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Genotypic Differences in Effects of Arsenic on Growth, and Concentration of Arsenic in Rice (*Oryza sativa*) genotypes

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Abstract

By using two Oryza sativa genotypes (Mahsuri and CN1035-60) a green house experiment was carried out to study i) genotypic variation in Arsenic (As) tolerance, and ii) As concentrations in different plant- parts at Agricultural Research Station, Bilaspur Chhattisgarh, India during 2010-2011. Plants were grown under controlled climatic conditions and subjected to increasing As supply in the form of Na_2HAsO_4 *@ 0, 10, 20,30,40,50 mg As/kg soil. Mahsuri showed greater sensitivity to As toxicity than CN1035-60. Increasing As supply markedly reduced the stem and root dry weight of both genotypes (Mahsuri and CN1035-60) and these decreases were more marked in Mahsuri. Increase in As concentration of about 5 times in stem and 9 times in root was noted with an application of 20 mg As/ kg soil than the control. As was accumulated in the roots in much higher amounts than in the stem, especially in the case of CN1035-60 indicating that there is limited transport of As from the root system to the above ground plant parts in CN1035-60. Genotype CN1035-60 had significantly less uptake of As than genotype Mahsuri.*

Key words: Arsenic, growth, genotypic variation, Oryza *sativa,* shoot and toxicity.

Introduction

Arsenic (As) is not essential for plant growth. Because of chemical similarities to P, As is able to replace P in many cell reactions and shows many harmful toxicities to plants including wilting of new-cycle leaves and retardation of root and top growth[1,2]. It should be noted that very little is known about the chemical forms of arsenic (e.g., inorganic and organic) in crop/vegetable, which in turn is needed for estimating its toxicity. Some recent studies suggest that a significant portion of arsenic in rice and vegetable exists as As (V)[3,4]. Plant species and even genotypes differ greatly in their ability to take up, transport and accumulate As within the plants[5]. Plant mechanisms affecting the root uptake and shoot transport of As can also affect the expression of As toxicity in plants[6]. The amounts of As taken by the plant genotypes had increased with increasing As levels. Therefore, the selection of plant genotypes with high ability to repress root uptake and shoot transport of As is a reasonable approach to alleviate adverse effects of As toxicity in crop plants. To our knowledge, there is no study in the literature dealing with As tolerance in rice genotypes. In the present study, using two rice genotypes, the effect of increasing As supply on the root and stem growth, and concentration of As in root and stem was studied.

Material and Methods

Soil Sampling: The soil used in this study was collected from Bilaspur, Chhattisgarh, India. Physico-chemical properties of the soil were measured by the standard methods of soil chemical analysis[7]. The soil had 0.78% organic carbon, 198 kg / ha available nitrogen, 194 kg/ ha K_2O_5 and Zn, B and Mo 0.50, 0.31 and 0.04 mg/ kg soil respectively, available sulphate-sulphur 9.1 mg/kg soil, available P 24.5 mg/ kg soil, and Cd 0.40 mg/ kg soil. The mean arsenic concentration in the top soil layer (top 75 mm) was 6.9 mg/ kg, while that at the bottom layer was 3.07 mg/ kg.

Greenhouse Experiment: The experiment was carried out with two genotypes of *Oryza sativa* (Mahsuri and CN1035-60) at Agricultural Research Station, Bilaspur Chhattisgarh, India during 2010-2011. The plants were grown in the green house under controlled environmental conditions (light/dark regimes of 16/8 h, temperature 30/22ºC, humidity 55/60% and photosynthetic photon flux 750 µmol m^{-2} s^{-1}).

