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Effect of arsenic stress on growth parameters and antioxidant responses (proline and ascorbic acid) in maize cultivars.

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ABSTRACT

Arsenic (As) is a naturally occurring metalloid that poses significant environmental and agricultural challenges due to its widespread contamination in soils and water sources worldwide. Contamination of soil by arsenic arises from both natural geological sources and anthropogenic activities, such as mining, industrial processes, and the extensive use of arsenic-containing pesticides and fertilizers. Among staple crops, maize (*Zea mays* L.) is critically important globally for food security, animal feed, and industrial uses, making the study of arsenic accumulation in maize tissues vital for understanding potential risks to human and animal health. Arsenic uptake by plants is complex, influenced by the arsenic species present in the soil (mainly arsenate (AsV) and arsenite (AsIII)) and the plant's physiological and biochemical mechanisms. The study aimed to explore the inherent genetic variability among the genotypes and their physiological and biochemical adaptations when exposed to arsenic stress at concentrations (0,50, and 100 mg/kg). Conversely, there was a substantial increase in oxidative stress indicators and antioxidant enzyme activities. Specifically, the levels of Proline and hydrogen peroxide (H_2O_2) increased, indicating enhanced lipid peroxidation and oxidative damage. In response to this stress, plants upregulated their antioxidant defense mechanisms, as observed by elevated activities of enzymes such as superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and increased ascorbic acid (ASA) levels. Among the varieties, Pusa HM-9 exhibited the highest tolerance to arsenic stress. This variety showed minimal reduction in growth and chlorophyll levels compared to the others, suggesting better adaptability and efficient stress mitigation mechanisms. The toxic impact of arsenic on maize extends beyond accumulation, adversely affecting plant growth, seed germination, root development, and photosynthesis, while inducing antioxidative responses such as proline and ascorbic acid accumulation. Understanding these biochemical responses is essential for evaluating maize tolerance mechanisms and food safety in arsenic-contaminated environments.

Key Words - Arsenic, Maize, Ascorbic Acid, Proline

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INTRODUCTION

Arsenic (As) is a naturally occurring metalloid that poses significant environmental and agricultural challenges due to its widespread contamination in soils and water sources worldwide. Contamination of soil by arsenic arises from both natural geological sources and anthropogenic activities such as mining, industrial processes, and the extensive use of arsenic-containing pesticides and fertilizers

(Abbas *et al.,* 2018). This contamination has become a serious issue, particularly affecting the safety of food crops grown on arsenic-polluted soils. Among staple crops, maize (*Zea mays* L.) is of critical importance globally for food security, animal feed, and industrial uses, making the study of arsenic accumulation in maize tissues vital for understanding potential risks to human and animal health (Yu *et al.,* 2009).

Arsenic uptake by plants is complex, influenced by the arsenic species present in the soil (mainly arsenate, As(V), and arsenite, As(III)) and the plant's physiological and biochemical mechanisms. Arsenate, chemically similar to phosphate, is taken up primarily through phosphate transporters, whereas arsenite enters via aquaporin channels (Abbas *et al.,* 2018). Once absorbed, arsenic undergoes transformation processes within the plant, resulting in various arsenic species, such as inorganic arsenite, arsenate, and organic methylated forms like monomethylarsenic acid (MMA) and dimethylarsenic acid (DMA) (Yu *et al.,* 2009). This phenomenon, known as arsenic speciation, is crucial because different arsenic species differ dramatically in their toxicity and mobility within the plant and to the consumers of these crops (Mahmood *et al.,* 2024).

In maize, arsenic accumulation is distributed unevenly across different tissues, with leaves, stems, bracts, and kernels showing variable concentrations. Studies have reported that leaves often contain the highest arsenic levels, followed by stems and bracts, with kernels usually showing comparatively lower accumulation, which is relevant for food safety considerations (Zhang *et al.,* 2017). However, even low arsenic levels in edible grain can pose chronic health risks upon prolonged consumption due to arsenic's carcinogenicity, immunotoxicity, and systemic toxicity in humans (FDA, 2024). Therefore, understanding the mechanisms that control the uptake, translocation, and accumulation of arsenic in maize is foundational for developing strategies to mitigate its presence in food products.

