentrations of arsenic in rice grain beyond the safe limit of 200 µg kg^{-1} of FAO in polished rice pose a great risk as well as a ban on rice export (Codex Alimentarius Commission report, 2014, 2016). Thus, there is an urgent need to evaluate the arsenic toxicity in rice and strategies to develop less arsenic accumulating rice varieties. However, currently there is no information available regarding the uptake and accumulation of arsenic in rice grain and related dietary toxicity in Pakistan. The objectives of this study were, therefore, to compare potential dietary toxicity of arsenic and the effect of various soil amendments on arsenic accumulation in rice grain in two rice genotypes that contrast in arsenic sensitivity.

2. Material and Methods

2.1. Soil collection and contamination

Soil was obtained from a non-contaminated area near river bank. It was air dried, spread on plastic sheets and then artificially contaminated by spraying it either with distilled water or with four levels of arsenic i.e. 10, 25, 50 and 100 mg kg^{-1}. Soils were equilibrated for 6-weeks, undergoing several cycles of saturation with distilled water and then air-drying. Sodium arsenate ($Na_2HAsO_4.7H_2O$) was used as a source of arsenic. After 6 weeks soil was filled in plastic pots of about 7 kg capacity for pot experiments.

Soil was collected from a well-known arsenic contaminated area, i.e. Manga-Mandi, was used to grow the plants with soil amendments as iron (Fe), phosphate (PO_4) and farmyard manure (FYM) at the rate of 80 kg ha^{-1}, 50 mg kg^{-1} and 10 t ha^{-1} respectively.

2.2. Physico-chemical properties of soil

Soil used in experiments was analyzed to determine its physicochemical properties. The Bouycous hydrometer method was used to determine the soil texture (Bouyoucos, 1962) whereas organic matter was analysed by the Walkley method (Walkley and Black, 1934). For chemical analysis of soil samples, suspensions were prepared in l:2.5 ratio of soil to water. The suspension was shaken at 200 rpm for 30 minutes.

The filtrate was then used for analysis of electrical conductivity (EC) and pH. To measure the total arsenic, phosphorous, and iron, soil was sieved by sieve size 425 μm and acid digested using nitric acid. Briefly, about 1 g soil was weighed and concentrated HNO_3 and H_2SO_4 (5:1) was added to it for digestion. Soil was digested at 100- 175 ^oC for 6 hours by gradual increase of temperature and the digests were diluted with de-ionized water and then concentration of arsenic, iron and phosphorous were analyzed using ICP-OES. For the measurement of bioavailable arsenic, phosphorous and iron, DTPA extraction was carried out. Briefly, about 5 g of soil was weighed and 10 ml of 5 mM Diethylene Triamine Pent acetic Acid (DTPA) with pH 7.3 was added in a flask. The flask was shaken at 200 rpm for 2 hrs and after centrifugation at 3000 rpm, supernatant was collected, filtered and analyzed using inductively coupled plasma - optical emission spectrometry (ICP-OES, iCAP 7000 series, Thermo Scientific).

2.3. Germination and early seedling studies

Seed germination and early seedling growth experiment were conducted to screen the rice varieties for their ability to germinate and grow under arsenic stress. Twelve popular rice genotypes named as BR-1, BR-18, BR-23, BAS-PAK, SUP-BAS, BAS-385, GSR-1, GSR-2, IR-6, PK-386, PS-2, KS-282 were used in this study. Prior to germination, seeds were surface-sterilized with 1% sodium hypochlorite ($NaOCl_4$) for 5 min and then washed with distilled water. Seeds were sown with four levels of arsenic in petri plates (50, 250, 500 and 1000 µg L^{-1}) and special germinators having soil (10, 25, 50 and 100 mg kg^{-1}) and young seedlings were grown for three weeks in a greenhouse with controlled growth conditions in the season of May-June having natural light, day/night humidity of 70-90% and day/night temperature of 25-30 ^{0}C. Germination count was taken five days after sowing whereas seedling growth parameters such as plant height, root length, fresh and dry weights were recorded after three weeks. Germination index was calculated from the formula as given in equation (1).

$$\mathbf{GI\%} = \frac{\mathbf{G_T * L_T}}{\mathbf{G_C * L_C}} \times \mathbf{100} \qquad \mathbf{(1)}$$

where G_T and G_c are numbers of germinated seeds, while L_T and L_c are the average of root length in arsenic treatment and control, respectively (Fatima et al., 2018). Based on this experiment, two promising genotypes i.e. BR-1 and Basmati-385 were selected and grown in large pots (7 kg capacity) for detailed studies including metal uptake by rice grains.

2.4. Pot experiment of rice and growth observation

Healthy seeds of rice genotype BR-1 and Basmati-385 were surface sterilized and sown in germination trays for 3-4 weeks. After that, uniform and healthy seedlings were transplanted

in pots prepared for rice transplants. Five seedlings/pot were transplanted and thinning was done after 2 weeks keeping 2 plants per pot for growth till grain stage. Plants were grown in the greenhouse for approximately 5 months with a 12/12 h light/dark cycle.

Water levels were regularly adjusted by arsenic free irrigation water whenever needed and fertilizer was applied as per rice plant requirement with the dosage of nitrogen-phosphate-potassium at the rate of 140-80-65 kg h^{-1}. Growth parameters such as plant biomass, fresh and dry weights, number of panicles, panicles weight, and grain yield were measured at the time of harvest. Different plant tissues were separated as root, shoot and grain and oven dried at 70 ^{o}C for 72 hour.

2.5. Determination of photosynthesis

Photosynthesis parameters such as leaf CO_2 assimilation rate, stomatal conductance (gs) and transpiration rate (E) were determined using a porometer (LI-1600 System, Li-COR Company). Data was recorded before the flowering stage and flag leaf was used to record the photosynthesis parameters. All data was recorded during day time in full sunlight exposure (10.00-12.00).

2.6. Arsenic concentrations, translocation factor and soil arsenic thresholds for potential dietary toxicity

Oven dried plant parts (root, shoot and grain) of rice were finely ground in a stainless steel mill while grain was dehusked prior to grinding. The powdered dry materials (0.4 g) were digested by single acid digestion using concentrated HNO_3. The digests were diluted with de-

ionized water, stored in 15ml falcon tubes and then concentration of arsenic, iron, phosphorous and zinc were analyzed using ICP-OES.

