The Biobrio ISSN : 2393-9508 e-ISSN: 2582-4902
An International Quarterly Journal of Life Sciences
Website: www.thebiobrio.in SJIF 2024:8.259

12(1&2): 1364-1372, 2025

Germination and Physiological responses of *Zea mays* L. to arsenite stress and its possible amelioration by Salicylic acid

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Received : 01st June, 2025 ; Accepted : 29th June, 2025
DOI:- https://doi.org/10.5281/zenodo.17264364

ABSTRACT

The present study investigates the effect of arsenic [As (III)] stress and its mitigation on germination, morphology and physiology of maize (*Zea mays*) plants through petriplate experiments. Maize seeds were treated with different concentrations (0, 25, 50 and 100 µM) of sodium arsenite. Increasing accumulation of arsenic in the water and soil leads to heavy losses in agricultural productivity. Salicylic acid, a phytohormone, has been found to be quite effective in combating the arsenic toxicity in the plants. In the current study, 5 µM of salicylic acid dose was used to study its mitigating effect on arsenite stress. Arsenite stress inhibited the growth of plants through reduction of root length, shoot length, and fresh weight in a dose-dependent manner. Highest dose of arsenite greatly decreased the pigment and protein level in the plants. Salicylic acid application reverted the stress toxicity in the plants by improving plant growth and also increased chlorophyll and protein content in the leaves.

Key Words - arsenite, salicylic acid, maize, mitigation, abiotic stress

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INTRODUCTION

Arsenic (As), a potentially poisonous metalloid and a group I carcinogen, occurs primarily in inorganic forms as arsenite (AsIII) and arsenate (AsV) (Rathinasabapathi, *et al.,* 2006; Meharg & Hartley-Whitaker, 2002). In recent years, various human activities such as prolonged use of arsenic-based pesticides, fuel use, mining and so on have culminated in arsenic contamination in numerous countries (Lee, *et al.,* 2008; Heikens, *et al.,* 2007). Arsenic level has been found to exceed the WHO safe limit of 0.05 mg L^{-1} in China, Bangladesh, Argentina, Mexico, Chile, Vietnam, Hungary and India (Panda, *et al.,* 2010; Smith, *et al.,* 1998; Chakraborti, *et al.*, 2002). Irrigation of agricultural fields with arsenic-laden water causes its accumulation in the soil which at higher levels could hinder plant growth. Plants growing in arsenic contaminated land display symptoms such as delay in seed germination, reduction in plant height, decrease in yield, lowered photosynthetic rate and reduced shoot and root growth (Meharg & Rahman, 2003; Jiang & Singh, 1994; Tsutsumi, 1980; Stoeva, *et al.,* 2003/4; Paliouris & Hutchinson, 1991). Furthermore, arsenic can build up and move around in plant tissues, particularly in the consumable parts, endangering human health and food security (Panda, *et al.,* 2010).

Maize (*Zea mays* L.) a member of the Poaceae family, is one of the important cereal crops grown around the globe. In India, maize is largely grown in states of Andhra Pradesh, Bihar, Karnataka, Maharashtra, Madhya Pradesh, Telangana and Tamil Nadu. *Zea mays* has been found to be a non – hyperaccumulator of arsenic and also shows sensitivity to arsenic stress. Arsenic has ben found to adversely affect the growth, development and yield quality of the maize plants (Bianucci, *et al.,* 2020; Mandal, *et al.,* 2019). Therefore, it is crucial to find efficacious strategies to mitigate As toxicity and accumulation in maize plants and improve their tolerance and adaptation to As stress. Salicylic acid (SA), a phytohormone, plays an important role in combating biotic and abiotic stress in plants. SA is synthesized via the shikimic acid pathway. Studies have reported different protective role of salicylic acid for instance, it helps in DNA repair processes and also assists in antioxidants and osmolyte generation. It also enhances the abiotic stress tolerance of the plants while also contributing in biogenesis of photosynthetic pigments (Singh, *et al.,* 2015; Saleem, *et al.,* 2021; Rafique, *et al.,*2023; Kaur, *et al.,*2021).

