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Evaluation of Germination Attributes, Metal Tolerance Index and Phytotoxicity Index in Green Gram Cultivars [*Vigna radiata* (L) Wilczek] under Arsenic Toxicity

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ABSTRACT

Arsenic is a heavy metal that causes severe damage to the crop plants. It has effects on different growth parameters including germination attribute of seedlings. Mung bean is one of the most important crops in the country. Due to heavy metal toxicity in field, the crop reduces its production potential to great extent. An experiment was conducted in the laboratory of Department of Plant Physiology, Institute of Agricultural Sciences, Banaras Hindu University using petri plates and germination papers with two green gram genotypes HUM1 and HUM16 during February 2020. The Mung bean varieties were subjected to six different arsenic treatments (0µM, 0.5µM, 1µM, 5µM, 10µM and 20µM). Germination attributes were observed at 3rd and 7th day after germination. There was significant difference in germination attributes like percent germination, radical and plumule length, seedling vigour index, metal tolerance index, phytotoxicity index observed in relation to the different treatments. Gradual changes in germination attributes in relation to arsenic stress were observed for both HUM-1 and HUM-16. However, there was no significant difference observed between the two genotypes in relation to the arsenic treatments in some parameters.

***Key words*:** Mung bean, Germination, Arsenic, Stress, Metal tolerance index

Mung bean [*Vigna radiata* **(**L.**)** Wilczek] is one of the most important pulse crops. It is grown throughout the southern Asia including India, Pakistan, Bangladesh, Malaysia, China etc. It is also grown in the parts of Africa and U.S.A and has recently been introduced in Australia. In India, it is grown in almost all parts of the country, on an area of about 3 million hectares with the production of about 1 million tonnes. The major green gram grown states are Odisha, Maharashtra, Andhra Pradesh, Telengana, Rajasthan, Madhya Pradesh, Bihar, Karnataka and Uttar Pradesh. Odisha is the state that is highest in green gram production as well as productivity followed by Maharashtra and Andhra Pradesh. Although Andhra Pradesh is higher in productivity compared to Maharashtra.

The element arsenic (As) is an environmental toxin that is found naturally in all type of soils. The main environmental exposure to As for humans is through contaminated drinking water, for example on the Indian sub-continent [1]. Arsenic becomes part of the human solid food chain when crops and fodder become contaminated. The metalloid enters into farming systems through a variety of means that include natural geochemical processes [2], the past and present use of As-based pesticides, mining operations, irrigation with As-contaminated groundwater, and fertilization with municipal solid wastes [3].

Arsenic is non-essential and generally toxic to plants. Roots are usually the first tissue to be exposed to As, where the metalloid inhibits root extension and proliferation. Upon translocation to the shoot, As can severely inhibit plant growth by slowing or arresting expansion and biomass accumulation, as well as compromising plant reproductive capacity through losses in fertility, yield, and fruit production [4]. At sufficiently high concentrations, As interferes with critical metabolic processes, which can lead to death. Most plants possess mechanisms to retain much of their As burden in the root. However, a genotype-dependent proportion of the As is translocated to the shoot and other tissues of the plant. Numerous physiological processes are susceptible to As toxicity. Cellular membranes become damaged in plants exposed to As, causing electrolyte leakage [5]. Membrane damage is often accompanied by an increase in malondialdehyde, a product of lipid peroxidation, pointing to the role of oxidative stress in As toxicity.