Translocation factor refers to translocation of arsenic from root to shoot and was determined by the formula given in equation (2):

$$\mathbf{TF = C_{shoot}/C_{root}} \quad \mathbf{(2)}$$

where C_{shoot} and C_{root} are arsenic concentrations in dry weight of shoot and root of plant, respectively. TF > 1 represent that effective translocation of arsenic was made to the shoot from root (Baker and Brooks, 1989). Bioaccumulation factor was also determined to evaluate the arsenic accumulation efficiency of each rice genotype according to formula in equation (3).

$$\mathbf{TF = C_{plant}/C_{soil}} \quad \mathbf{(3)}$$

Where C_{plant} and C_{soil} are arsenic concentrations in dry weight of plant and soil, respectively.

To determine the soil threshold for arsenic, safe limits of arsenic in rice as developed by Codex Alimantarious Commission and FAO were used and soil thresholds for potential dietary toxicity were calculated from regression equation as described by Long et al., (2003) using arsenic concentration in soil and grain.

2.7. Quality control

Arsenic analyses were validated using a standard reference material (SRM) for rice. Certified rice floor ERM-BC211 from European commission supplied by Sigma Aldrich was used as SRM for total arsenic. ICP-OES analysis showed the average arsenic concentration 257.51±4.02 µg kg^{-1} DW very close to the ERM certified value (260±13 µg kg^{-1} DW) showing 99.04% recovery.

2.8. Statistical analysis

All data was analyzed by statistical software SPSS (IBM version 24.0). Reported values are means of three replicates. In each rice genotype, means were compared by one way analysis of variance and two way analysis of variance (ANOVA) followed by Tukey's test at significance level of $P < 0.05$, while graphical work was carried out by Sigma Plot software (v.10). Correlation matrices were generated using corrplot library in R software (version 3.4.0). Correlations were stated statistically significant if P value was < 1%. Pearson correlation was considered positive for the value of correlation coefficient >0.5 while it was negative if the value for coefficient was <0.5.

3. Results

3.1. Physico-chemical properties of soil

The texture of soil used in study was clay loam with EC 920 µS/cm, while pH was 7.02. Organic matter of the soil was recorded to be 0.81%. Detailed physicochemical properties of soil before and after amendments are given in supplementary table 1. Total and bioavailable concentrations of arsenic, phosphorous and iron in both control and Manga-Mandi soil (MMS) are given in Fig.1, while concentrations of arsenic, phosphorous and iron in Manga-Mandi soil after amendments are given in supplementary Fig. 1.

3.2. Effect of arsenic on seed germination, hypocotyl and radical lengths

Arsenic treatment caused variation in seed germination among different genotypes with stimulatory effect in most cases. At 50 µg L^{-1} and 250 µg L^{-1} arsenic concentration observed in water in contaminated region- unpublished results and 500 µg L^{-1} arsenic treatment, both basmati

and coarse grain rice exhibited stimulation in germination except Bas-385 that showed a negative effect at 50 µg L^{-1} and then showed an improvement in germination at 250 µg L^{-1} and 500 µg L^{-1} arsenic. Treatment of seeds with 1000 µg L^{-1} arsenic led to a decrease in germination percentage in all the basmati genotypes. A similar trend was observed for hypocotyl and radical lengths (Table1). Based on germination index (Table1) and early seedling studies, two contrasting basmati genotypes BR-1 and Basmati-385 (Bas-385) were selected for pot experiments to study the toxicity of arsenic in details.

3.3.Effect of arsenic on growth and yield of rice in pot experiment

It was noted that arsenic treatment caused early flowering in BR-1 where it was started first in 25 mg kg^{-1} treatment followed by 50 mg kg^{-1} treatment and then in remaining treatments. While in Bas-385 all levels of treatments showed simultaneous early flowering as compared to control. Low concentration of arsenic in soil showed a positive effect on growth in genotype BR-1 with an increase in plant height and shoot fresh weight. At the highest arsenic concentration, a decrease of 19% and 21% in plant height and 36% and 60% in shoot fresh weight was observed in both BR-1 and Bas-385 genotypes respectively (Table. 2).

Number of tillers was also affected by soil arsenic concentration with more pronounced effects in Bas-385. Effect on yield parameter was significant among the treatments and genotypes with more severe impact on BR-1 showing 40-50% decrease in grain yield (50 and100 mg kg^{-1} soil arsenic). Application of soil amendments in Manga-Mandi soil (MMS) caused significantly different responses in various parameters (Table. 2).

In BR-1, plant height was stimulated by iron and farmyard manure, while in the case of Bas-385 it was phosphate and farmyard manure. Plant biomass and yield showed variation due to

application of different soil amendments in both genotypes with a significant stimulatory effect of iron and phosphate amendment in Bas-385 while reduction in yield was observed in BR-1 after these amendments.

3.4.Effect of arsenic on photosynthesis

In spiked soil experiments, photosynthesis parameters such as transpiration rate (E) and stomatal conductance (gs) exhibited significant variation (P<0.05) in both genotypes at different levels of arsenic in soil, while leaf CO_2 assimilation rate was significantly (P<0.05) different among both genotypes but remained unaffected by soil arsenic concentration (Fig. 2A, B, C). Transpiration rate (E) showed a significant decrease in Bas-385 at initial arsenic treatments of 10 and 25 mg kg^{-1} and while in BR-1 it remained unaffected and then showed a significant decline.

However, in Bas-385 it showed a significant improvement at highest treatment (P<0.05). Stomatal conductance followed a similar trend as the transpiration rate in Bas-385, while in case of BR-1 it showed an increase at 10 mg kg^{-1} treatment and then remained unaffected. There were no significant differences in transpiration rate (E), stomatal conductance (gs) and leaf CO_2 assimilation rate between genotypes grown in Manga-Mandi soil with various amendments (Fig. 2D, E, F).

3.5. Arsenic concentration in grain, shoot and root

Arsenic concentration was significantly (P<0.05) different among different tissues of the two genotypes growing at various levels of arsenic. An increase in the uptake in concentration of arsenic in grain was observed in both genotypes with increasing soil arsenic treatment up to 25 mg kg^{-1}, while at higher soil treatment, arsenic concentrations increased in BR-1 but the opposite

was observed in Bas-385 (Fig. 3A). Both genotypes exhibited consistent increases in arsenic uptake in shoot and root (Fig. 3B and C) with increases in soil arsenic except BR-1 which exhibited a decrease in shoot arsenic at arsenic level of 100 mg kg^{-1} (Fig. 3B).

Application of amendments in Manga-Mandi soil showed significant (P<0.05) difference among genotypes. Both genotypes showed lower arsenic concentration in grain with iron amendment followed by farmyard manure with more profound effects in Bas-385. Genotype Bas-385 showed 24% reduction in grain arsenic, while the reduction was 14% in case of BR-1 compared to growth in Manga-Mandi soil without any amendment (Fig.3D). Soil amendments also affected root and shoot arsenic concentration with significant reduction in shoot arsenic in BR-1 while an increase was observed in Bas-385. On the other hand, root arsenic concentration was increased with iron and remained unaffected with phosphate in both genotypes, while farmyard manure caused an increase in arsenic concentration of root in Bas-385 (Fig.3E and F).

