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Original Research

Effects of arsenic toxicity on morphological characters in blackgram (*Vigna mungo* L.) during early growth stage

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Abstract: Blackgram is an important pulse crop of the tropic and sub-tropic area and has been identified as a potential crop in many countries. In the south-East Asia arsenic toxicity in soil and water is one of the most environmental problems. Crop productivity is highly affected by cultivation in arsenic polluted soil or irrigation through arsenic polluted water. The present study was conducted to evaluate the effect of arsenic (As) on fresh shoot length, fresh shoot weight, fresh root length, fresh shoot weight and total fresh biomass, The results indicate that root length is more affected than shoot length due to arsenic toxicity. The fresh shoot weight observed was more affected than fresh root weight. This study indicates that arsenic toxicity causes the deleterious effect on blackgram growth. The toxic effect of blackgram depends on the genotypic variability. Some blackgram genotypes show very less toxic effect of arsenic due to its genetic makeup. Experimental findings of study indicate that longer root length and more shoot weight in arsenic stress condition may be tolerant blackgram genotype to arsenic toxicity.

Key words: Arsenic; Blackgram; Pulse; Tolerance.

Introduction

Blackgram (*Vigna mungo* L.) or mash or Urd bean belongs to family *Leguminosae* and the subfamily *Papilionaceae*. It is an annual and important short duration pulse crop growing in many parts of India and cultivated both in *Kharif* and *Rabi* season. Urd bean has been reported to have originated in India by several workers too like De Candolle (1) and Vavilov (2). The optimum temperature for better growth of blackgram is 25-35^{o}C, but it can tolerate up to 42^{o}C which permit to cultivate during summer and winter season. Blackgram is cultivated both in hilly and plain regions with short life cycle (90-120 days) and high nutritive value (3, 4). Blackgram is quite drought resistant but intolerant of frost and prolonged cloudiness. It is cultivated in most of the soils, but it can grow better on heavier soils (pH 5.5-7.5) with an annual rainfall of 600-1000 mm (4, 5). Among pulses blackgram occupies a prominent place in India, covering an area of about 3.26 million hectares with a production of 1.76 million tonnes (6, 7). Blackgram contributes 13% in total pulses area and 10% in total pulses production of India (8). India is the world's largest producer and consumer of blackgram, but it is not able to fulfil the blackgram demand (7). Blackgram is used for human food, green manure, forage silage, hey and chicken pasture (9, 10). Blackgram is an excellent source of essential amino acids. Heavy metal stress is an important abiotic stress of crop species that are grown in the vicinity of heavy industries particularly in developing countries (10-12). The toxicity of heavy metals is a problem for ecological, evolutionary and environmental reasons (4, 13).

Arsenic (As) is a metalloid, which is highly toxic for all forms of life. Arsenic is a group I carcinogen and occurs predominantly in inorganic form as arsenate (As V) and arsenite (As III) (14-17). The soil is contaminated by arsenic through production/use of arsenic-based pesticides manufacture of arsenic based compounds, smelting of arsenic ores, mining process and fuel utilization (17-19). Water and soil polluted with arsenic are the major sources of drinking water and food chain contamination in numerous countries (17, 20). Arsenic poisoning has become epidemic worldwide and arsenic cause various types of disease like cancer, multiples bones disease and keratosis on palm and sole (21). The major arsenic pollution affected states in India are West Bengal, Bihar, Jharkhand, Uttar Pradesh, Assam, Chhattisgarh and Manipur (18). The Ganga-Brahmaputra plain is the most affected area for arsenic pollution (18). Irrigation using arsenic (As) polluted water causes a hazardous effect on soil environment and crop quality (17). Arsenic concentration (20 ppm) in the soil causes 20% loss of crop production (22). The various hazardous effects of arsenic is reported on different plants like yield losses, inhibition of seed germination, decrease in plant height, lower fruit and grain yield, reduction in root and shoot growth, wilting and necrosis of leaf blades and reduction in leaf area and photosynthesis (17). The arsenic is absorbed by roots through various channels like NIP (Nodulin 26-like Intrinsic Proteins) subfamily of aquaporins and phosphate transporter system (23). Keeping all the above-mentioned points in view, the present study was performed to investigate the effect of arsenic on blackgram during the early growth stage.

