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Estimation of Tolerance and Toxic Limits of Sodium Arsenate in *Hordeum vulgare* (Barley) Seedlings

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ABSTRACT

Heavy metal pollution, primarily driven by escalating human and industrial activities, poses a significant threat to ecosystems. Arsenic (As), a prominent toxic metalloid, is a major environmental contaminant with detrimental effects on plant health and crop productivity. In this study, the tolerance and toxic thresholds of sodium arsenate were assessed in four accessions of Hordeum vulgare (barley). A range of thirteen molar concentrations (10^{-13} M to 10^{-1} M) of sodium arsenate was used to treat seven-day-old barley seedlings. Radicle length was measured as the primary parameter to determine arsenic toxicity and tolerance levels. Results indicate that 10^{-3} M concentration marks the threshold nearing toxicity, while lower concentrations allowed radicle growth, revealing varying degrees of tolerance among barley accessions. This study provides critical insights for arsenic phytotoxicity and offers a foundation for identifying tolerant barley varieties for cultivation in arsenic-affected regions.

Keywords- Arsenic, Barley, *Hordeum vulgare*, Heavy metal toxicity, Sodium arsenate, Tolerance limit, Radicle length.

I. INTRODUCTION

Arsenic (As), a naturally occurring metalloid, has become a major environmental pollutant due to both geogenic processes and anthropogenic activities such as mining, use of arsenical pesticides, and industrial discharge (Mandal & Suzuki, 2002; Smedley & Kinniburgh,2002; Genchi et al., 2022,; Patel et al., 2023). As an element with properties intermediate between metals and non-metals, it ranks as the 20th most abundant trace element in the Earth's crust, 14th in seawater and 12th in the human body Jomova, et al. 2011). Arsenic commonly exists in four oxidation states: arsenate (As^{5+}), arsenite (As^{3+}), elemental arsenic (As^{0}), and arsine (As^{3-}). Among these forms, arsenate (As^{5+}) is considered the most stable (Sharma and Sohn, 2009).

Arsenic (As) contamination in soil and water represents a serious global environmental and public health issue, particularly in agricultural regions relying on groundwater for irrigation (Naujokas et al., 2013; Bhattacharya et al., 2014).). Once released into the environment, arsenic easily contaminates soil and water, posing serious threats to both ecological systems and agricultural productivity (Zhao et al., 2009). Its solubility and mobility in the environment are largely influenced by pH and the surrounding ionic conditions. Among various arsenic species, sodium arsenate [$Na_2HAsO_4 \cdot 7H_2O$] is a highly soluble and bioavailable form of arsenate (As^{5+}), which poses a significant threat to crop productivity and food safety. In agricultural systems, arsenate (As^{5+}), the dominant form under aerobic conditions, competes with phosphate for uptake due to its structural similarity, leading to disruptions in phosphate metabolism and plant development (Meharg & Hartley-Whitaker, 2002; Finnegan & Chen, 2012; Sinha et al. 2023). Exposure to arsenic adversely affects plant physiological and biochemical processes. In barley (Hordeum vulgare L.), arsenic exposure has been shown to inhibit seed germination, reduce root and shoot elongation, and impair photosynthetic efficiency (Sanal et al., 2014)

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II. MATERIALS AND METHODS

2.1 Plant Material

Four genetically diverse accessions of Hordeum vulgare were selected for the experiment. Certified seeds were procured from agricultural research stations. The three accessions B.G. 105, HBL-87, were procured from Directorate of Wheat Research (DWR) Karnal and accession IC 108098 was procured from National Bureau of Genetic Resources.

2.2 Preparation of Arsenic Treatments

Sodium arsenate ($Na_2HAsO_4 \cdot 7H_2O$) was used as the arsenic source. Thirteen molar concentrations (10^{-13} M to 10^{-1} M) were prepared in distilled water to assess both tolerance and toxic thresholds.

2.3 Experimental Design

Seeds were surface sterilized using 0.1% $HgCl_2$ solution for two minutes and washed thoroughly with distilled water. Sterilized seeds were placed in Petri dishes lined with moistened filter paper. Each dish received one of the thirteen arsenic concentrations, with three replicates per treatment. Both the control and treated sets were raised in Calton's Seed Germinator in total darkness at 20°C.

2.4 Data Collection

After seven days, radicle lengths of the seedlings were measured. Mean radicle length was calculated for each accession and treatment concentration. The concentration at which radicle growth began to decline significantly was considered the toxic limit, while the highest concentration supporting normal growth was regarded as the tolerance limit. A parameter called Response Cofficient (RC) was calculated using the following formula for estimating the toxicity imposed by As+5

RC= VT-VC/ VC

VT= (value of treated set; VC= value of control set)

The negative values for RCs indicated inhibition while the positive values indicated stimulation.