3.6. Effect of arsenic on grain phosphorous, zinc and iron

Arsenic treatment had a significant effect on iron and phosphorous concentration in rice grain, while it was non-significant for zinc. Also, a significant effect of genotype was observed for phosphorous concentration in grain (Suppl. Fig.2). The combined effect of soil treatment×genotype was non-significant for grain zinc while it was significant for iron and phosphorous as analyzed by ANOVA at $P \leq 0.05$ (Suppl.Table.2). From Pearson correlation analysis, BR-1 showed a strong and significant positive correlation between grain arsenic and phosphorous (r =0.81) and moderate but non-significant correlation between grain zinc and iron (r =0.69) respectively (Fig.4A).

293 On the other hand, a strong positive correlation of grain arsenic with zinc, iron and
294 phosphorous (r =0.76, 0.82 and 0.81 respectively) and between grain zinc and iron (r =0.95) was
295 observed for genotype Bas-385 (Fig.4B), however except for the correlation between grain
296 arsenic and zinc, all these correlations were significant ($P \leq 0.01$) in Bas-385.
297
298 **3.7. Soil thresholds for arsenic toxicity**
299 Total arsenic thresholds of soil that cause potential dietary toxicity were 12 mg kg^{-1} and
300 10 mg kg^{-1} for Bas-385 and BR-1 respectively, while the bioavailable thresholds were 0.96 mg
301 kg^{-1} and 0.79 mg kg^{-1} respectively. Bioavailable arsenic was significantly correlated with total
302 arsenic concentrations in soil ($P \leq 0.01$). A strong positive and significant correlation was
303 observed for soil total arsenic with root and grain arsenic concentration in genotype BR-1 (r =
304 0.81, 0.93). Furthermore, a non-significant but moderate positive correlation (r = 0.54, 0.56 and
305 0.52) was observed for shoot arsenic with grain arsenic, zinc and phosphorous content
306 respectively (Fig. 4A).
307 Arsenic concentration of soil was strongly and significantly correlated with root and
308 shoot arsenic content of genotype Bas-385. Furthermore, there was a week to moderate
309 correlation of grain arsenic concentration with arsenic content of root, shoot and soil in Bas-385
310 (Fig. 4B). Arsenic concentration in root of Bas-385 was found both positively and significantly
311 correlated with soil arsenic concentration (r = 0.89, $P < 0.01$).
312
313 **4. Discussion**
314 Exposure to arsenic led to disruption of several physiological mechanisms and affected
315 plant growth, yield and uptake. However, these effects vary among the plants depending on the

316 type of plants, genetics, translocation properties and level of exposure (Suriyagoda et al., 2018).
317 Arsenic in rice is of utmost concern due to heavy consumption of rice by human population and
318 its use in different baby foods. Selection of rice genotypes that can avoid arsenic uptake or
319 accumulate less arsenic in grain can be a useful strategy to reduce its exposure in food chain
320 (Zhu et al., 2006). Amendment of soil with nutrients or organic matter is another way to reduce
321 the arsenic accumulation in rice grain.
322
323 **4.1. Effect of arsenic on germination**
324 Arsenic has been shown to cause a reduction in seed germination for example in
325 *Trigonella foenum-graecum L. and Lathyrus sativus L* (Talukdar 2011). Shri et al. (2009)
326 reported the sensitivity of rice seed germination upon exposure to arsenic can be attributed to the
327 toxicity due to interaction of arsenic with enzyme of starch metabolism, thus affecting the
328 germination. However, low concentrations of arsenic, Cd and Cu can stimulate germination due
329 to the generation of reactive oxygen and nitrogen species caused by the metal(loid) (Kjaer et al.,
330 1998; Li et al., 2007; Lefevre et al., 2009). In the present study, stimulation in germination was
331 observed in most of the genotypes at arsenic treatment from 50 µg L^{-1} to 500 µg L^{-1} (see
332 germination index in Table.1). In contrast, at higher concentration of arsenic, a significant
333 decrease was observed in all genotypes suggesting ~250 µg L^{-1} arsenic treatment as an
334 "optimum" level with no negative effect on germination of seeds.
335
336 **4.2. Effect of arsenic on plant growth**
337 Toxicity of arsenic was observed at increasing arsenic concentration in both genotypes.
338 Furthermore, a significant effect of soil and treatment interaction ($P < 0.05$) was observed for all
339 growth parameters when analyzed by two way analysis of variance (Suppl.Table.2). Geng et al.

(2005) observed a drop in rice plant height and biomass by increasing the arsenic concentration and similar results were observed by Rahman et al. (2007). The toxicity of arsenic is likely due to the anaerobic environment in paddy fields where reducing redox conditions favour the bioavailability of arsenite which is more toxic than arsenate (Zia et al., 2017). This rice specific aspect affects both arsenic translocation and seed setting and consequently overall yield (Finnegan and Chen, 2012; Wang et al., 2018; Islam, S. et al., 2017).

4.3. Effect of arsenic on photosynthesis

Photosynthesis is an important parameter for plant growth that provides the energy for all essential functions. Arsenic being a phytotoxic element can impact on photosynthesis by affecting the chlorophyll contents and structure of chloroplast (Rahman et al., 2007). As an analogue of phosphate it interferes with photophosphorylation (Meharg, 1994). In bean plants, photosynthesis was not affected by low concentrations of soil arsenic up to ~25 mg kg^{-1} but inhibitory effects were observed at higher concentrations of 50 and 100 mg kg^{-1} (Miteva and Merakchiyska, 2002). In a sand culture experiment of bean plants, Stoeva et al. (2005) reported a negative effect of arsenic at 5 mg L^{-1} treatment. In this study, arsenic treatment did not alter CO_2 assimilation rate (Fig. 2C), but a negative effect was observed on transpiration (E) and stomatal conductance (gs) as showed in Fig. 2A and 2B.

Stoeva and Bineva (2003) reported that in stress condition, limitation of mesophyll and stomatal cells due to metal induced changes in pigment apparatus and biochemical pathway of Calvin cycle, can cause a reduction in photosynthesis activity. In contrast to our findings with spiked soil, no significant change in transpiration rate, stomatal conductance or CO_2 assimilation was observed when plants were grown in Manga-Mandi soil with various soil amendments. This

can be attributed to the fact that all these amendments were in Manga-Mandi soil having the same arsenic concentration. It could also be due to the activation of antioxidant defense system and high concentration of glutathione that has been reported to ameliorate the effects of stress, thus helping to sustain the activity of important photosynthetic enzymes under stress conditions (Alexieva et al., 2001; Pietrini et al., 2003).