The toxic impact of arsenic on maize plants extends beyond accumulation. Arsenic exposure adversely affects plant growth, reducing seed germination rates, stunting root development, interfering with photosynthesis by degrading chlorophyll content, and damaging cellular membranes due to oxidative stress (Gupta, 2022; Singh *et al.,* 2012). These physiological disruptions not only compromise maize yield and quality but also induce biochemical pathways that plants use in response to arsenic stress, including activation of antioxidant enzymes such as superoxide dismutase and catalase, and synthesis of thiol-containing compounds like glutathione and phytochelatins that detoxify arsenic via chelation (Abbas *et al.,* 2018).

Arsenic contamination in agricultural soils poses a significant threat to crop growth and productivity, particularly in maize (*Zea mays* L.), a staple food crop worldwide. Elevated arsenic levels induce oxidative stress in plants, triggering physiological and biochemical alterations that affect plant health. One key response to arsenic stress is the modulation of osmoprotectants such as proline, an amino acid that accumulates to protect cells against oxidative damage by stabilizing proteins and membranes and scavenging reactive oxygen species (ROS) (Adamipour *et al.,* 2025).

Concurrently, ascorbic acid, a vital antioxidant, plays a crucial role in mitigating oxidative stress by detoxifying ROS and maintaining redox homeostasis under metal toxicity. Experimental evidence demonstrates that increasing soil arsenic concentrations (0, 50, 100 mg/kg) leads to a significant rise in proline content in maize, suggesting an adaptive protective mechanism, while ascorbic acid content also shows changes indicative of an activated antioxidative defense system (Adamipour *et al.,* 2025). Understanding these biochemical responses at varying arsenic levels is essential for evaluating maize tolerance mechanisms and developing strategies to enhance crop resilience in arsenic-contaminated environments.

The ecological and health implications of arsenic accumulation in maize are profound, especially in regions with arsenic-contaminated soils used for agriculture. Chronic exposure through dietary intake activates arsenic toxicity in humans, leading to diseases such as arsenicosis, skin lesions, cardiovascular disorders, and an increased risk of various cancers (Bangladesh studies, 2025). The presence of arsenic in maize grain thus presents a direct food safety concern, necessitating rigorous assessment of arsenic levels in soil and crops, and evaluation of the speciation of arsenic compounds in edible tissues to accurately assess toxicological risk (Yu *et al.,* 2009).

Research efforts have increasingly focused on genetic and agronomic approaches to address arsenic contamination in maize. Quantitative trait loci (QTL) mapping has identified genetic regions associated with arsenic accumulation in maize tissues, pointing toward the potential for breeding or engineering arsenic-tolerant varieties that limit arsenic uptake or promote detoxification (Zhang *et al.,* 2016). Additionally, biotic interventions such as the inoculation with arbuscular mycorrhizal fungi have demonstrated effects on arsenic uptake and speciation, reducing the more toxic arsenite accumulation in shoots while enhancing organic arsenic forms that may be less harmful (Yu *et al.,* 2009). These insights contribute to sustainable agricultural practices that aim to reduce arsenic bioavailability and translocation within maize plants, thereby enhancing food safety.

The speciation of arsenic within maize tissues is equally important for food safety evaluations. Inorganic arsenic species, particularly As(III) and As(V), are more toxic compared to their methylated organic counterparts. Methylation processes within plants and soil microbes can transform arsenic into MMA and DMA, which are generally less toxic but can still pose health concerns depending on concentration and exposure duration (Mahmood *et al.,* 2024). Therefore, speciation analyses a detailed profiling of arsenic chemical forms are integral components of risk assessments and regulatory standards for arsenic in food products.