III. RESULTS AND DISCUSSION

The results indicated that barley seedlings exhibited differential sensitivity to sodium arsenate concentrations. Data related to lengths of roots in control and treated sets and their corresponding RCs for treated sets are given in table 2.1 and table 2.2. Response coefficients for root length in various treated sets are also represented diagrammatically in figure 2.1. At extremely low concentrations (10^{-13} to 10^{-9} M), radicle growth was comparable to the control, indicating no toxic effect. However, beginning at 10^{-6} M, a gradual reduction in radicle length was observed, suggesting the onset of arsenic stress. A marked decline in radicle elongation was evident at 10^{-3} M concentration, indicating proximity to the toxic limit. Molar concentration 10^{-2} M and 10^{-1}M were lethal for seedling.

Table 2.1: Effect of sodium arsenate on radicle length (mean ± SE), range, and response coefficient (RC%) in *Hordeum vulgare*

	B.G. 105			HBL-87		
TREATMENT	Mean ± SE	Range	RC	Mean ± SE	Range	RC
	5.16 ± 0.13	4.30 - 6.00		5.30± 0.14	4.30-6.30	
10^{-13}	12.09 ± 0.18	11.00 - 14.00	1.34	9.63 ± 0.10	8.80-10.50	0.84
10^{-12}	11.66± 0.17	10.00 - 12.60	1.26	9.66 ± **0.11**	9.00-10.80	0.82
10^{-11}	10.47 ± 0.50	10.80 - 12.60	1.03	9.32 ± 0.19	8.60-10.00	0.76
10^{-10}	11.49 ± 0.15	10.00 - 12.69	1.23	8.03 ± 0.14	7.20-9.20	0.52
10^{-9}	11.57 ± 0.17	10.20-12.60	1.24	7.88± 0.18	6.20-9.60	0.49
10^{-8}	11.06 ± 0.12	9.80 -11.80	1.14	9.31 ± 0.13	3.00-5.30	0.19
10^{-7}	10.81 ± 0.14	9.80 -12.00	1.09	5.81 ± 0.14	4.80-6.90	0.1
10^{-6}	10.65 ± 0.12	10.00 -11.70	1.07	6.35 ± 0.11	5.20-7.50	0.2
10^{-5}	6.37 ± 0.16	5.20 -7.70	0.24	7.06 ± 0.11	6.00-8.20	0.33
10^{-4}	5.73 ± 0.12	5.00-6.90	0.11	6.87 ± 0.14	6.00-8.20	0.3
10^{-3}	2.56 ± 0.10	1.80-3.20	-0.5	1.52± 0,06	1.00-2.00	-0.71
10^{-2}	0.00 ± 0.00	0.00-0.00	-1	0.00 ± 0.00	0.00-0.00	-1
10^{-1}	0.00-0.00	0.00-0.00	-1	0.00 ± 0.00	0.00-0.00	-1
	K-144			IC 108098		
	5.26 ± 0.13	4.30-6.30		7.43± 0.14	6.00-8.50	
10^{-13}	10.98 ± 0.12	8.20-9.80	1.09	9.21± 0.10	9.00-11.80	0.94
10^{-12}	10.99 ± 0.13	7.00-9.10	0.94	12.31± 0.19	11.40-14.40	0.78
10^{-11}	8.03 ± 0.13	9.80-11.90	0.53	8.66 ± 0.16	8.30-9.80	0.73
10^{-10}	7.83 ± 0.10	7.00-8.60	0.49	10.44 ± 0.13	8.10-10.00	0.73