4.4. Arsenic concentrations in grain, shoot and root

Uptake and accumulation of arsenic in different tissues of rice is of utmost concern when considering food chain toxicity. There were significant differences in arsenic concentration in grains, shoots and roots. In both genotypes the highest concentration of arsenic was observed in roots followed by shoots and grains. Grain arsenic levels were genotype and soil amendment-dependent. Although the both genotypes have high accumulation factor at various levels of treatment but high grain and shoot concentrations of arsenic and translocation factor of BR-1 suggest that this genotype is sensitive to arsenic toxicity. This may be due to the difference in uptake, defense mechanism and metabolic pathways among BR-1 and Bas-385. A number of processes are involved in arsenic translocation from root to grain that differ considerably among genotypes (Islam, S. et al., 2017). Arsenic tolerant rice lines balanced the stress by antioxidants, phytochelation and scavenging of reactive oxygen species (ROS) through glutathione (Tripathi, P. et al., 2012). Change in expression level of genes that involves in phytochelation, transport pathways and detoxification of arsenic can play a plausible role in differential uptake between genotypes. Zvobgo et al. (2018) reported the upregulation of phosphate and silicon transporter genes under arsenic stress in barley. Differential response in activities of antioxidants was also observed in various genotype of rice (Rai et al., 2011).

386 **4.4.1. Effect of arsenic on grain phosphorous, zinc and iron**

387 Contamination of arsenic in rice grain can cause the restricted uptake of other
388 micronutrients, thus disturbing the nutrient value of grain. It was reported that low soil arsenic
389 concentration support the uptake of iron, zinc and phosphorous, while high levels of arsenic in
390 soil can hampered the uptake of essential micronutrients in rice (Dwivedi et al., 2010). In our
391 experiment, a strong positive correlation was observed for grain arsenic with phosphorous and
392 iron with zinc in BR-1 (Fig.4A) while Bas-385 showed a strong positive correlation of grain
393 arsenic with zinc, iron and phosphorous (Fig.4B). However, it was noted that the correlation was
394 significant only between grain arsenic and phosphorous for genotype BR-1, while in Bas-385 it
395 was significant with both iron and phosphorous, showing non-significant correlation with zinc at
396 $P<0.01$. Punshon et al. (2018) reported a positive trend for iron, zinc and arsenic abundance in
397 rice grain, exposed to high concentration of arsenic at grain filling stage. These findings might
398 suggest the difference in nutrient uptake efficiency and interaction among various nutrients
399 across different genotypes. Beesley et al. (2018) also found that rice genotypes played substantial
400 role for variation in grain phosphorous and iron uptake with a significant correlation between
401 genotype and micronutrients.
402
403 **4.5. Effect of soil amendments**

404 Iron can promote formation of root iron plaque that sequesters most of the soluble arsenic
405 and thus reduces arsenic uptake and ultimately its accumulation in grain. The use of 2% iron
406 oxide as a soil amendment was reported to be effective to lower rice grain arsenic (Farrow et al.,
407 2015). Supplementation of soil with iron at grain filling stage led to a decrease in arsenic
408 accumulation (Yu et al., 2017). Other amendments such as pine sawdust and biochar550

409 (prepared from pine sawdust at 550^0C) have been reported to increase the arsenic mobility and
410 plant availability, possibly because of an increase in pH. Furthermore, studies also revealed that
411 amendment of soil with biochar can change the soil metagenomics that influence the availability
412 of arsenic in rice fields (Qiao et al., 2017; Qiao et al., 2018).

413 With variable results, it is crucial that amendments should be selected carefully,
414 especially in paddy field applications where soil properties fluctuate considerably (Beiyuan et al.,
415 2017a). Findings in this study illustrate significant effect of soil amendments during flowering
416 stage, with iron sulfate ($FeSO_4$) being more effective than farmyard manure and phosphate the
417 least effective. Application of Fe(II) enhances opportunity for Fe(II)-sulfide formation
418 sequestering As on its surface or As(III)-sulfide formation which are stable under reduced paddy
419 soil conditions (Niazi and Burton 2016). The efficacy of amendments was influenced by the rice
420 genotype with more profound effects observed in Bas-385 in comparison to BR-1. In genotype
421 Bas-385, addition of phosphate caused a significant increase in shoot arsenic concentration while
422 in grain this increase was non-significant. This increase in shoot arsenic can be supported by the
423 findings that competitive mobilization of arsenic in paddy soils in presence of phosphate can
424 results in high root to shoot translocation that also depend on other factors such as rice genotype,
425 soil redox status, dose of phosphate and type of soil (Lee et al., 2016). Hossain et al. (2009) also
426 observed that addition of phosphate in soil used to grow rice increased the concentration of
427 arsenic in straw and grain.

428

429 **4.6. Soil thresholds for arsenic toxicity**

430 With growing concerns of arsenic toxicity, it is important to determine the soil threshold
431 arsenic value and its bioaccumulation in crops in order to avoid contamination of edible parts.

According to the definition by Islam et al. (2007) the soil threshold is the highest permissible limit of heavy metal/metalloid in the soil without potential dietary toxicity in humans. The maximum limit for inorganic arsenic in rice is 200 µg kg^{-1} and 350 µg kg^{-1} for polished and husked rice respectively (Codex alimantarious commission report-2016).

Soil threshold for potential dietary toxicity as calculated from the regression equation between soil and grain arsenic concentrations (Long et al., 2003) was ~10 mg kg^{-1} and 12 mg kg^{-1} (considering maximum limit of inorganic arsenic in rice) for BR-1and Bas-385 respectively. Threshold values for potential toxicity are related to the translocation and accumulation factor of the genotype (Table 3). Overall, translocation factors were higher for genotype BR-1, making it more sensitive. The results are supported by the findings of Long et al. (2003) where available zinc threshold was low for pakchoi due to its high accumulation and translocation compared to Chinese cabbage and celery. Soil amendments also changed the TF and BF (Table. 3) which could be due to the changes in pH and organic matter, leading to change in arsenic uptake among both genotypes.

5. Conclusion

Genotype dependent effects of arsenic on the growth and yield of rice plants were observed and both genotypes have notable differences is accumulation and translocation of arsenic with variable growth and yield responses. Soil thresholds for potential dietary toxicity suggest that genotype Bas-385 can be used safely for rice production in areas with soil arsenic contamination up to 12 mg kg^{-1} and that iron sulfate amendment can be used effectively to reduce the translocation of arsenic to rice grain, allowing cultivation in soils with arsenic content as high as 15 mg kg^{-1}. Though this is a considerable improvement, costs of amendments are still a big

challenge in many farming communities (Punshon et al., 2018), However, considering the genotype dependent response towards iron sulfate amendments, an appropriate and cautious use of iron sulfate is required to reduce the arsenic translocation. For BR-1 the values are less encouraging, reflecting its sensitivity for arsenic due to high translocation factor and grain arsenic concentration. The difference in uptake can be attributed to variation in antioxidants, uptake mechanism, and regulation of detoxification and transport pathways that need to be investigated.