METHODOLOGY

Plant Material and Growth Conditions

Two Maize (*Zea mays* L.) varieties, specifically the widely grown variety DMH-117, PUSA HM-9, were brought from Bihar Krishi Bhawan, used for the study. Seeds were grown in pots containing 1 kg of soil per pot mixed with river sand in a 3:1 ratio, air-dried, and sieved through a 2 mm mesh. The soil was collected from the uppermost ploughed layer (0-20 cm) of a test field and characterized for organic matter, hydrolyzable nitrogen, available phosphorus, potassium, and pH (Ci *et al.,* 2012).

Arsenic Treatment

Soil arsenic contamination was simulated by amending soil or hiking soil with sodium arsenate ($Na_3AsO_4 \cdot 12H_2O$) to achieve arsenic concentrations of 0, 50, and 100 mg/kg soil (Ci *et al.,* 2012). Each treatment had three replicates, and a control without arsenic was included. The water-holding capacity was maintained between 70-80% to avoid leaching. Fertilization was done with ammonium dihydrogen phosphate (5.5 g $NH_4H_2PO_3$ per pot).

Plant Sampling and Preparation

At physiological maturity, plants were harvested and separated into roots, stalk, leaves, sheath, tassel, cob, bract, and kernel. Roots were gently washed to remove soil particles. Plant tissues were killed by heating at 105°C for 30 minutes and then dried at 65°C until constant weight. Dry tissues were pulverized and sieved to less than 250 µm for analysis. Soil samples were also collected, air-dried, and sieved to less than 150 µm (Ci *et al.,* 2012).

Growth and Yield Measurements

Plant growth, like root height and shoot height, was measured using from scale. Yield parameters included ear length, number of rows per ear, kernels per row, ear diameter, kernel weight, and grain yield per plant (Ci *et al.,* 2012).

Proline Estimation

Fresh plant tissue (usually 0.5 g to 1 g) is homogenized in 3-5 mL of 3% sulphosalicylic acid to extract free proline. The homogenate is centrifuged to obtain a clear supernatant.1 mL of the supernatant, 2 mL of acid ninhydrin reagent, and 2 mL of glacial acetic acid are added. The mixture is incubated at 100°C for 60 minutes in a water bath. After cooling in an ice bath, 4 mL of toluene is added, and the mixture is vortexed; the chromophore-containing toluene (upper layer) is separated. The absorbance of the toluene layer is read at 520 nm using a spectrophotometer, using toluene as a blank. Proline concentration is calculated from a standard curve prepared with known concentrations of proline and expressed on a fresh weight basis (Bates, 1973).

Ascorbic Acid

Ascorbic acid was extracted from 100 mg of leaf tissue using 6% trichloroacetic acid, according to Oser's (1979) method. One drop of 10% thiourea solution (in 70% ethanol) was added after four millilitres of the extract were combined with two millilitres of 2% dinitrophenyl hydrazine. After boiling the mixture for 15 minutes in water bath and allowing it to cool to room temperature, 5 millilitres of 80% (v/v) H_2SO_4 were added at 0°C. In a Spectrochem spectrophotometer, the absorbance of the hydrazone complex solution was measured at 530 nm.

RESULT & DISCUSSION

The presented Figure 1A and 1B compare the effects of varying treatments (control, 50 mg/kg, and 100 mg/kg) on root and shoot lengths in two maize varieties, DHM-117 and PUSA HM-9. Results indicate that both root and shoot lengths decrease as treatment concentration increases, but the extent of reduction and varietal response differ notably.

Root Length Comparison

For root length, the control groups in both varieties show the highest values, with PUSA HM-9 exhibiting a longer root system than DHM-117 under all treatments. Specifically, PUSA HM-9 control plants have roots around 25 cm, while DHM-117 controls are approximately 17 cm. When exposed to 50 mg/kg and 100 mg/kg, root lengths decrease in both varieties, but the reduction is more severe in DHM-117. At 100 mg/kg, DHM-117 roots drop to about 12 cm, compared to PUSA HM-9, which decreases to roughly 22 cm. This suggests that PUSA HM-9 is more tolerant to the imposed stress, maintaining relatively higher root lengths at increasing treatment levels.