Materials and Methods

Plant materials and arsenic treatment

The thirty-two genotypes of blackgram (*Vigna mungo* (L.) Hepper) were procured from IIPR Kanpur, India. The seeds were surface sterilized with mercuric chloride (0.1% w/v). The sand was thoroughly washed with water containing potassium permanganate ($KMNO_4$) (1mg/L) and dried. These seeds were sown in the pots. All plants were irrigated by 500 ml Hoagland solution in 4Kg sand (24) weekly. After 21 days of sowing control group plants were irrigated with only 500 ml of Hoagland solution and treatment group plants were irrigated with 500 ml Hoagland solution which contains 150μM of sodium arsenite ($NaAsO_2$).

Morphological studies

After 30 days of sowing plants, growth parameters were estimated after uprooting the plants. The root length and shoot length were measured in centimetre (cm), whereas the fresh shoot weight, fresh root weight and total biomass were measured in gram (gm). Arsenic tolerance trait index (ATTI) at the seedling stage was calculated following the formula Ali et al (25).

$$\text{ATTI} = \frac{\text{Value of trait under stress condition}}{\text{Value of trait under controlled condition}} \times 100$$

Statistical analysis

The experiment was conducted in a completely randomized design (CRD) and observations were recorded in three replications. The data were analyzed by one-way analysis of variance (ANOVA) (26) to determine the level of significance at $P \leq 0.01$. The significant difference between control and treatment for each parameter in every blackgram genotypes were estimated through student's t-test.

Results

The effect of arsenic toxicity on shoot length, fresh shoot weight, root length, fresh root weight and total biomass is presented in Table 1 & Fig 1. The calculated t-value and ATTI is presented in Table 2. It was observed that shoot length, fresh shoot weight, root length, fresh root weight and fresh total biomass was affected by the arsenic toxicity. The calculated F-value of ANOVA (Table 1) for shoot length, fresh shoot weight, root length, fresh root weight and total biomass indicates that experiment was significant at 1% probability level in control and arsenic stress condition. Effect of arsenic toxicity on different morphological parameters is presented in Table 2 & Fig 1.

Effect of arsenic on fresh primary root length

It has been observed that the roots of all genotypes were affected by As toxicity. The highest ATTI was observed in the case of genotype IPU 2K-99-224, whereas lowest ATTI was in IPU 99-235, indicates that primary root of IPU 99-235 is much affected and IPU 2K-99-224 was less affected by arsenic toxicity. All the genotypes, except IPU 2K-99-226 show significant difference between control and As treated plants root length. In the control condition, the shortest root length was observed for genotypes SPS 38 and longest root length was observed in IPU 99-176. After arsenic treatment, the longest root was observed in IPU 25 and shortest in IPU 99-235.

