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Full Length Research Article

Effect of Arsenic on Germination and Early Seedling Growth in Rice (*Oryza sativa* L.)

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Abstract

Arsenic (As), a ubiquitous metalloid and potential carcinogen. Arsenic sensitivity and toxicity to plants are influenced by not only the concentration and the toxicant types, but also by the life-stage or biological process (germination, seedling survival, vegetative growth). The morphological changes in germinating rice seedlings were investigated under arsenic stress. The objective of the study was to evaluate the germination performance and initial growth habits of rice genotype HUR-105 in response to the different As concentration in the growing solution. Therefore, the effects of As (0, 20, 50, 100, 150, 200 µM) on germination percentage, α-amylase activity, shoot and root length, fresh and dry weight of root and shoot, leaf number, leaf area and their percentage reduction were determined. A noticeable decrease in germination percentage, shoot, and root elongation as well as plant biomass was observed with arsenic treatments, as compared to control. From the results obtained in the present study, it can be concluded that rice genotype HUR-105 is sensitive to As stress.

***Key words*:** *Oryza sativa* L., Arsenic, Germination percentage, α-amylase activity seedling growth

Arsenic (As) toxicity has been known for centuries, and has recently received increased attention because of its chronic and epidemic effects on human health. As can be present in the terrestrial, marine, and freshwater environments in various chemical forms. Organic arsenic species are less toxic than inorganic species to aquatic plants, animals and humans, and this has been presumed to be true for terrestrial plants also [1]. There is a general agreement that arsenic contamination in the groundwater of south and southeast Asia has resulted due to release of arsenic from solid phases under anaerobic conditions [2]. Widespread use of arsenic contaminated groundwater for irrigation in rice field elevates its concentration in surface soil and eventually into rice plants and grains [3]. Numerous studies have been carried out in relation to uptake and translocation of arsenic from soil to plants [4].

Plants normally take up arsenic predominantly in trivalent (AsIII) and pentavalent (AsV) forms, which are known to interfere with various metabolic pathways in cells like, interaction with sulfhydryl groups and replacement of phosphate from ATP. Hence, plants not tolerant to arsenic show toxic patterns such as decrease in plant growth and crop yield. Heavy metals including metalloid arsenic have been reported to stimulate the formation of free radicals and reactive oxygen species leading to oxidative stress [5]. As exposure on maize (*Zea mays* L) root proteome causes induction of oxidative stress is the main process underlying arsenic toxicity in plants [6].

Paddy rice (*Oryza sativa* L.) is the most widely consumed cereal in Southeast and East Asia [7]. Unfortunately, rice is more prone to arsenic uptake than other cereals such as wheat (*Triticum aestivum*) and barley (*Hordeum vulgare*) [8]. Metal toxicity to plants is influenced by the concentration of metal and the life-stage of plants. Seedling and seed germination stage of plant life is sensitive to environmental factors such as heavy metals pollution [9]. Germination inhibition is among the best-known effects of toxic impact of heavy metals. 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 has become an important index to evaluate plant's heavy metals tolerance. In comparison, seed germination and the early seedling growth are more sensitive to metal pollution because some of the defense mechanisms have not developed; these two stages are important considerations in toxicity assessment [10]. Reactive oxygen species (ROS) are produced as the earliest response to arsenic toxicity in plants, which can affect metabolism via oxidative cell damage [11]. The phytotoxicity of arsenic has been deeply studied, and researches show that the seed germination percentage, shoot length, root length, and biomass of rice were affected by arsenic treatments [10], [12]. As also alters photosynthetic activity by destroying cell membranes through oxidative damage, also resulting in DNA damage, particularly in arsenic (III)

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treatments [13-14]. So, a sustainable method is needed to alleviate the toxicity of arsenic for effective remediation.

In this study the apparent toxic symptoms of arsenite including seed germination, α-amylase activity and early seedling growth, were investigated. The sensitive symptoms, which could be used as indicators for arsenic toxicity, were evaluated.