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MATERIALS AND METHODS

The experiment was conducted in the laboratory of Department of Plant Physiology at Institute of Agricultural Sciences, Banaras Hindu University using petri plates with two green gram cultivars HUM1 and HUM16 procured from Department of Genetics and Plant Breeding, Institute of Agricultural Sciences, Banaras Hindu University consisting of six treatments and three replications during February 2020. The chemical used for the treatment was $Na_2HAsO_4.7H_2O$. Thirty-six numbers of petri plates were obtained from the Department. The petri plates were washed by using lab detergent and rinsed initially with tap water then finally with distilled water. The washed petri plates were placed in oven at 100°C for 24 hours for dry sterilization. Bold and uniform seeds were selected where as small, discolored, infected seeds were discarded. Seedlings were supplied with Hoagland and sodium arsenate solutions two times every day *viz*. morning and late evening. Six different concentrations of arsenic (control, 0.5μM, 1μM, 5μM, 10μM and 20μM) were taken. Green gram seeds obtained were washed with tap water initially then surface sterilization of seeds was carried out using 0.1% $HgCl_2$ for 2-3 minutes. Subsequently it was washed in sterilized distilled water for 5-6 times and then the seeds were dried with the help of blotting paper. After sterilization one part of the seeds were placed in germination papers, and the other in the petri plates. The germination papers, containing the seeds were placed in 500ml sized beakers, in which the Hoagland solution and the arsenic solution ($Na_2HAsO_4.7H_2O$) were added. Whereas small sized thin germination papers were made for the petri plates in which the already prepared solutions were added. The morphological parameters of roots, shoots and leaves were analyzed separately. The morphological data were taken at 3 and 7 days after sowing. Germination percentage was calculated at 72 hours after sowing. The plumule length of seedlings was measured by using a thread from each replication under each treatment. The plumule length is measured from the point of the base of plumule up to the growing tip using a thread. The threads were then placed on a meter scale and the length of shoot is expressed in cm. The plumule length per plant in each treatment is obtained by taking the average of plumule length of three seedlings obtained from the 3 replications under each treatment. Plumule length was measured for each treatment carefully and with utmost precaution. Radicle length was obtained by taking average of radicle length of three plants obtained from the three replications under each treatment. Radicle length is expressed in centimeters. The radicle length was measured using a thread and measuring from the base of the radicle to the main growing tip and expressed in centimeters.

Seedling vigor index was calculated on three days as well as seventh day after germination by using the below given formula:

Seedling vigor index = % germination × radicle length

Per cent phytotoxicty was calculated on three days as well as seventh day after germination using the formula of [6].

$$\%\ \text{Phytotoxicity} = \frac{[\text{Radicle length of seed (control)} - \text{Radicle length of seed (treatment)}]}{\text{Radicle length of control}} \times 100$$

Metal tolerance index was calculated on five day as well as seventh day after germination from the radicle length using the formula given by [7].

$$\text{MTI} = \frac{\text{Radicle length of seed T}}{\text{Radicle length of seed C}} \times 100$$

Shoot and root samples of three seedlings from each replication were blot dried to remove any moisture present on the surface then were placed inside an envelope for drying inside an oven at 100°C for an hour to kill the metabolic activities followed by constant temperature of 70°C for a period of 72 hours. Weighing was made regularly till a constant weight is achieved using an electronic weighing balance. The seedling dry weight was obtained by sum of averaged shoot and root dry weights per plant.

RESULTS AND DISCUSSION

Many plants at seed germination and seedling stages are sensitive to environmental factors. Therefore, the change of plant growth at the germination and seedling stage under heavy metal stress is often regarded as an important index to evaluate plant tolerance to heavy metals. Germination percentage showed significant differences among different treatments at 3th and 7th day after germination. Among the treatments, maximum germination percentage was found in control and T_2 (0.5 μM) on 3th day after sowing for both the varieties. However, at 7th day, maximum germination percentage was observed in the control followed by T_2 for both HUM-1 and HUM-16, whereas the least germination percentage was observed in T_6 (20μM). Per cent germination did not decrease significantly up to arsenic levels of 1μM but decreased significantly at 5μM, 10μM and 20μM concentrations in moongbean. The maximum reduction in germination was observed at the highest concentration. There was lesser reduction in germination per cent in moongbean genotype HUM-16 as compared to HUM-1 at 7 days after germination. There was some recovery in per cent germination from 3 days after germination to 7 days after germination in both genotypes. Similar results of decrease in per cent germination with increasing arsenic concentrations has been reported in wheat [8] and *Cicer arietinum* [9]. Heavy metal arsenic might have adverse effect on enzymes such as α-amylase and others responsible for seed germination and thus reducing per cent germination.

There was gradual decrease in radical length from the control to the T6 observed for both HUM-1 and HUM-16. It was found that 27.4% and 33.6% reduction in radical length at T6 in relation to the control on 3 days after germination in HUM-1 and HUM-16 respectively. There was 32.5% and 30.2% reduction in radical length observed in HUM-1 and HUM-16 respectively after 7 days of germination. The least radical length was found in T_6 (20μM) for both HUM-1 and HUM-16 on 3th as well as 7th day of germination. Radical length was more inhibited than plumule length and HUM-16 performed better than HUM-1 upto arsenic concentration of 5 μM but at 10 μM and above there was drastic reduction in radical length and plumule length in both moongbean genotypes. [10] observed that arsenic reduced the elongation of wheat seedling and reported that the root growth inhibition was stronger than shoot growth inhibition. [11] reported that germination and early seedling growth can also decrease significantly with increasing concentrations of arsenic in rice. The inhibitory effect of arsenic on growth could be due to hinderance in cell growth. Arsenic in cells get associated with middle lamella and increase the cross linking that eventually lead to reduced cellular expansion and growth [12].