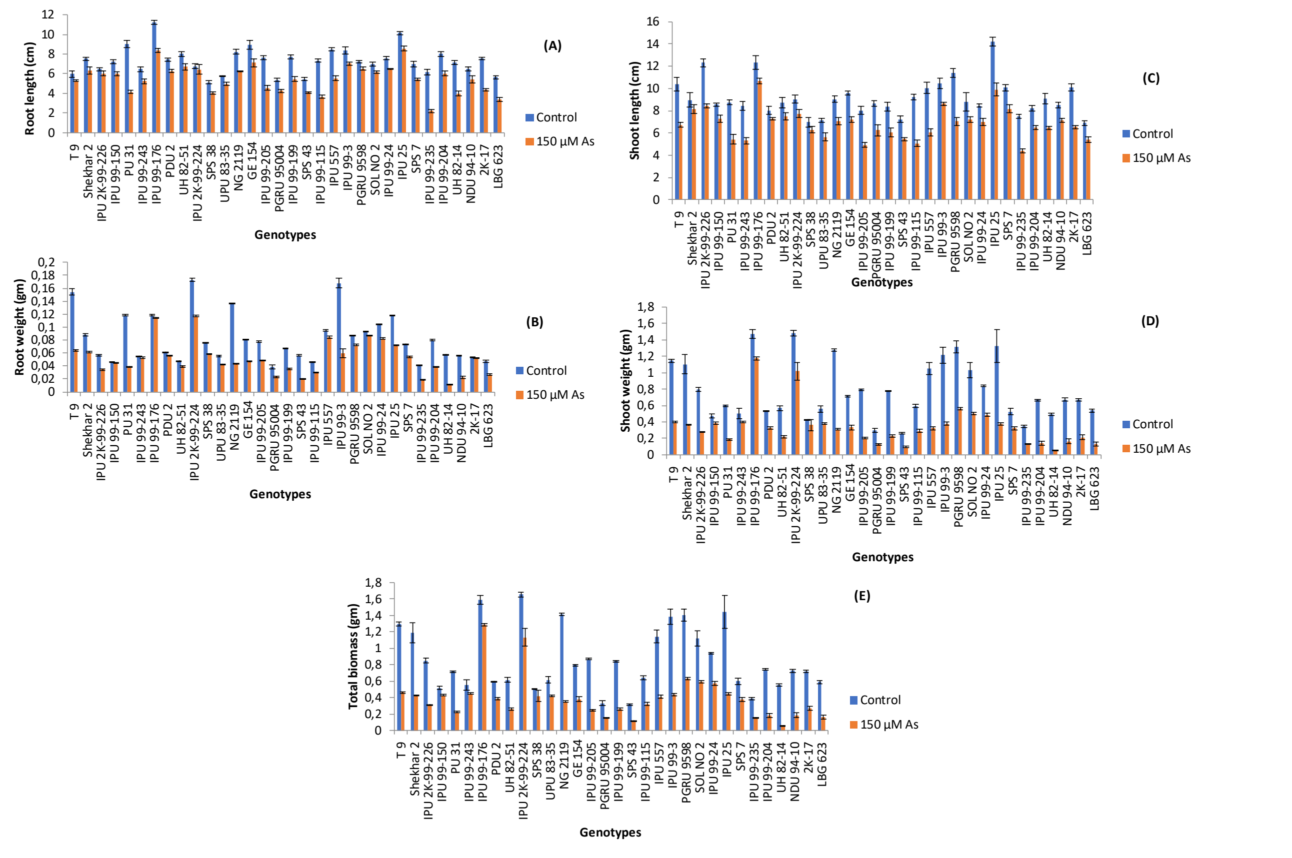

Figure 1. Effect of Arsenic toxicity on different morphological traits (Root length (A), Root weight (B), Shoot length (C), Shoot weight (D) and Total biomass (E)) in the genotypes of Blackgram (*Vigna mungo* L.Hepper).

Table 1. Performance of different morphological parameters of 32 blackgram genotypes at early growth stage in control and arsenic stress condition.