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Figure Captions

Fig.1: Bioavailable (A) and total (B) Arsenic (As), Iron (Fe) and phosphorous (P) concentrations in control (CK) and Manga-Mandi soil (MMS). Error bars show ±S.E of means of three replicates (n=3). Different bars for a same element (i.e. filled with different color) labeled with different alphabet are significantly different from each other (Tukey; $P<0.05$).

Fig.2: Transpiration rate (E), Stomatal conductance (gs) and leaf CO_2 assimilation rate of two rice genotypes grown in soil having different arsenic concentrations (0 mg kg^{-1}, 10 mg kg^{-1}, 25 mg kg^{-1}, 50 mg kg^{-1}, and 100 mg kg^{-1}) and arsenic contaminated soil from Mangamandi (MMS) along with iron (Fe), phosphate (PO_4) & farmyard manure (FYM) as an amendment. Error bars show ±S.E of means of three replicates (n=3). Similar bars (i.e. filled with similar color) labeled with different alphabet are significantly different from each other (Tukey; $P<0.05$).

Fig.3: Arsenic concentration in grain (A&D), shoot (B&E) and root (C&F) of two rice genotypes grown in soil having different arsenic concentrations (0 mg kg^{-1}, 10 mg kg^{-1}, 25 mg kg^{-1}, 50 mg kg^{-1}, and 100 mg kg^{-1}) and arsenic contaminated soil from Manga-Mandi (MMS) along with iron (Fe), phosphate (PO_4) & farmyard manure (FYM) as an amendment. Error bars show ±S.E of means of three replicates (n=3). Similar bars (i.e. filled with similar color) labeled with different alphabet are significantly different from each other (Tukey; $P<0.05$).

Fig.4: Pearson's correlation matrix between concentration of soil total As (ST.As), soil bioavailable As (SB.As), shoot (S), root (R) & grain (G) As, Zn, Fe and P of two rice genotypes (A&B). Genotypes are represented as G1 for BR-1 & G2 for Bas-385. Correlation was statistically significant with P value <1%. All non-significant correlations were crossed.

Table 1: Effect of arsenic on seed germination, radical & hypocotyl length and germination index in two genotypes of rice in different concentrations on Arsenic. Values are means ±SE **(**n = 3). Values with different alphabet are significantly different from each other (Tukey; $P < 0.05$).

	As Treatment (mg L^{-1})	BR-1	BR-18	BR-23	BAS-PAK	SUP-BAS	BAS-385	GSR-1	GSR-2	IR-6	PK-386	PS-2	KS-282
Germination %	0	91.7±4.2ab	95.8±4.2ab	87.5±0.0b	91.7±4.2a	91.7±4.2ab	83.3±4.2a	87.5±0.0a	83.3±4.2a	91.7±4.2a	100.0±0.0a	100.0±0.0a	95.8±4.2ab
	0.05	100.0±0.0a	100.0±0.0a	100.0±0.0a	100.0±0.0a	83.3±4.2ab	79.2±4.2a	87.5±0.0a	95.8±4.2a	95.8±4.2a	100.0±0.0a	100.0±0.0a	91.7±4.2ab
	0.25	100.0±0.0a	95.8±4.2ab	100.0±0.0a	95.8±4.2a	100.0±0.0a	91.7±4.2a	91.7±4.2a	91.7±4.2a	95.8±4.2a	100.0±0.0a	100.0±0.0a	83.3±4.2b
	0.5	100.0±0.0a	87.5±0.0b	100.0±0.0a	100.0±0.0a	75.0±7.2b	95.8±4.2a	91.7±4.2a	91.7±4.2a	95.8±4.2a	87.5±0.0b	95.8±4.2a	100.0±0.0a
	1	87.5±0.0b	87.5±0.0b	91.7±4.2ab	95.8±4.2a	83.3±4.2ab	83.3±4.2a	87.5±0.0a	95.8±4.2a	100.0±0.0a	91.7±4.2ab	95.8±4.2a	100.0±0.0a
Hypocotyl length (cm)	0	0.84±0.07b	1.22±0.16a	1.42±0.16c	2.37±0.12ab	1.23±0.20a	1.68±0.08ab	4.30±0.07a	2.45±0.11ab	2.61±0.17a	2.16±0.08b	3.01±0.06a	2.92±0.13a
	0.05	1.26±0.14a	1.26±0.21a	2.44±0.07a	2.35±0.21ab	1.37±0.14a	1.26±0.11bc	4.69±0.25a	2.44±0.13ab	3.30±0.07a	2.75±0.16ab	3.52±0.10a	3.20±0.12a
	0.25	0.97±0.10ab	1.77±0.12a	2.28±0.15ab	2.80±0.29a	1.24±0.07a	1.91±0.11a	4.20±0.12a	2.20±0.11b	2.75±0.19a	2.95±0.06a	3.63±0.31a	2.78±0.34a
	0.5	1.35±0.03a	2.04±0.28a	2.48±0.04a	2.67±0.17ab	1.03±0.02a	1.21±0.12c	4.34±0.17a	3.67±0.22a	2.73±0.10a	2.65±0.12ab	3.58±0.24a	3.25±0.17a
	1	1.09±0.00ab	1.57±0.28a	1.62±0.31bc	1.92±0.08b	1.35±0.17a	1.04±0.07c	3.81±0.38a	3.47±0.57ab	3.10±0.23a	2.49±0.29ab	3.10±0.25a	3.22±0.11a
Radical length (cm)	0	1.44±0.10b	1.73±0.20b	2.07±0.25b	2.50±0.30b	2.05±0.33a	3.07±0.13a	3.85±0.31a	1.80±0.07b	2.56±0.21b	2.12±0.07b	2.98±0.17b	2.53±0.06a
	0.05	1.79±0.10b	2.26±0.08ab	3.87±0.10a	2.82±0.16b	1.60±0.21a	2.26±0.25ab	4.04±0.49a	2.05±0.06b	3.74±0.14a	3.15±0.08a	4.59±0.13ab	2.92±0.21a
	0.25	2.98±0.41a	2.75±0.41ab	3.87±0.25a	4.13±0.29a	2.49±0.10a	3.42±0.24a	3.77±0.27a	2.12±0.18b	2.78±0.33ab	3.42±0.19a	5.00±0.13a	3.01±0.48a
	0.5	2.89±0.21a	3.53±0.33a	4.45±0.01a	4.28±0.34a	1.62±0.19a	2.66±0.64ab	3.90±0.43a	3.77±0.22a	2.81±0.09ab	3.20±0.15a	4.41±0.84ab	3.62±0.04a
	1	2.94±0.16a	2.85±0.37ab	3.76±0.49a	3.32±0.17ab	2.36±0.28a	1.42±0.17b	2.88±0.28a	3.10±0.28a	3.41±0.19ab	2.90±0.26a	3.92±0.11ab	3.62±0.13a
Germination Index %	0.05	136.4±5.7b	139.5±11.6a	221.9±35.4a	124.8±4.3c	82.4±30.7a	69.3±2.7ab	108.3±20.9a	131.2±7.0b	155.1±15.5a	148.5±4.6ab	154.6±4.8a	111.9±13.5a
	0.25	224.1±19.1a	161.8±24.1a	220.7±34.1a	175.0±11.3ab	144.4±34.8a	124.4±16.9a	103.6±10.1a	129.2±10.6b	114.2±13.1a	161.2±8.0a	168.8±6.4a	102.7±12.1a
	0.5	222.9±28.3a	190.1±17.4a	253.7±32.7a	188.8±2.5a	68.8±16.1a	103.3±27.7ab	108.8±18.1a	230.6±15.1a	116.7±10.6a	132.9±10.7ab	138.8±18.9a	150.7±11.8a
	1	196.1±8.5ab	158.0±36.3a	190.9±14.8a	142.2±14.5bc	119.2±41.9a	46.3±4.7b	77.1±13.9a	197.5±14.9a	146.2±1.3a	124.2±4.9b	126.5±6.0a	151.1±15.4a