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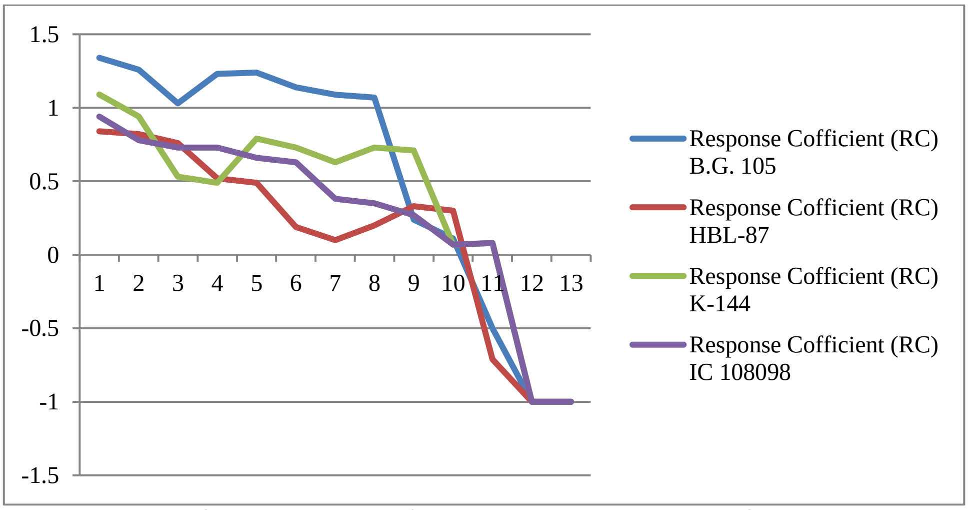
10^{-9}	9.42 ± **0.13**	8.40-10.30	0.79	12.35 ± 0.15	11.00-13.50	0.66
10^{-8}	9.08 ± 0.13	8.10-10.00	0.73	8.81 ± 0.13	8.00-9.40	0.63
10^{-7}	8.59 ± 0.09	8.00-9.40	0.63	10.25± 0.12	9.10-11.20	0.38
10^{-6}	9.11 ± 0.09	8.30-9.80	0.73	10.03 ± 0.12	9.00-11.00	0.35
10^{-5}	10.22 ± 0.18	9.00-11.80	0.71	9.47± **0.17**	8.40-10.30	0.27
10^{-4}	5.52 ± 0.14	4.60-6.80	0.07	4.36 ± 0.07	4.60-6.80	0.07
10^{-3}	5.68 ± 0.13	4.90-6.80	0.08	2.34± 0.11	4.90-6.80	0.08
10^{-2}	0.00 ± 0.00	0.00-0.00	-1	0.00 ± 0.00	0.00-0.00	-1
10^{-1}	0.00 ± 0.00	0.00-0.00	-1	0.00 ± 0.00	0.00-0.00	-1

Table 2.2: Effect of different concentration of sodium arsenate on the redicle length expressed as Response Coefficient (RC)

	Response Cofficient (RC)			
ACCESSIONS	B.G. 105	HBL-87	K-144	IC 108098
10^{-13}	1.34	0.84	1.09	0.94
10^{-12}	1.26	0.82	0.94	0.78
10^{-11}	1.03	0.76	0.53	0.73
10^{-10}	1.23	0.52	0.49	0.73
10^{-9}	1.24	0.49	0.79	0.66
10^{-8}	1.14	0.19	0.73	0.63
10^{-7}	1.09	0.1	0.63	0.38
10^{-6}	1.07	0.2	0.73	0.35
10^{-5}	0.24	0.33	0.71	0.27
10^{-4}	0.11	0.3	0.07	0.07
10^{-3}	-0.5	-0.71	0.08	0.08
10^{-2}	-1	-1	-1	-1
10^{-1}	-1	-1	-1	-1



13=10^{-13}M, 11=10^{-11},9=10^{-9}M, 7=10^{-7}M, 5=10^{-5}M, 3=10^{-3} 1=10^{-1}M

Figure 2.1 Effect of different concentration of sodium arsenate on the redicle length expressed as Response Cofficient (RC)

The data demonstrate that sodium arsenate has a pronounced phytotoxic effect on barley radicle growth,even at low concentrations. The study reveals a sharp decline in radicle elongation beyond the 10^{-5} M sodium arsenate concentration. At higher concentrations (10^{-3} M and above), the toxic effects were pronounced, ultimately halting growth at 10^{-1} M. Reduction of both root and shoot length is a typical of response to toxic metals (Kabata and Pendias 1984). Reduced root length growth in response to arsenic exposure has been reported

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by a number of investigators [Whitaker et al. 2001; Sneller et al. 2000). Reduction of root length growth with increasing concentration of arsenic is due to the fact that plant roots were the first point of contact for these toxic arsenic species in the nutrient media. Several author reported that the inhibition of root length caused by heavy metals may be due to metal interference with cell division, together with inducement of chromosomal aberrations and irregular mitosis (Jiang et al 2001, Liu, et al 2003) , which can be effected on seedling growth (Samantaray et al. 1999)in a study by means of chromite mine pollute soil in five mung bean cultivars, noted that root growth was significantly affected 28th days after root emergence as seedling are more sensitive than seed germination for measurement of the toxic effect of chromium pollution. Arsenate toxicity can interfere with ATP production, disrupt phosphate metabolism, and generate reactive oxygen species (Meharg & Hartley-Whitaker, 2002; Finnegan & Chen, 2012), all of which may lead to impaired cell division and elongation in the root meristem. These findings align with previous studies that report growth inhibition in cereals due to arsenic exposure, primarily through interference in nutrient uptake, oxidative stress, and metabolic dysfunction.

IV. CONCLUSION

This study demonstrates that sodium arsenate exerts concentration-dependent phytotoxic effects on barley seedlings. A concentration of 10^{-3} M is identified as approaching the toxic limit for most accessions, making it a critical threshold for future tolerance screening. Variability among accessions indicates the potential for selecting arsenic-tolerant genotypes, which can be used for cultivation in contaminated areas or further breeding programs.

Further research involving physiological, biochemical, and molecular analyses is recommended to elucidate the tolerance mechanisms and enhance phytoremediation strategies using barley.

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