MATERIALS AND METHODS

Plant materials and growth conditions

Mature rice (*Oryza sativa* L.) seeds of genotype HUR-105 with uniform size were washed and soaked in distilled water for 24 h then surface sterilized with 1% (v/v) sodium hypochlorite (NaOCl) solution for 10 min. Seed germination was tested on moist filter paper in Petri plates (150 mm, Riviera TM) at 30°C in the dark. Fifty seeds were placed in each dish, covered by lid. Sodium arsenite ($NaAsO_2$) was used as source of arsenic and different arsenite concentrations (20µM 50µM, 100µM, 150µM and 200µM) prepared. The treatments studied were expressed as (As concentration (µM): (A_1-As 20, A_2- As 50, A_3- As 100, A_4- As 150 and A_5- As 200) and Control (A_0). To analyze the changes in morphological seedling growth under arsenic treatments 7 days of old seedlings with uniform length of respective treatments, were transferred to PVC cups (12 cm diameter and 11 cm high, forty plants per cup) containing coco peat and grown in modified Hoagland's nutrient solution [15] under different arsenic concentrations. The seedlings were allowed to grow under control environment in growth chamber (Caltan-193, NSW, New Delhi) with photon flux of 400 µM m^{-2} s^{-2} PAR at 14/10 h photoperiodic settings. The temperature was set at 35 °C / 28 °C (day/night) with a relative humidity set point of 70%. The pH of the nutrient solution was adjusted to 5.8. Plants were harvested after 20 days, washed with milli-Q, separated into roots and shoots, blotted and used for the study of various parameters.

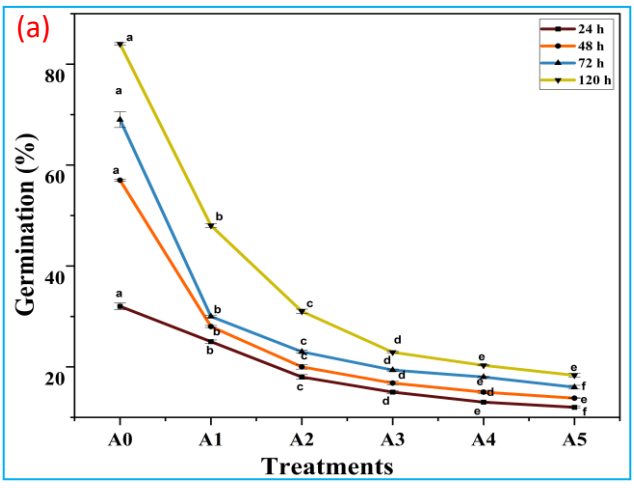
Germination and seedling growth assay

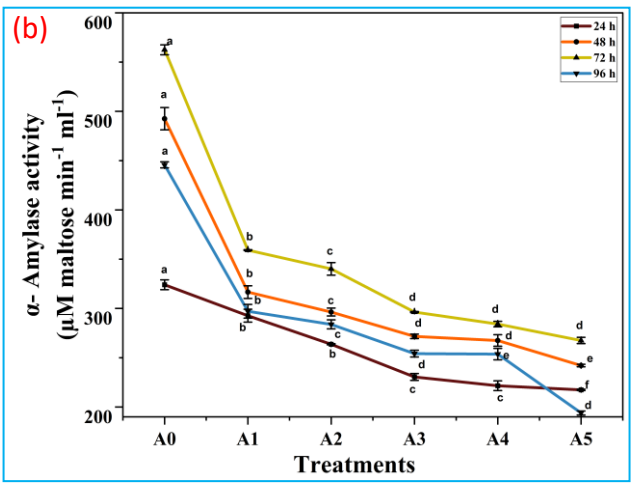
Seed germination test was performed and data was recorded at 24 h (1 day), 48 h (2 days), 72 h (3 days), 5 days, 7 days and 10 days after incubation. Seeds were considered germinated when both the plumule and radicle were extended from their junction and extended up to 2mm. Seedlings were measured for their shoot length (SL) using measurement scale and root length (RL) and root volume (RV) via Epson Biovis Root scanner at every 10 days till 30 days. The fresh weight (FW) of intact seedling (root + shoot) was taken individually, immediately after harvesting and the samples were oven-dried at 70 °C to measure dry weight (DW) by using electronic weighing balance (Sartorius BT-224S). The activity of α-Amylase enzyme was assayed from the saccharifying activity as per the protocol described by [16]. The enzyme activity calculated by the formula given by [17]. The leaf number and leaf area recorded after 10, 20 and 30 days after germination.