	SL (cm)				FSW (gm)				RL (cm)				FRW (gm)				FTBM (gm)			
G	C	AST	ATTI	T	C	AST	ATTI	T	C	AST	ATTI	T	C	AST	ATTI	T	C	AST	ATTI	T
T 9	10.40	6.73	64.74	10.00**	1.145	0.399	34.82	56.40**	5.97	5.30	88.83	3.16*	0.154	0.064	41.53	29.94**	1.3	0.463	35.619	53.13**
Shekhar 2	8.97	8.13	90.71	1.87	1.103	0.365	33.04	10.82**	7.53	6.33	84.07	5.43**	0.088	0.061	69.03	19.51**	1.192	0.426	35.708	11.04**
IPU 2K-99-226	12.30	8.43	68.56	17.10**	0.795	0.277	34.83	30.45**	6.47	6.03	93.30	2.55	0.057	0.034	60.48	23.69**	0.852	0.310	36.435	30.89**
IPU 99-150	8.57	7.27	84.82	6.59**	0.473	0.386	81.61	5.85**	7.20	6.03	83.80	7.00**	0.046	0.045	97.74	2.08	0.518	0.430	83.053	6.15**
PU 31	8.73	5.43	62.21	11.51**	0.599	0.187	31.17	54.85**	9.03	4.13	45.76	22.16**	0.118	0.039	32.67	80.26**	0.717	0.225	31.417	65.44**
IPU 99-243	8.43	5.30	62.85	10.78**	0.502	0.398	79.37	2.84*	6.47	5.23	80.93	6.00**	0.055	0.052	95.62	2.84*	0.557	0.451	80.966	2.88*
IPU 99-176	12.30	10.70	86.49	4.42*	1.472	1.175	79.77	9.42**	11.2	8.40	75.00	17.15**	0.118	0.114	96.67	4.26*	1.591	1.289	81.03	9.77**
PDU 2	8.03	7.30	90.87	3.48*	0.537	0.329	61.31	25.54**	7.43	6.27	84.30	9.35**	0.061	0.056	90.98	9.25**	0.598	0.385	64.338	27.32**
UH 82-51	8.70	7.50	86.21	3.80*	0.569	0.223	39.19	17.96**	8.03	6.70	83.40	5.25**	0.048	0.040	82.91	12.03**	0.617	0.263	42.581	17.94**
IPU 2K-99-224	9.03	7.73	85.61	4.53*	1.483	1.016	68.50	7.28**	6.77	6.43	95.07	4.25*	0.173	0.117	67.69	30.76**	1.656	1.133	68.417	8.15**
SPS 38	6.97	6.30	90.43	2.25	0.426	0.361	84.77	1.62	5.13	4.03	78.57	8.82**	0.076	0.058	77.21	29.22**	0.502	0.420	83.634	2.08
UPU 83-35	7.13	5.63	78.97	6.78**	0.559	0.379	67.68	7.63**	5.77	4.97	86.13	8.49**	0.055	0.043	76.9	19.37**	0.615	0.421	68.514	8.08**
NG 2119	9.03	7.07	78.23	7.88**	1.278	0.31	24.25	83.91**	8.23	6.23	75.71	13.42**	0.136	0.044	31.94	140.82**	1.414	0.353	24.987	97.31**
GE 154	9.60	7.23	75.35	12.75**	0.715	0.335	46.83	21.11**	8.97	7.13	79.55	5.24**	0.081	0.047	58.4	54.80**	0.795	0.382	48.005	22.46**
IPU 99-205	8.03	4.93	61.41	13.15**	0.792	0.202	25.50	73.72**	7.60	4.57	60.09	16.34**	0.078	0.049	62.57	40.97**	0.87	0.251	28.808	84.49**
PGRU 95004	8.67	6.27	72.31	7.39**	0.295	0.129	43.78	11.16**	5.37	4.27	79.50	8.82**	0.038	0.023	60.14	7.56**	0.334	0.153	45.668	11.03**
IPU 99-199	8.37	6.07	72.51	6.97**	0.775	0.228	29.38	63.11**	7.70	5.47	71.00	12.03**	0.068	0.036	52.71	54.97**	0.843	0.263	31.257	63.14**
SPS 43	7.23	5.47	75.58	8.96**	0.262	0.095	36.06	23.73**	5.47	4.10	75.00	12.97**	0.057	0.020	36.16	68.03**	0.319	0.115	36.082	26.88**
IPU 99-115	9.23	5.07	54.87	18.23**	0.595	0.291	48.97	49.00**	7.33	3.67	50.00	29.40**	0.046	0.030	65.76	19.99**	0.64	0.321	50.167	20.35**
IPU 557	10.00	6.07	60.47	11.67**	1.050	0.325	30.94	16.51**	8.47	5.53	65.35	17.26**	0.095	0.084	88.97	9.03**	1.145	0.409	35.747	16.58**
IPU 99-3	10.50	8.63	82.48	6.67**	1.213	0.376	30.98	14.38**	8.37	7.03	84.06	5.35**	0.168	0.059	35.34	19.12**	1.381	0.435	31.513	17.67**
PGRU 9598	11.40	7.03	61.70	14.21**	1.314	0.561	42.65	17.10**	7.23	6.50	89.86	5.50**	0.087	0.073	83.12	17.81**	1.401	0.633	45.172	17.49**
SOL No 2	8.80	7.23	82.20	3.24*	1.028	0.503	48.94	9.86**	7.00	6.17	88.1	5.74**	0.093	0.087	93.53	9.02**	1.122	0.591	52.653	9.91**
IPU 99-24	8.47	7.00	82.68	7.55**	0.840	0.487	58.05	23.74**	7.57	6.47	85.46	11.67**	0.104	0.083	79.18	19.29**	0.944	0.570	60.388	24.26**
IPU 25	14.20	9.90	69.72	10.86**	1.325	0.375	28.34	8.14**	10.10	8.57	84.54	9.22**	0.117	0.072	61.23	64.97**	1.442	0.447	31.017	8.50**
SPS 7	10.10	8.17	81.13	7.07**	0.526	0.323	61.33	8.13**	7.00	5.43	77.62	8.06**	0.073	0.055	74.26	23.96**	0.6	0.377	62.91	8.88**
IPU 99-235	7.50	4.40	58.67	18.98**	0.345	0.137	39.60	23.27**	6.17	2.23	36.22	23.14**	0.041	0.018	44.96	55.43**	0.385	0.155	40.163	26.61**
IPU 99-204	8.23	6.50	78.95	9.34**	0.664	0.141	21.20	32.14**	8.03	6.03	75.10	9.73**	0.080	0.039	48.17	51.09**	0.744	0.179	24.100	33.12**
UH 82-14	9.10	6.47	71.06	9.44**	0.495	0.052	10.56	50.54**	7.17	3.97	55.35	16.97**	0.058	0.012	20.00	82.56**	0.552	0.064	11.549	54.01**
NDU 94-10	8.53	7.13	83.59	8.24**	0.672	0.164	24.40	26.44**	6.50	5.43	83.59	4.57*	0.055	0.022	40.17	31.78**	0.727	0.186	25.605	27.10**
2K-17	10.10	6.53	64.69	18.35**	0.668	0.214	32.10	27.55**	7.57	4.37	57.71	28.95**	0.053	0.052	97.87	1.59	0.721	0.267	36.959	28.26**
LBG 623	6.90	5.43	78.74	7.90**	0.541	0.134	24.72	22.34**	5.63	3.37	59.76	15.21**	0.047	0.027	56.05	14.58**	0.588	0.160	27.24	25.25**