Table 2: Effect of arsenic on plant growth/biomass in two genotypes of rice grown in arsenic contaminated soil for six months. Values are means **±SE (n =** 3). MMS is Manga-Mandi soil, with amendments of Iron, phosphate and farmyard manure respectively. Values with different alphabet are significantly different from each other (Tukey; $P < 0.05$).

	Soil As Treatment ($mg\ kg^{-1}$)	Plant height (cm)	Shoot Fresh Wt.(g)	No. of Tillers	1000 grain weight (g)	Grain yield(g)
BR-1	**0**	98.21±0.85b	35.17±0.74b	16.00±0.29a	18.95±0.39a	14.12±0.42a
	10	102.45±1.12ab	40.78±0.45a	12.50±0.20b	16.12±0.11cd	11.48±0.09b
	25	93.13±2.24b	33.62±0.14b	16.00±0.29a	17.02±0.08bc	9.51±0.10c
	50	93.39±1.50b	23.43±0.07c	13.50±0.76b	15.42±0.28d	6.82±0.11e
	100	79.33±1.70c	22.23±0.36c	14.00±0.50ab	18.10±0.42ab	8.09±0.22d
	MMS	87.21±2.58ab	20.03±0.61a	12.00±0.29a	16.33±1.09a	8.65±0.55a
	MMS+Fe	92.35±1.91ab	20.88±0.32a	11.17±0.60ab	20.45±0.59a	8.37±0.13a
	MMS+P	85.99±1.45b	21.23±0.42a	11.67±0.44a	16.03±1.44a	4.63±0.39b
	MMS+FYM	96.01±2.33a	22.27±0.61a	9.67±0.17b	17.40±1.75a	7.42±0.52a
Bas-385	**0**	124.63±0.56a	41.87±0.52a	12.00±1.32a	21.55±0.34b	5.87±0.14b
	10	120.23±1.85a	37.45±0.58b	11.17±0.60ab	26.23±0.57a	9.18±0.18a
	25	113.20±0.75b	31.01±0.30c	9.00±0.58ab	17.68±0.37c	3.91±0.08d
	50	104.99±1.12c	18.47±0.19d	8.44±0.22b	21.05±0.75b	4.40±0.18cd
	100	97.37±2.24d	16.73±0.11d	8.33±0.33b	19.40±0.27bc	4.98±0.13c
	MMS	106.60±1.09a	22.58±0.94a	10.17±0.33a	15.53±0.34a	3.42±0.07c
	MMS+Fe	105.51±1.89a	20.73±0.41ab	9.67±0.44a	16.69±0.32a	6.49±0.20a
	MMS+P	110.79±0.97a	22.38±0.82a	10.50±0.00a	15.47±1.49a	4.86±0.35b
	MMS+FYM	108.91±0.80a	17.95±0.67b	9.67±0.17a	16.52±0.63a	3.13±0.03c

Table 3: Translocation factors[a] (TF) and bioaccumulation factors[b] (AF) of Rice grown in soil with various treatments of As for 180 days.

	TF		BAF	
Treatments (mg kg^{-1})	BR-1	Bas-385	BR-1	Bas-385
0	0.252	0.067	3.549	5.036
10	0.034	0.049	7.975	4.483
25	0.046	0.023	5.069	4.820
50	0.050	0.014	3.079	3.479
100	0.019	0.014	2.176	4.648
MMS	0.100	0.008	3.523	3.952
MMS+Fe	0.002	0.011	8.191	10.530
MMS+P	0.010	0.025	3.487	4.093
MMS+FYM	0.029	0.002	3.702	14.491

[a] Translocation factor is calculated as As concentrations in shoots/As concentrations in roots.

[b] Bioaccumulation factor is calculated as As concentrations in plant/As concentrations in soil