Two 30-d-old seedlings of rice (*Oryza sativa*) varieties were transplanted in earthenware pots packed with 5 kg of air-dry soil. As was supplied as Na_2HAsO_4 in concentrations of 0 (control treatment), 10, 20, 30, 40 and 50 mg As/ kg to the experimental soil. Phosphorus as $CaH_2PO_4.H_2O$ at 13 mg P/ kg, K as KCl at 24 mg K/ kg, and N as CO $(NH_2)_2$ at 60 mg N/ kg were supplied as solution (in distilled water) at the start of the experiment to ensure adequate mineral nutrition. Application of all nutrient solutions and first application of arsenate treatment to dry soil were conducted before transplantation of rice seedlings. The experimental design was completely randomized with each treatment replicated three times. The plants were watered daily as needed. At harvest, the roots and stems were separated and dried at 70˚C in order to determine dry weight and as concentration.

Determination of Arsenic in Plant: The Plant samples (approximately 0.2000–0.5000 g) were digested by 10 ml of concentrated Analar HNO_3. The digestion tubes were heated on a heating block at 180°C for 1 h and then at 200°C to evaporate the samples to dryness. The residue was taken up in 10 ml of 10% (w/v) HCl containing 10% (w/v) KI and 5% (w/v) ascorbic acid. As concentrations in the samples were then determined by hydride generation atomic absorption spectrophotometry.

Statistical Analysis: The results were statistically analyzed by Duncan's Multiple Range test at a 5% probability level[8].

Results and Discussion

An increasing supply of As resulted in significant decreases in the stem and root growth of both genotypes. The growth of Mahsuri and CN1035-60 were significantly reduced at the 0-10 mg/kg and 30-40 mg/kg of As treated soil, respectively (figures 1 and 2). The decreases were more distinct in Mahsuri. for example, with a 20 mg/ kg As supply, stem dry weight was reduced by around 20% in CN1035-60 and 30% in Mahsuri. Similar decreases were also noted for root dry weight (figures 1 and 2). The reduction in stem and root growth of plants due to application of as are in agreement with the findings of other workers[9].

Arsenic phytotoxicity was shown by growth retardation of Mahsuri and CN1035-60. Mahsuri appeared to have a higher susceptibility to as toxicity than CN1035-60 and this higher sensitivity was associated with corresponding decreases in stem and root growth (figures 1 and 2). The present study has shown reduced growth of plants grown in soil containing high concentration of arsenic. Similar results were also reported by previous workers[10, 11]. It seems that the effect of As on stem and root growth varies depending on the genotypes, level of contamination and plant tissue ability to tolerate As. Different investigators widely identified the symptoms of As toxicity in rice plants[2]. Some workers investigated and found the rice appear to be more sensitive than other plants in experiments with toxic levels of As[1]. It concluded that As concentration appearing toxicity was widely varied with plant species.

Significant differences among both genotypes were noted in As accumulation. High arsenic in soil appears to result in higher concentration of arsenic in root and stem of rice plants (table 1 and 2). This result is in agreement with that reported by several workers based on a greenhouse study[10]. Some recent studies suggest that there is a linear relationship between As content of plant and soluble As concentration in soils that plants take up passively with the water flow[12, 13]. Therefore, it seems that rice plants accumulate more As due to higher water demand[14]. Some previous workers also reported similar results5.[5, 14]. Increase in As concentration of about 5 times in stem and 9 times in root was noted with an application of 20 mg As/ kg than the control (table 1 and 2). Table 1 and 2 show that Arsenic concentration in roots of rice plants varied from 9.0 to 368.4 µg/g (with a mean of 130. 33 µg/g), while that in the stem varied from 7.5 to 158.1 µg/g (with a mean of 53. 24 µg/g). Table 1 and 2 also show that As was accumulated in the roots in much higher amounts than in the stem, especially in the case of CN1035-60. For example, with 40 mg/ kg As supply, the As concentration was about 4 times and 2 times higher in the roots than in the stem of CN1035-60 and Mahsuri respectively indicating that there is limited transport of As from the root system to the above ground plant parts in CN1035-60 genotype. Genotype CN1035-60 had significantly less uptake of As than genotype Mahsuri (table 3). With these results, Mahsuri plants accumulate more As than CN1035-60 indicating that it has better absorbing ability and has higher potential for removing As from moderately contaminated soils. Previous workers also reported similar results[15]. The amount of As accumulated and translocated in plants varies with species.