RESULTS AND DISCUSSION

Effect of arsenic on germination and growth parameters

Seed germination is one of the most sensitive processes to metal pollution because of lack of defense mechanisms and germination of rice seeds proved to be very sensitive to arsenic contamination and hence is an important consideration while studying effects of arsenic on seedling growth [10]. Rice seeds were exposed to different concentrations of arsenic to determine their effects on germination. Inhibition of seedling growth under arsenic treatment including seed germination, root and shoot length (RL and SL) was observed when compared with untreated seedlings as a preliminary experiment, and found that arsenic was highly toxic to germination of rice seeds. The results are in agreement with [14], [18] who studied the toxic effects of arsenic in rice germination and growth. The germination decreased significantly with the increase in arsenic concentration (Fig 1a). Approximately, 45% to 65% inhibition over control at lower arsenic levels was observed whereas at higher arsenic levels more than 60% and upto 80% inhibition was evidenced in seed germination at 24h, 48h, 72h, 5 d, 7d and 10 d after incubation (Fig 1a). Energy for germination of seeds and for growth of roots and shoots is provided by sugars metabolism and for this purpose α-amylase converts endospermic stored starch into metabolizable sugars which is not adequate in arsenic stress condition [19] attributed to poor cell wall metabolism, inhibited α-amylase activation and poor signaling of ROS and hormones [20]. The α-amylase enzyme activity was measured in terms maltose produced by the α-amylase (µM maltose min^{-1} mL^{-1}). Initially after 24 h the α-amylase activity was 324.01µM maltose min^{-1} mL^{-1} in control and found to be significantly decreased in arsenic treatments with lowest activity observed in 200 µM i.e., 217.35 µM maltose min^{-1} mL^{-1} (Fig 1b). The enzyme activity negatively affected and drastically inhibited with increasing arsenic concentrations. The highest activity was noticed after 72 h of incubation in all treatments. Toxic effects of 150 and 200 µM arsenic at 48 h and 72 h was very prominent as the enzyme activity decreased by almost 51% and 53% respectively when compare with control ($p < 0.01$). Hence the present study suggests that arsenic toxicity greatly inhibits germination in rice seeds by suppressing the activity of α -amylase, considered as a crucial starch hydrolyzing enzyme.