Seedling vigour index (SVI) is the potential of seed germination and seedling size against the toxicity and tolerance of metals. Seedling vigour index decreased with increasing arsenic doses with drastic reduction at sodium arsenate @ 5.0 μM and above and the least seedling vigour index was recorded at the highest arsenic concentration of 20 μM, used in this study in both moongbean genotypes. HUM-16 showed lesser decrease in vigour index as compared to HUM-1 at the same arsenic concentrations. Several authors reported that, the inhibition of root elongation caused by heavy metals may be due to metal interference with cell division, including inducement of chromosomal aberrations and abnormal mitosis [13-14], which can affect seedling growth and explain the inhibition of seedling growth and decrease in seedling vigour index. The presence of heavy metals in soil can function as stresses which causes physiological and biochemical constraints thereby decreasing plant vigour and inhibiting plant growth [15].

Table 1 Effect of different arsenic concentrations on germination attributes of green gram at three days after germination

Arsenic treatments \ Parameters	Germination %		Radical length (cm)		Plumule length (cm)		Seedling vigour index		Phytotoxicity index (%)		Metal tolerance index (%)	
	HUM1	HUM16	HUM1	HUM16	HUM1	HUM16	HUM1	HUM16	HUM1	HUM16	HUM1	HUM16
T_1 (0 µM As)	91.67	87.50	3.17	3.42	8.85	6.20	290.59	299.25	0.00	0.00	100.00	100.00
T_2 (0.5 µM As)	91.67	87.50	2.90	3.02	9.23	6.25	265.84	264.25	4.73	11.69	91.48	88.30
T_3 (1 µM As)	87.50	83.33	3.07	3.23	8.38	5.93	268.62	269.16	3.15	5.55	96.84	94.44
T_4 (5 µM As)	83.33	83.33	2.93	2.65	8.05	5.87	244.16	220.82	7.57	27.51	92.42	77.48
T_5 (10 µM As)	79.17	79.17	3.00	2.27	7.37	4.85	237.51	179.72	5.36	33.62	94.64	66.37
T_6 (20 µM As)	66.87	87.50	2.30	2.27	6.83	4.99	153.80	198.62	27.44	33.62	72.55	66.37
Mean	83.33	82.64	2.89	2.81	8.12	5.68	240.82	234.22	8.83	17.83	91.16	82.16
	SEm±	CD at 5%	SEm±	CD at 5%	SEm±	CD at 5%	SEm±	CD at 5%	SEm±	CD at 5%	SEm±	CD at 5%
Treatments (T)	2.41	7.02	0.10	0.30	0.13	0.39	15.02	43.83	3.458	10.09	3.10	9.05
Varieties (V)	1.39	4.05	0.059	0.17	0.08	NS	8.67	25.31	2.00	5.83	1.80	5.22
Interaction (T × V)	3.4	NS	0.14	0.42	0.19	NS	21.24	NS	4.27	12.52	4.38	12.87

NS- Non-Significance, CD- Critical Difference, SEm±- Mean Standard Error, cm- Centimeter, As- Arsenic

Table 2 Effect of different arsenic concentrations on germination attributes of green gram at seven days after germination