Salicylic Acid application on the heavy meal stressed plants have been reported to relieve toxicity in the plants. Hence, taking into consideration the progress in arsenic stress and related field with respect to maize, this work lays a brief outline on the germination and physiological aspects of the selected plant due to arsenic induced toxicity and its mitigation through SA.

MATERIAL & METHODS

Experimental Design

To examine the effects of arsenite stress on maize, a petriplate experiment was conducted in the Plant Physiology lab of Department of Botany, Patna University. Maize seeds were procured from the local farmer's market. Maize seeds were surface sterilized with 0.01% $HgCl_2$ and then rinsed with distilled water. The seeds were then put on sterilized Petri plates lined with cotton and Whatman filter paper. Seeds were subjected to different concentration of sodium arsenite solutions – 0 µM (control), 25 µM, 50 µM and 100 µM with 5 µM salicylic acid solution in 100 µM As treatment. The entire setup was arranged in completely randomized design with 3 replications. The seedlings were harvested after 15 days for the subsequent studies.

Morphological attributes:

Germination percentage (G%): The germination percentage of the treated and non-treated plants was calculated as follows:

Germination percentage = Total number of seeds germinated / Total number of seeds in all replicates x 100 (ISTA, 2010)

Root length (RL), Shoot length (SL) and plant fresh weight (PFW): Three seedlings were selected at random from each replicate. Root and shoot lengths were measured using a measuring scale while fresh weights were estimated using an electronic balance. Average length (shoot + root length) of all the three seedlings was calculated in cm while the average fresh weight was calculated in gms.

Seedling Vigour Index I: Seedling vigour index was calculated as follows:

Seedling vigour index I = Germination (%) x Average seedling length (Kumar, *et al.,* 2011)

Physiological attributes:

Pigment estimation: Pigment content was measured by Arnon method (Arnon, 1949). 0.1g of fresh leaves were taken after removing the midrib and grounded in 10 ml 80% (v/v) acetone using a mortar and pestle. The homogenate was centrifuged and the optical density of the supernatant was spectrophotometrically measured at wavelengths 480, 510, 652 and 663 nm. The pigment content was expressed in mg/g tissue fresh weight. The pigment content was calculated using the following formulas:

Chlorophyll a (mg/g f w) = [12.7(A663)-2.69(A645)] x V/ 1000xW

Chlorophyll b (mg/g fw) = [22.9(A645)-4.68(A663)] x V/ 1000xW

Total Chlorophyll (mg/g fw) = [20.2(A645)-8.02(A663)] x V/ 1000xW

Carotenoid (mg/g fw) = [7.6(A480)-1.49(A510)] x V/1000xW

Protein estimation: The proteins in enzyme extracts were evaluated using the Lowry *et al.* (1951). Protein was precipitated with 20% (w/v) chilled TCA and the mixture was allowed to stand for atleast 24 hours at 4°C in a refrigerator. The contents were centrifuged and the residue was washed and dissolved in 0.1N NaOH. It was then reacted with 0.5 ml Folin Ciocalteau reagent. The optical density of the mixture was measured at 640 nm using a spectrophotometer. Protein concentrations in samples were evaluated using the bovine serum albumin standard curve and expressed as µg protein / 100 mg tissue fresh weight (Lowry, *et al.,* 1951).

Stress Tolerance Indices: Germination stress tolerance index (GSI), Root length stress index (RLSI) and Shoot length stress index (SLSI) were calculated as follows (Kandil, Sharief, & EL-Fatah, 2019):

Germination stress tolerance index (GSI) = G% of stressed seeds/ G% of control seeds *100

Root length stress index (RLSI) = Root length of stressed seeds/ Root length of control seeds *100

Shoot length stress index (SLSI) = Shoot length of stressed seeds/ Root length of control seeds*100

Statistical analysis: All the data analysis were carried out using MS-Excel.