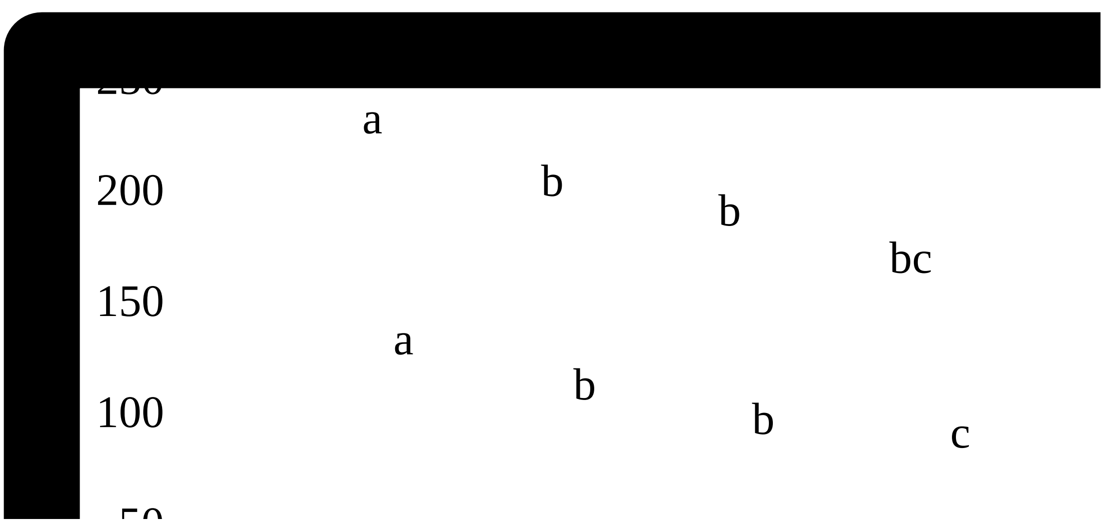

Figure-1
Effect of increasing As supply on stem and root dry weight of Mahsuri. The data represent means ± SD of three independent replications. Means followed by similar letters are not significantly different from each other at 0.05 level of probability