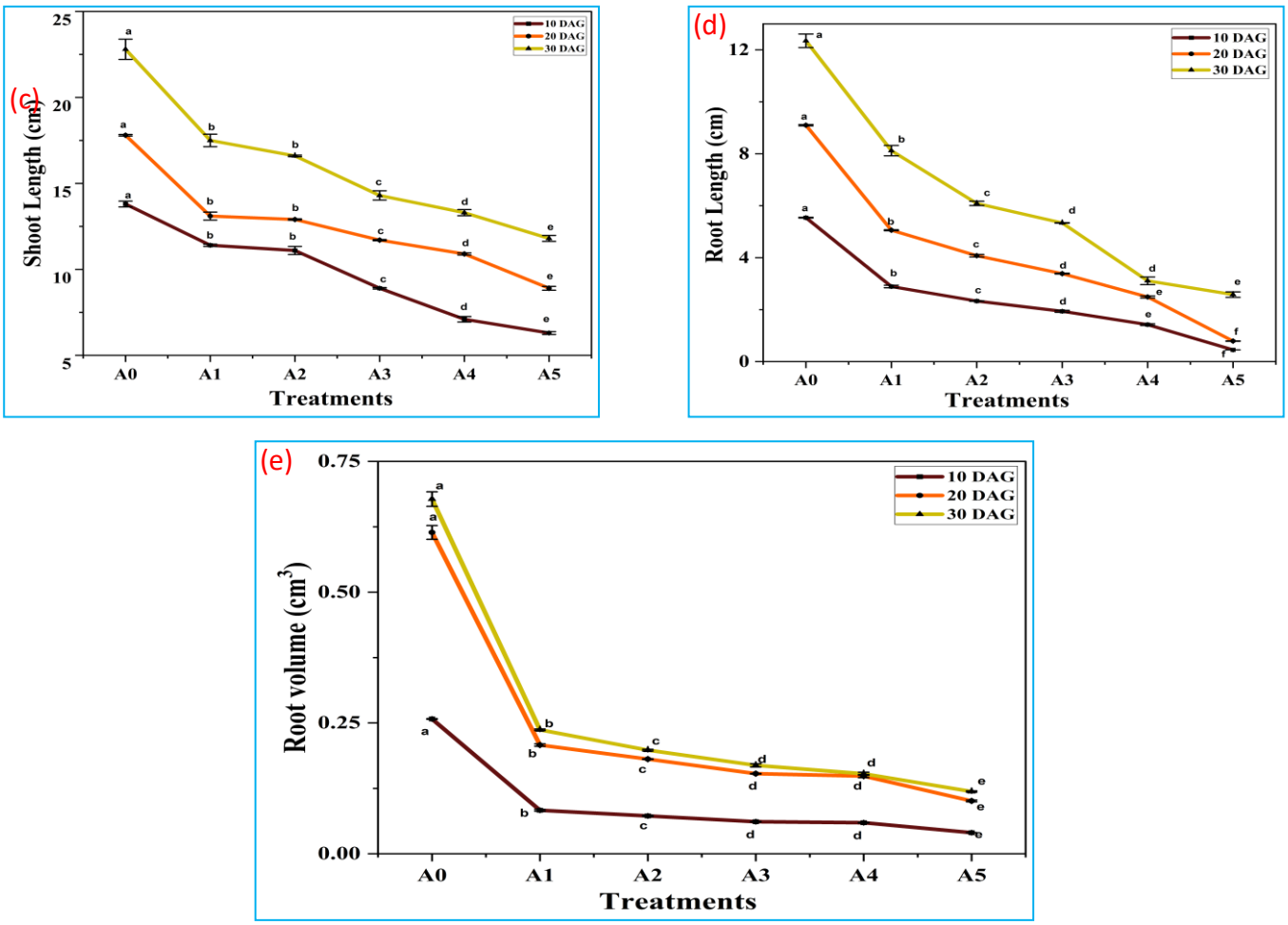


Fig 1 Effect of sodium arsenite ($NaAsO_2$) on (a) Germination percentage, (b) α-amylase activity, at different intervals after sowing and seedling growth including (c) Shoot length (cm) (d) Root length (cm) and (d) Root volume (cm^3) in rice genotype HUR-105 at 10, 20 and 30 days after germination

Table 1 Effect of sodium arsenite ($NaAsO_2$) on fresh weight (g) and dry weight (g) of shoot, root and total seedling of rice genotype HUR-105 at 10, 20 and 30 days after germination

Treatments	Days after germination																	
	10						20						30					
	Shoot		Root		Seedling		Shoot		Root		Seedling		Shoot		Root		Seedling	
	FW	DW	FW	DW	FW	DW	FW	DW	FW	DW	FW	DW	FW	DW	FW	DW	FW	DW
A_0	3.38^a	0.15^a	0.79^a	0.061^a	4.16^a	0.20^a	8.56^a	0.89^a	1.84^a	0.54^a	10.4^a	1.43^a	14.03^a	1.51^a	4.61a	0.74^a	18.67^a	2.25^a
A_1	2.48^b	0.12^b	0.68^b	0.053^b	3.16^b	0.17^b	6.28^b	0.74^b	0.94^b	0.47^b	7.22^b	1.21^b	10.01^b	1.35^b	3.05^b	0.58^b	13.06^b	1.93^b
	(-26)	(-14)	(-14)	(-20)	(-24)	(-16)	(-27)	(-17)	(-49)	(-27)	(-31)	(-15)	(-29)	(-11)	(-34)	(-22)	(-30)	(-14)
A_2	1.49^c	0.10^c	0.61^c	0.051^c	2.11^c	0.15^c	5.91^c	0.68^c	0.84^c	0.41^c	6.75^c	1.09^c	9.45^c	1.24^c	2.85^c	0.52^c	12.32^c	1.76^c
	(-56)	(-27)	(-22)	(-25)	(-49)	(-26)	(-31)	(-24)	(-54)	(-24)	(-35)	(-24)	(-33)	(-18)	(-37)	(-30)	(-34)	(-22)
A_3	0.78^d	0.09^d	0.26^d	0.043^d	1.03^d	0.13^d	4.67^d	0.63^d	0.61^d	0.37^d	5.28^d	1.00^d	9.37^c	1.19^c	2.84^c	0.49^c	12.21^c	1.68^d
	(-77)	(-37)	(-67)	(-29)	(-75)	(-35)	(-45)	(-29)	(-67)	(-31)	(-49)	(-30)	(-33)	(-21)	(-58)	(-34)	(-35)	(-25)
A_4	0.70^e	0.08^e	0.21^e	0.040^e	0.88^e	0.11^e	3.29^e	0.57^e	0.54^e	0.25^e	3.83^e	0.82^e	9.01^c	1.08^d	1.87^d	0.43^d	10.88^d	1.51^e
	(-79)	(-46)	(-73)	(-35)	(-79)	(-42)	(-62)	(-36)	(-71)	(-54)	(-63)	(-43)	(-36)	(-28)	(-59)	(-42)	(-42)	(-33)
A_5	0.53^f	0.05^f	0.19^f	0.031^f	0.74^f	0.09^f	2.97^e	0.48^f	0.41^f	0.18^f	3.38^e	0.66^f	7.86^d	0.89^e	1.52^e	0.38^e	9.38^e	1.27^f
	(-84)	(-60)	(-77)	(-50)	(-82)	(-57)	(-65)	(-46)	(-78)	(-67)	(-68)	(-54)	(-44)	(-41)	(-67)	(-49)	(-50)	(-44)
±SE(d)	0.05	0	0.012	0.001	0.03	0.02	0.106	0.015	0.013	0.01	0.134	0.025	0.233	0.025	0.053	0.015	0.282	0.039
CD at 1%	0.14	0.01	0.036	0.002	0.09	0.04	0.325	0.045	0.04	0.029	0.408	0.077	0.711	0.077	0.161	0.045	0.863	0.119