RESULT & DISCUSSIONS

Effect of arsenite and SA on germination:

The obtained data (table: 1 and Fig: 1) revealed a significant effect of arsenite on germination of maize seeds. At the highest concentration of arsenite (100 µM), germination was inhibited with germination percentage of only 33.33% in comparison to control with 100 % of germination. With the increase in arsenite concentration, a decrease in germination percentage was observed in all the treatment groups. Salicylic acid treatment showed an increase in germination percentage with 53.33%. Similar results have been reported in *Cicer arientum* (Bhattacharya, *et al.,* 2012), wheat (LI, *et al.,* 2007) and rice (Abedin & Meharg, 2002). The first physiological activity that metals affect is seed germination (Shanker, *et al.,* 2005). Higher Heavy metal concentration has been found to show decrease in germination period, seedling vigour and vitality of the seeds. Although in some cases at lower doses, it may enhance the germination of the seeds (Ma & Hong, 1998). Salicylic acid has also shown similar results in mitigating As, Zn and Ni toxicity (Kotapati, *et al.,* 2017; Mabrouk, *et al.,* 2019).

Effect of arsenite and SA on morphological parameters:

Root lengths of the treated and the control plants are shown in table: 1 and Fig.: 2 and 3. Mean root length of the control plants were recorded to be the highest (12.21 cm) and the arsenite treated plants showed reduced root lengths. At 25 µM As root length was measured to be 8.50 cm, which further decreased to 3.70 cm at 50 µM As concentration and was lowest (1.42 cm) at 100 µM As concentration. SA application increased the mean root length at 100 µM to 2.34 cm. Shoot length showed varying results. It was found to be highest (11.40 cm) at 25 µM As while control was found to be 11.35 cm. At 100 µM As shoot length was observed to be the lowest at 6.73 cm. Likewise, plant fresh weight was also found to be significantly affected by increasing dose of arsenite as shown

Table 1: Effect of arsenite and SA on different growth and physiological parameters of maize seeds

Treatment	Control	25 µM As	50 µM As	100 µM As	100 µM As + 5 µM SA
G% (Mean ± SD)	100.00 ± 0.00	96.67 ± 5.77	70.00 ± 10.00	33.33 ± 11.55	53.33 ± 5.77
SL (Mean ± SD)	11.35 ± 0.14	11.40 ± 0.56	8.30 ± 0.46	6.73 ± 0.12	8.60 ± 0.35
RL (Mean ± SD)	12.21 ± 0.07	8.50 ± 0.46	3.70 ± 0.20	1.42 ± 0.11	2.34 ± 0.02
SVI (Mean ± SD)	2356.33 ± 20.53	1925.33 ± 168.05	837.33 ± 93.52	272.27 ± 95.60	584.57 ± 77.81
PFW (Mean ± SD)	1.03 ± 0.01	0.85 ± 0.04	0.74 ± 0.03	0.47 ± 0.02	0.65 ± 0.03
Chl. A (Mean ± SD)	0.84 ± 0.02	0.47 ± 0.03	0.34 ± 0.02	0.13 ± 0.02	0.31 ± 0.01
Chl. B (Mean ± SD)	0.46 ± 0.03	0.37 ± 0.02	0.24 ± 0.01	0.08 ± 0.02	0.20 ± 0.003
Total Chl. (Mean ± SD)	1.28 ± 0.02	0.81 ± 0.01	0.57 ± 0.02	0.22 ± 0.02	0.53 ± 0.01
Car. (Mean ± SD)	0.41 ± 0.005	0.69 ± 0.008	0.33 ± 0.01	0.25 ± 0.004	0.32 ± 0.005
Protein (Mean ± SD)	70.17 ± 0.76	60.33 ± 0.58	50.75 ± 0.65	28.05 ± 0.05	39.06 ± 0.58
GSI (Mean ± SD)	0.00 ± 0.00	96.67 ± 5.77	70.00 ± 10.00	33.33 ± 11.55	53.33 ± 5.77
RLSI (Mean ± SD)	0.00 ± 0.00	69.59 ± 3.63	30.29 ± 1.52	11.65 ± 0.84	19.19 ± 0.02
SLSI (Mean ± SD)	0.00 ± 0.00	100.50 ± 5.66	73.17 ± 4.87	59.33 ± 1.1	75.78 ± 3.32