Arsenic treatments \ Parameters	Germination %		Radical length (cm)		Plumule length (cm)		Seedling vigour index		Phytotoxicity index (%)		Metal tolerance index (%)	
	HUM1	HUM16	HUM1	HUM16	HUM1	HUM16	HUM1	HUM16	HUM1	HUM16	HUM1	HUM16
T_1 (0 µM As)	95.83	95.83	3.69	4.47	24.11	24.52	353.61	428.36	0.00	0.00	100.00	100.00
T_2 (0.5 µM As)	91.67	91.67	3.63	3.83	23.97	24.35	332.76	351.09	1.62	14.31	98.37	85.68
T_3 (1 µM As)	87.50	91.67	3.58	3.75	22.52	23.77	313.25	343.76	2.98	16.11	97.01	83.89
T_4 (5 µM As)	83.33	87.50	3.47	3.13	20.06	23.28	289.15	273.87	5.96	29.97	94.03	70.02
T_5 (10 µM As)	79.17	83.33	3.18	3.20	20.99	18.56	251.76	264.99	13.82	28.41	86.17	71.58
T_6 (20 µM As)	75.00	95.83	2.49	3.12	19.09	16.50	186.75	298.99	29.52	30.20	67.47	69.79
Mean	85.42	88.89	3.34	3.58	21.79	21.83	285.30	318.22	9.48	19.91	90.51	80.09
	SEm±	CD at 5%	SEm±	CD at 5%	SEm±	CD at 5%	SEm±	CD at 5%	SEm±	CD at 5%	SEm±	CD at 5%
Treatments (T)	2.95	8.60	0.089	0.26	0.51	1.49	16.29	47.56	2.14	6.25	2.15	6.27
Varieties (V)	1.70	4.96	0.052	0.15	0.30	0.86	9.41	27.46	1.24	3.61	1.24	3.62
Interaction (T × V)	4.17	NS	0.13	0.37	0.72	1.33	22.45	NS	3.03	8.89	3.04	8.92

NS- Non-Significance, CD- Critical Difference, SEm±- Mean Standard Error, cm- Centimeter, As- Arsenic

Biological systems have been adversely affected by heavy metals as reported by various authors. In our experiment, per cent phytotoxicity was not severe in HUM- 1 upto sodium arsenate concentration of 10µM but HUM-16 showed greater per cent phytotoxicity beyond 5µM concentration. Similar to our results [10] reported that phytotoxicity of shoot and root was decreased at lower concentration (0.5mg/L) and increased at higher concentration (20 mg /L) of arsenic. Phytotoxicity index shows significant difference among the treatments at 3days as well as 7 days after sowing. There was significant difference observed between the two genotypes in relation to the arsenic treatments in both HUM-1 and HUM-16. Highest phytotoxicity index was found in treatment T6 followed by T_5 for both HUM-1 and HUM-16 at 3rd as well as 7th day after germination. There was gradual increase in phytotoxicity index observed with increasing arsenic concentration. The treatment T2 and the control showed the least phytotoxicity index in both the genotypes on 3rd as well as 7th day after germination.

Significant interaction observed among the genotypes and the treatments at 7 days after germination. However, there was no significant interaction observed on 3 days after germination. There was 27.4% and 33.6% increase in phytotoxicity index found in HUM-1 and HUM-16 respectively on 3th day of germination. Likewise, 29.5% and 30.2% increase in phytotoxicity index found on 7th day after germination in HUM-1 and HUM-16 respectively. The analysis of variance for the varieties as well as treatments was significant. The interaction between treatment and genotype was also significant.

The result on metal tolerance index was just opposite to per cent phytotoxicity. HUM -1 showed greater metal tolerance index than HUM-16 at the same arsenic concentrations except at 20µM arsenic, where both genotypes showed equal decrease in metal tolerance index. Tolerance index decreases in *Triticum aestivum* and *Albizia procera* due to increasing arsenic concentrations has also been reported by [16]. In the present study, there was decrease in seedling dry weight of mung bean seedlings and the highest decrease was recorded at 20µM arsenic. The HUM-1 genotype showed higher seedling dry weight as compared to the HUM-16 under arsenic treatment. The decrease in seedling dry weight could be due to decrease in overall growth of mung bean seedlings such as germination per cent, radical length and plumule length. [11] also reported that germination and early seedling growth can also decrease significantly with increasing concentrations of arsenic in rice. They observed that the growth of the whole plant was hampered and the plant biomass decreased finally. Their study also showed similar result in case of chickpea seedlings.

CONCLUSION

With increasing population, there is increasing demand for pulse production. Increasing pulse production needs a thorough study on the factors deteriorating the normal growth and development of the crop plants. Knowledge on the effect of heavy metal concentration in the soil to the growing crops and the finding out the tolerant genotypes give an idea for recommendation of specific variety to a specific land or area. From the experiment it is concluded that arsenic toxicity in a minute concentration has an adverse effect on different germination attributes like germination percentage, radical and plumule length, seedling vigour index, phytotoxicity index, metal tolerance index etc. of mung bean genotypes. HUM16 genotype showed more phytotoxicty index than HUM1 genotype. In contrast, HUM1 genotype has higher metal tolerance index than HUM16 genotype. So, it is suggested that growing HUM1 genotype over HUM 16 genotype in arsenic prone area would be better as toxic effect due to arsenic is comparatively less on the former one.

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