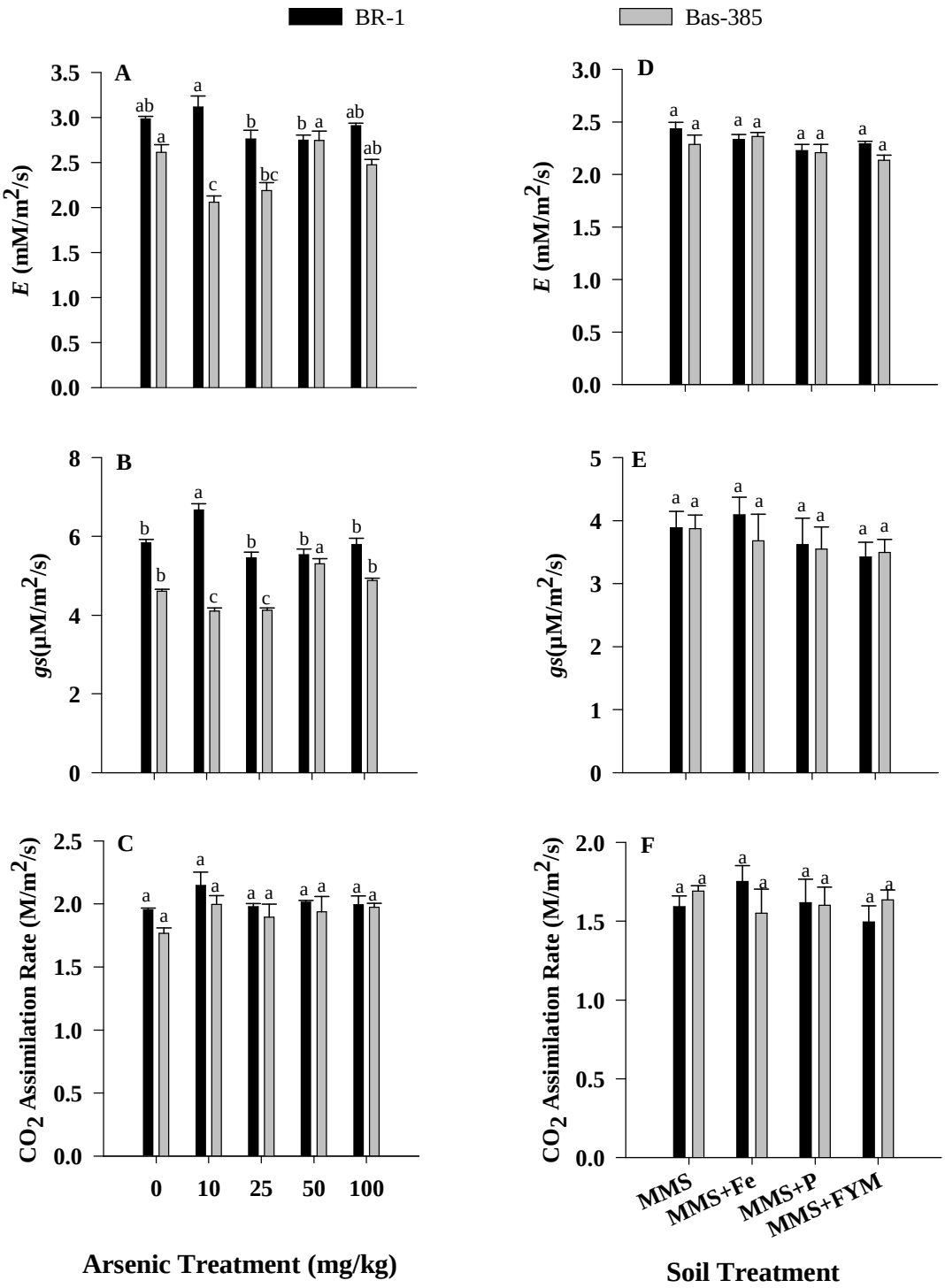
BR-1
Bas-385
A
B
C
D
E
F
E (mM/m2/s)
gs(µM/m2/s)
CO2 Assimilation Rate (M/m2/s)
0
10
25
50
100
Arsenic Treatment (mg/kg)
MMS
MMS+Fe
MMS+P
MMS+FYM
Soil Treatment