FW: Fresh weight; DW: Dry weight

*A_0, A_1, A_2, A_3, A_4, and A_5 represent 0, 20, 50, 100, 150 and 200 µM sodium arsenite (NaAsO2) respectively. Duncan's Multiple Range Test (DMRT) post-hoc test was performed at α = 0.01. Mean in each column followed by same letter are not significantly different. Values in the parentheses denotes percent decrease over control

Length, volume and biomass of root and shoot were recorded and found to be significantly affected due to arsenic stress (Fig 1c, d, e). The shoot and root growth in terms of root length, shoot length, root and shoot fresh weight was substantially reduced at lower arsenic levels (As 20 and As 50) as well as at higher As levels (As 100, As 150 and As 200).

Initially after 10 days of germination more than 35% at lower arsenic level and more than 55% inhibition at higher arsenic levels were evidenced in shoot length whereas in root length and root volume, significant reduction of more than 60% and 70% at lower arsenic levels and 90% and 85% reduction at higher arsenic levels was observed respectively when compared to control (Fig 1c-d). At 150 µM and 200 µM concentrations almost complete root inhibition was observed. The roots turned black in higher arsenic levels. Similarly, after 20 and 30 days of germination the shoot and root length along with root volume reduced significantly in all arsenic treatments (Fig 1e). Reduced root growth in response to arsenic exposure associated with inhibited supply of essential ions required for the growth of plant has been reported by a number of investigators in rice [18], [21-22]. The fresh weight and dry weight of root and shoot was affected more in comparison to root and shoot length (Table 1). The shoot fresh weight inhibited by more than 50% at lower arsenic levels and nearly 85% at higher arsenic levels during initial growth period (10 DAG), while at further stages (20 and 30 DAG) the growth inhibition was comparatively less, and showed almost 65% and 45% reduction over control. The inhibition was stronger in roots than in shoots when exposed to arsenic, because the plant roots are the first point of contact for these toxic arsenic species in the nutrient media. The root fresh weight showed greater inhibition compared to shoot. There was nearly 80% inhibition in initial period (10 DAG), while at further stages (20 and 30 DAG) the inhibition was 75% and 67% respectively at higher arsenic levels. The root growth was highly affected under arsenic stress showing typical sensitivity of roots towards arsenic toxicity. At higher arsenic (As 100, As 150 and As 200) concentrations, root dry biomass (70% and 50%) was affected more compared to shoot dry matter (50% and 40%) respectively at 20 and 30 days after germination. With increasing arsenic concentrations, the root growth relatively reduced where higher arsenic accumulation in roots by seedlings compared to shoots might be a reason and causing higher retention of arsenic in roots attributing to its compartmentalization in root vacuoles. At toxic concentrations (As 150 and As 200) ample inhibition in shoot and root dry biomass was observed and explained. Similar findings were obtained by [21]. Regardless of rice varieties, accumulation of arsenic was 28 folds higher in the root than that of the shoot [3].