Shoot Length Comparison

Similarly, the shoot length data demonstrate that PUSA HM-9 consistently outperforms DHM-117. Under control conditions, both varieties have comparable shoot lengths (approximately 34 cm). However, as treatment concentrations increase, the decline in shoot length is more pronounced in DHM-117. At 100 mg/kg, DHM-117 shoots measure about 20 cm; whereas PUSA HM-9 shoots remain near 29 cm, reflecting greater resilience.

The data collectively reveal that PUSA HM-9 maintains higher growth (both root and shoot) under stress compared to DHM-117. The relative decline in both traits is steeper in DHM-117, highlighting its greater sensitivity. This variation might be attributed to intrinsic genetic differences regarding stress adaptation, as previously discussed in recent maize stress physiology research.

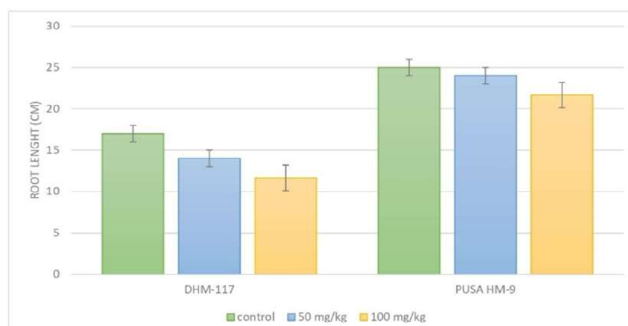

Fig.1A

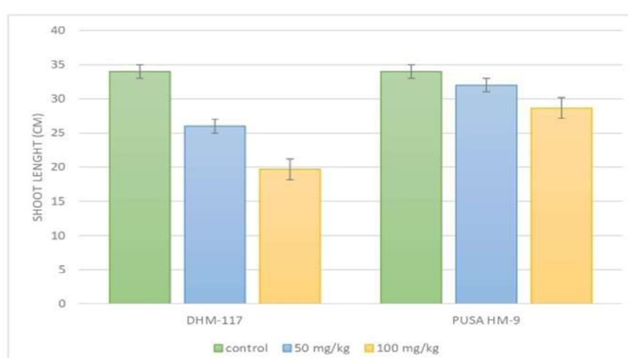

Fig.1B

Ascorbic Acid

The Fig.2 illustrates the ascorbic acid content (mg g^{-1} F. WT) in two maize varieties, DHM-117 and PUSA HM-9, under three conditions: control, 50 mg/kg, and 100 mg/kg treatments.

Under the control condition, both DHM-117 and PUSA HM-9 exhibit comparable ascorbic acid levels, approximately 20 mg/g F. WT. The error bars suggest a small standard deviation, indicating consistency within the measurements for both varieties. The results highlight both similarities and differences between DHM-117 and PUSA HM-9. While both varieties respond positively to increased treatment doses, DHM-117 displays a notably higher capacity for ascorbic acid accumulation at the highest administered concentration. These findings support the idea that DHM-117 could be more suitable for environments or agricultural practices aiming for maximized ascorbic acid content, especially under stress or supplementation regimes. Such differences may be attributable to genetic and physiological variation between the varieties, warranting further research into their respective stress-response pathways and metabolic networks.

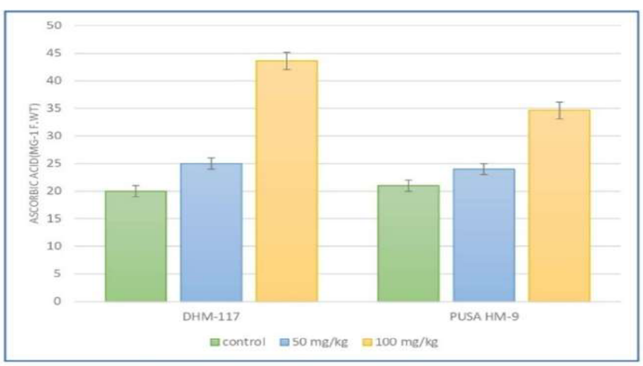

Fig. 2

Proline

The provided Fig.3 illustrates the impact of arsenic stress (at concentrations of 50 mg/kg and 100 mg/kg) on proline accumulation in two maize cultivars, DHM-117 and PUSA HM-9, compared to unstressed control conditions. There is a clear trend of increasing proline content with rising arsenic concentrations in both cultivars, though the absolute values differ slightly.