Fig.2

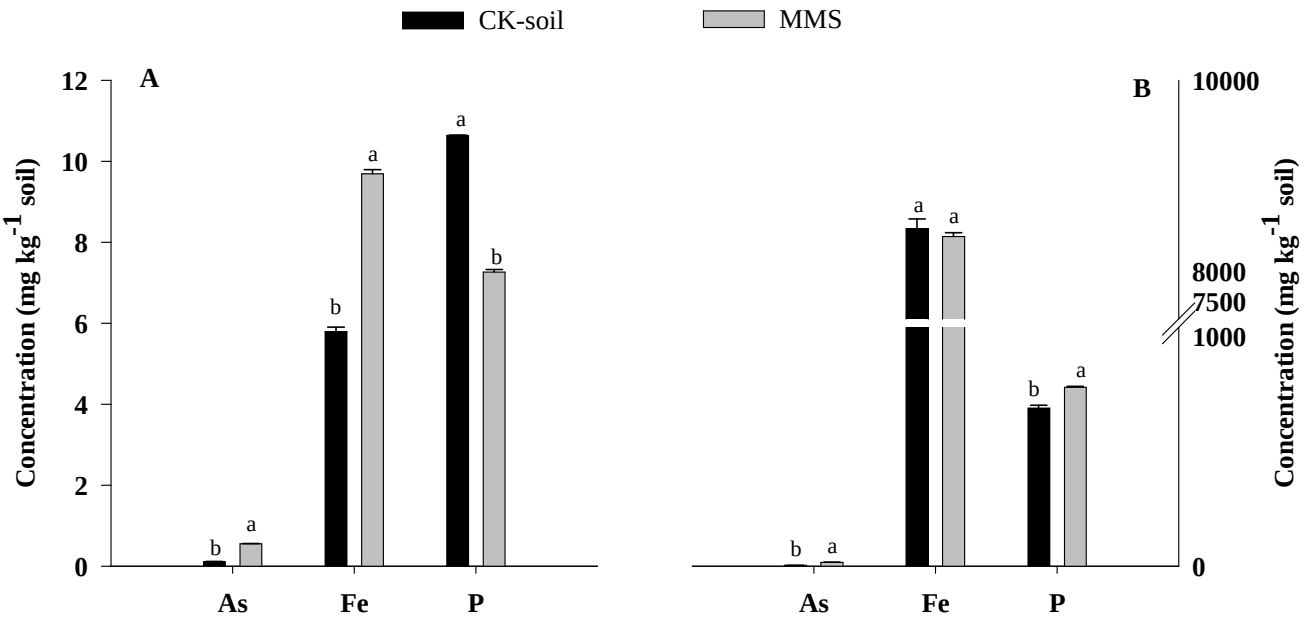

Fig.1

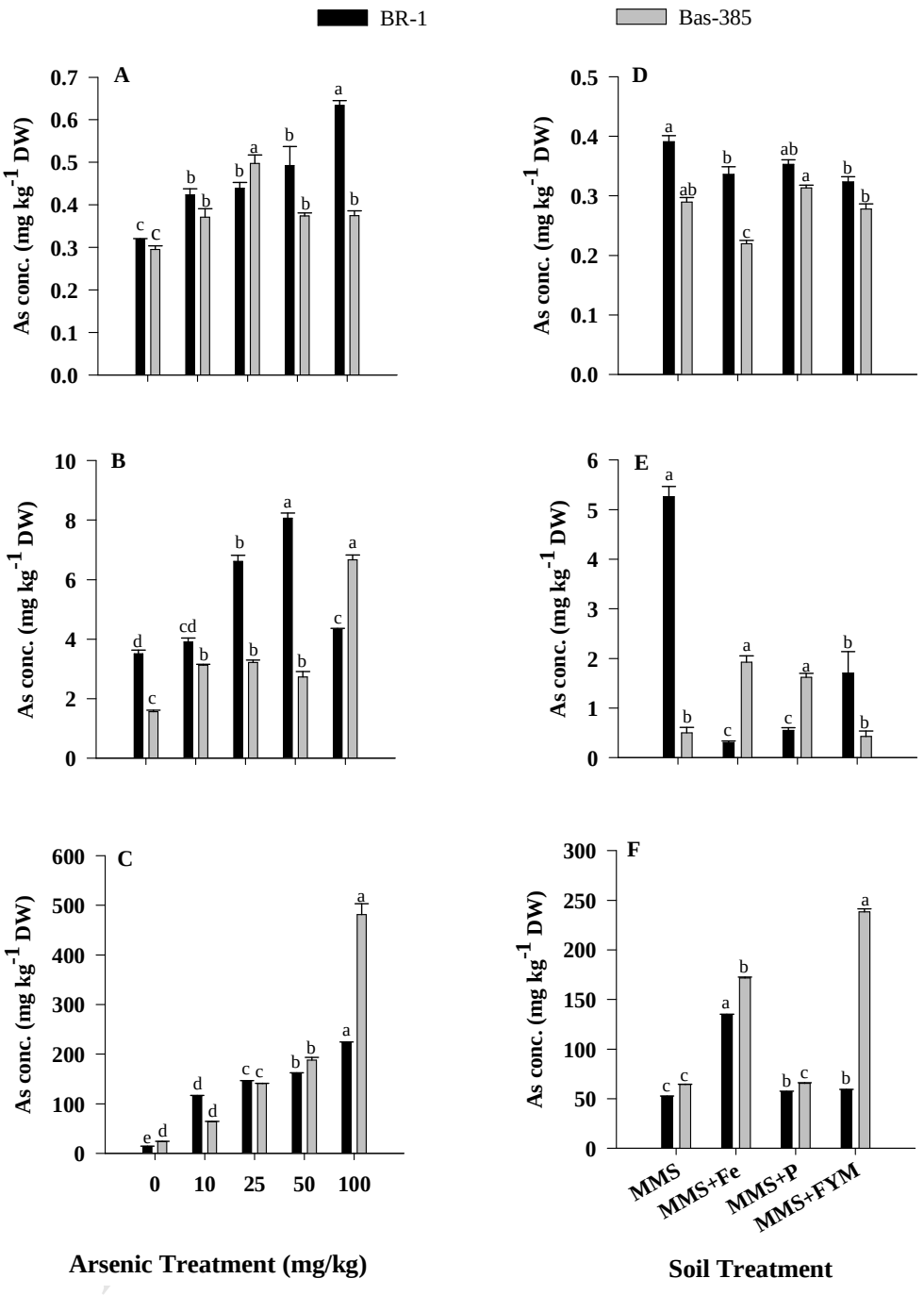
BR-1
Bas-385
A
B
C
D
E
F
As conc. (mg kg-1 DW)
Arsenic Treatment (mg/kg)
0
10
25
50
100
MMS
MMS+Fe
MMS+P
MMS+FYM
Soil Treatment

Fig.3

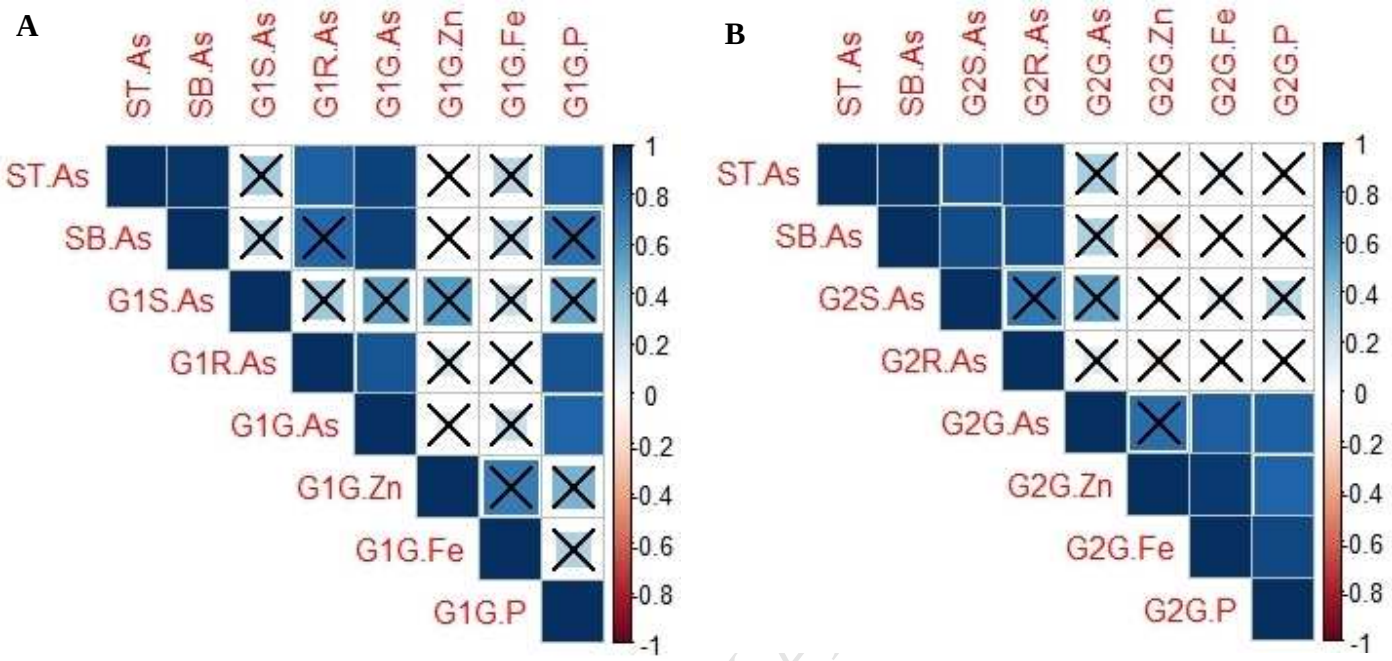

Fig.4

Highlights

- Arsenic (As) toxicity in basmati rice shows genotype dependent effects on growth
- Bas-385 showed substantial yield improvement at 10 mg kg^{-1} soil arsenic
- Arsenic concentration in rice followed the order roots > shoot > grain in both genotypes
- Iron sulfate amendment caused a significant reduction in grain arsenic
- High concentration of arsenic in soil led to 40%-50% reduction in grain yield