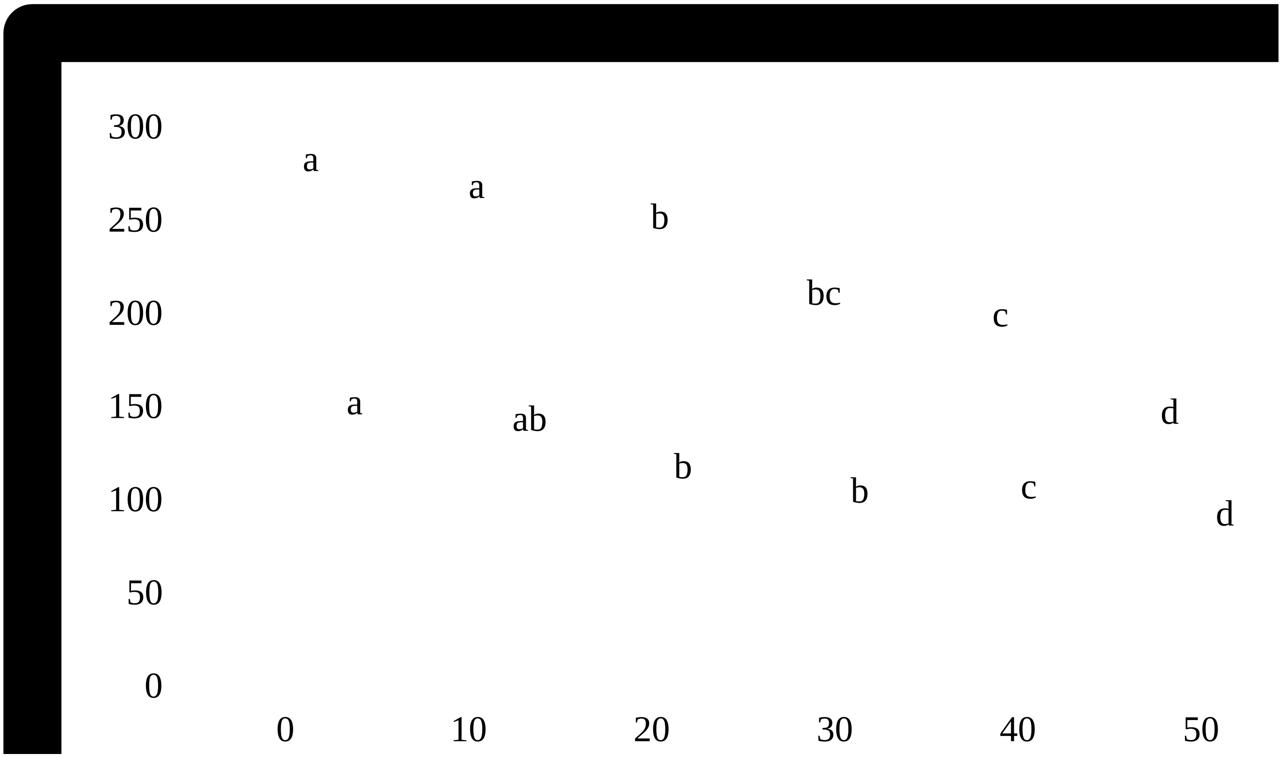

Figure-2

Effect of increasing as supply on stem and root dry weight of CN1035-60. The data represent means ± SD of three independent replications. Means followed by similar letters are not significantly different from each other at 0.05 level of probability

Table-1

Effect of increasing as supply on stem and root as concentrations of *Oryza sativa* genotype -Mahsuri. The data represent means ± SD of three independent replications. Means followed by similar letters are not significantly different from each other at 0.05 level of probability

As Supply (mg / kg)	**As conc. (µg/g) Stem**	**As conc. (µg/g)** Root
0	8.50 ± 1	9.00 ± 1
10	37. 20 ± 1	53. 01 ± 1
20	45. 14 ± 1	82. 21 ± 1
30	69. 04 ± 1	110. 04 ± 2
40	93. 51 ± 1	188. 50 ± 1
50	158. 11 ± 2	260. 34 ± 3
LSD (<0.05)	2. 21	17. 3

LSD: minimum significant difference. Details of treatments are given under Materials and Methods

Table-2

Effect of increasing as supply on stem and root as concentrations of *Oryza sativa* genotype - CN1035-60. The data represent means ± SD of three independent replications, Means followed by similar letters are not significantly different from each other at 0.05 level of probability

As Supply (mg / kg)	**As conc. (µg/g) Stem**	**As conc. (µg/g)** Root
0	7. 50 ± 1	9.00 ± 1
10	22. 20 ± 1	70. 30 ± 1
20	38. 50 ± 1	82. 60 ± 1
30	41. 20 ± 1	120. 40 ± 1
40	53. 70 ± 2	213. 10 ± 3
50	65. 50 ± 1	368. 40 ± 3
LSD (<0.05)	3.15	21. 7

LSD: minimum significant difference. Details of treatments are given under Materials and Methods

Table-3
Total as uptake in each plant of Mahsuri and CN1035-60 in soils of different as concentration

As conc. (mg/ kg)	Absorbed As conc. (µg / plant) Mahsuri	Absorbed As conc. (µg /plant) CN1035-60	Mean
Control	16 d	14 d	15
10	177 c	123 c	150
20	214 c	178 c	196
30	352 bc	217 b	384
40	484 b	318 b	401
50	610 a	498 a	554
Mean	308	224	
LSD (<0.05)	102	87	

Values followed by similar letters are not significantly different from each other at 0.05 level of probability. LSD: minimum significant difference. Details of treatments are given under Materials and Methods

Conclusion

In conclusion, the results presented show the existence of genotypic variations in the tolerance to As toxicity among *Oryza sativa* genotypes. The differential tolerance to As toxicity in *Oryza sativa* was related to As concentrations in the different plant parts and the retention of As in the roots. More data are needed to ascertain the findings of this study.

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