Proline is a well-known osmoprotectant that typically accumulates in plant tissues under abiotic stress conditions, including heavy metal toxicity. In this graph, both cultivars demonstrate a significant elevation in proline levels as the arsenic concentration in the soil increases. For DHM-117, proline levels progress from approximately 1.5 (control) to about 8 (50 mg/kg) and peak around 17 (100 mg/kg). Similarly, for PUSA HM-9, the values increase from about 2 (control) to 7 (50 mg/kg), reaching roughly 15 (100 mg/kg). Both maize cultivars DHM-117 and PUSA HM-9 exhibit the same qualitative response, with proline content rising sharply under arsenic stress. However, at both stress levels, DHM-117 shows a slightly higher proline accumulation compared to PUSA HM-9, indicating genotype-dependent metabolic adaptation. This suggests that DHM-117 may possess slightly greater tolerance to arsenic-induced oxidative stress or a more robust mechanism for osmotic adjustment, commonly linked with proline biosynthesis pathways in maize and other crops.

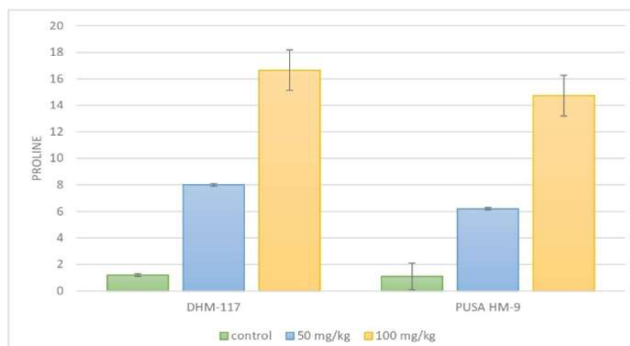

Fig. 3

CONCLUSION

The present study shows significant impacts on maize growth and physiology, with clear genotype-dependent differences in arsenic tolerance. Pusa HM-9 demonstrated greater tolerance to arsenic stress, maintaining higher growth and chlorophyll levels, while both cultivars showed increased proline and ascorbic acid in response to arsenic exposure, indicating activation of protective antioxidant mechanisms. The genotypic variability found suggests scope for breeding or selecting maize varieties with enhanced arsenic tolerance, crucial for food safety in contaminated soils. Furthermore, speciation analysis emphasized the importance of differentiating between arsenic forms due to varying toxicity, underlining the need for careful risk assessment and mitigation strategies for maize cultivation in arsenic-affected regions. The findings stress the necessity for continued research on physiological and biochemical maize responses, informed variety selection, and targeted agronomic practices to minimize arsenic health risks in agricultural products.

Biological and Food Safety Implications

Elevated proline levels generally reflect increased metabolic stress, which is consistent with prior reports on plants exposed to arsenic contamination. Higher proline can help scavenge free radicals and stabilize cellular structures, supporting plant survival under adverse conditions. These results align with literature showing similar increases in proline under heavy metal stress in maize, rice, and other major crops. Such metabolic adjustments are critical for maintaining growth and development when exposed to environmental pollutants, though sustained arsenic exposure poses a risk for food safety due to potential residue accumulation in edible tissues.

Statistical Analysis

Statistical analyses of the data were performed with GraphPad Prism 5.0 (GraphPad Software, Inc.) for a complete randomized block design. The experiments were performed in triplicate (n = 3). Data presented as mean ± standard error (SE).

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CONFLICT OF INTEREST

The authors declare no conflict of interest

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