Abbreviations: SL: Fresh shoot length (cm); FSW: Fresh shoot weight (gm); RL: Fresh root length (cm); FRW: Fresh root weight (gm); FTBM: Fresh total biomass (gm); G: Genotypes; C: Control; AST: Arsenic stress; ATTI: Arsenic tolerance index; T: t-value calculated; Asterisk (*) indicate that mean values are significantly different between the treatment and control ($p \leq 0.05$); Asterisk (**) indicate that mean values are significantly different between the treatment and control ($p \leq 0.01$).

Table 2. Analysis of variance (ANOVA) for different morphological characters in blackgram during control and arsenic stress condition.

Character	**Range**		**General mean**		**SD (m)**		**SE (m)**		**CV (%)**		**CD (5%)**		F-value	
	C	AST	**C**	AST	**C**	AST	**C**	AST	**C**	AST	**C**	AST	C	AST
Shoot length (cm)	6.9000-14.2000	10.6667-4.4000	9.1740	6.8448	0.3578	0.2812	0.225	0.174	4.239	4.398	0.636	0.492	53.342**	63.978**
Fresh shoot weight (gm)	0.2620-1.4831	0.0523-1.1746	0.7830	0.3398	0.03748	0.0198	0.032	0.016	7.139	8.285	0.091	0.045	118.326**	229.602**
Root length (cm)	5.1333-11.2000	2.2333-8.8567	7.3282	5.5125	0.2119	0.2118	0.132	0.135	3.111	4.242	0.373	0.382	105.033**	108.279**
Fresh root weight (gm)	0.0380-0.1730	0.0120-0.1170	0.0820	0.0516	0.0014	0.0012	0.001	0.001	2.404	3.120	0.003	0.003	1067.404**	764.468**
Total biomass (gm)	0.3185-1.6561	1.2888-0.0638	0.8651	0.3910	0.0375	0.019	0.032	0.016	6.459	7.039	0.091	0.045	140.025**	261.320**

Abbreviations: C: Control; AST: Arsenic stress; Asterisk (**) indicate that experiment is Significant at 1% probability level.