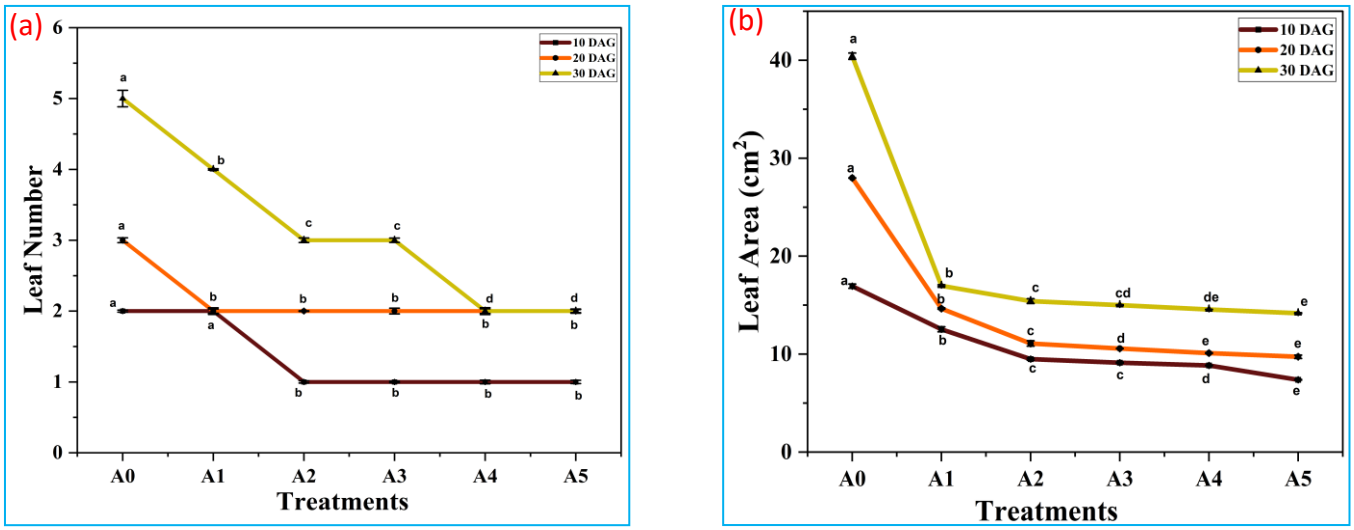


Fig 2 Effect of sodium arsenite ($NaAsO_2$) on (a) leaf number and (b) leaf area (cm^2) of rice genotype HUR-105 at 10, 20 and 30 days after germination

Leaf number was recorded at 10, 20 and 30 days after germination (Fig 2a). After 10 days of germination the seedling was in 2 leaves stage in control and treatment with 20 µM arsenic does not had any significant effect in leaf number but at remaining all other arsenic treatments i.e., 50 µM, 100 µM, 150 µM and 200 µM the leaf number remained 1 and all treatments performed at par ($p < 0.01$) showing no significant difference. After 20 and 30 days of germination leaf number in control increased by 2 and 4 respectively but in all arsenic treatments the number of leaves was less than control. Leaf area was calculated at 10, 20 and 30 days after germination (Fig 2b). Control showed maximum leaf area in comparison to all other treatments. As treatment at 20 µM showed 26%, 48% and 58% decrease over control at 10, 20 and 30 days after germination respectively ($p < 0.01$). At 10 days after sowing treatment 50 µM and 100 µM arsenic were found to be at par and showed an average 45% decrease in leaf area over control while at 20 and 30 days after germination almost 63% reduction over control ($p < 0.01$). After 10 days of germination there was more than 55 % reduction leaf area was observed at toxic arsenic concentrations, whereas after 30 days of germination almost 65% leaf area inhibition was observed over control ($p < 0.01$). Compared to other parameters studied, toxic effect on leaf number is not prominently observed. The present results are in agreement with [22]. At a higher concentration, arsenic interferes with various metabolic processes, adversely affects the plant metabolism, and consequences in death [22]. Early seedling growth of rice decreased significantly with increasing concentrations of arsenic [23].

CONCLUSION

The overall results showed that seeds of rice genotype HUR-105 had negative effect of arsenic treatments. Accelerated arsenic concentration significantly decreased the germination percentage, α-amylase activity and other growth parameters studied. The α-amylase, considered as a crucial starch hydrolyzing enzyme, negatively affected and drastically inhibited with increasing arsenic concentrations. Further, the fresh weight and dry weight of root and shoot was affected more in comparison to root and shoot length. Among all the morphological parameters of rice seedlings under arsenic stress evaluated, the inhibition was stronger in the root than in the shoot. From the results obtained in the present study, it can be concluded that rice genotype HUR-105 is sensitive to arsenic treatment.

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