Effect of arsenic on fresh root weight

The highest fresh root weight was observed in genotype IPU 2K-99-224 in control and arsenic stress condition whereas the lowest fresh root weight was observed in genotype IPU 99-235. The highest ATTI for fresh root weight was observed in genotype 2K-17 and lowest in UH 82-14. The difference between fresh root weight in control and arsenic stress condition was observed significant except genotype 2K-17 and IPU 99-150.

Effect of arsenic on fresh shoot weight

In control condition, the highest shoot weight was observed in IPU 2K-99-224 and lowest shoot weight was observed in SPS 43. In arsenic stress condition, the highest fresh shoot weight was observed for IPU 99-176 and lowest fresh shoot weight was observed in genotype Sol No 2. The highest arsenic tolerance index was observed in genotype SPS 38, whereas the lowest ATTI was observed in genotype UH 82-14. All the genotypes except SPS 38 show significant difference between shoot weight for control and arsenic stress condition.

Effect of arsenic on fresh shoot length

The longest shoot length was observed in genotype IPU 25 and shortest shoot length was observed in LBG 623 in the control condition. In arsenic stress condition, the longest shoot length was observed in IPU 99-176 and shortest shoot length was observed in IPU 99-235. The highest ATTI was observed in PDU 2 and lowest was observed in IPU 99-115. All genotypes except SPS 38 and Shekhar 2 shows the non-significant difference between shoot length in control and arsenic stress condition.

Effect of arsenic on total fresh biomass

In the control condition, the highest total fresh biomass was observed in IPU 2K 99-224 and lowest biomass was observed in SPS 43. In arsenic stress condition the highest biomass was observed in IPU 99-176 and lowest in UH 82-14. The highest ATTI was observed in SPS 38 and lowest in UH 82-14. All the genotypes except SPS 38 show significant difference in total biomass between control and arsenic stress condition.

Discussion

The reduction in shoot length, shoot weight, root length, root weight and total fresh biomass was reported by various researchers in various crops in response to different heavy metals exposure. Experimental findings indicate that arsenic (As) stress causes an adverse effect on the root & shoot growth, similar effects were also observed by nickel (Ni) (27, 28) and zinc (Zn) toxicity (27) in blackgram. The fresh shoot weight, fresh root weight and fresh total biomass reduction were observed in this experiment, similar findings was also reported by nickel (Ni) toxicity (10, 28) and due to lead (Pb) toxicity (10) in blackgram. The fresh shoot weight was more affected than fresh root weight indicating the higher metal sensitivity of shoot than root which have also been reported in blackgram and other plant species (10, 28, 29). It is speculated that absorption of root passes these metals to shoot through translocation. Thus, roots are less affected as compared to shoot (10, 30, 31). A wide range of ATTI in fresh shoot weight, fresh root weight and total biomass indicating that fresh biomass of shoot, root and total biomass was highly influenced by the arsenic toxicity. The shoot weight is more affected in comparison to root weight by arsenic toxicity. The ATTI of root length and shoot length indicates that root length is more affected by arsenic toxicity than shoot length. The wide ATTI range indicates that arsenic causes deleterious effects on *Vigna mungo* L. Our results showed that the shoot weight and root weight are more affected than shoot length and root length. These observations indicates that emphasis should be given to those genotype(s) which have high shoot weight and root weight in arsenic stress condition and may be more tolerant to arsenic toxicity. Further selection of arsenic-tolerant *Vigna mungo* genotype(s), the more emphasis should be given on the root length than shoot length. This experiment indicates that the genotype which has longer root length and more shoot weight in arsenic stress condition may be tolerant blackgram genotype(s) to arsenic toxicity. The reason for wide range of ATTI observed may be attributed to different morphological parameters due to genetic variation of different blackgram genotype(s). It indicates that genetic variability has significant effect to tolerate arsenic toxicity. Our result is in accordance with previous reports reported by various workers (32-34).

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