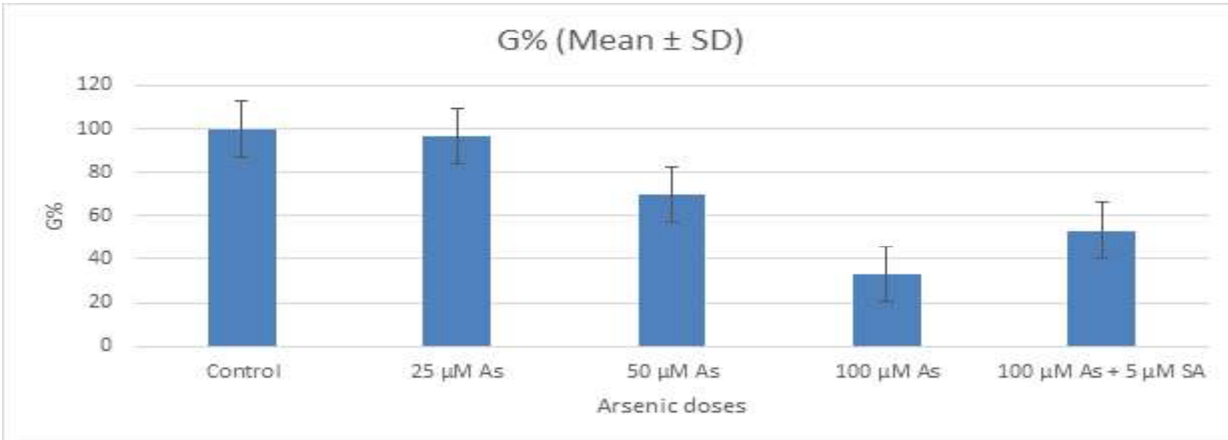

Fig. 1: Effect of Arsenite and SA on germination percentage (G%)

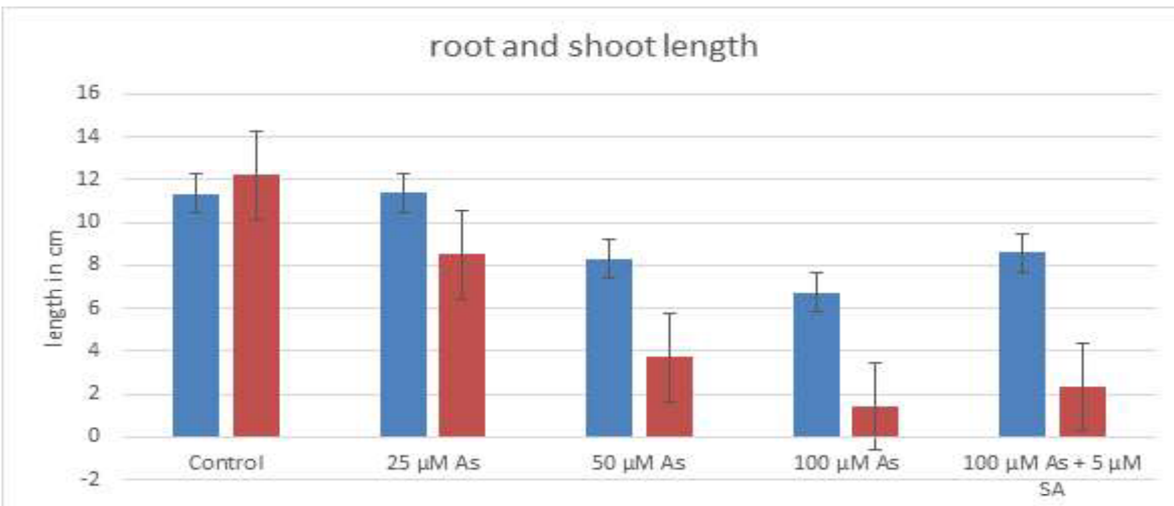

Fig. 2: Effect of Arsenite and SA on root and shoot length (cm)

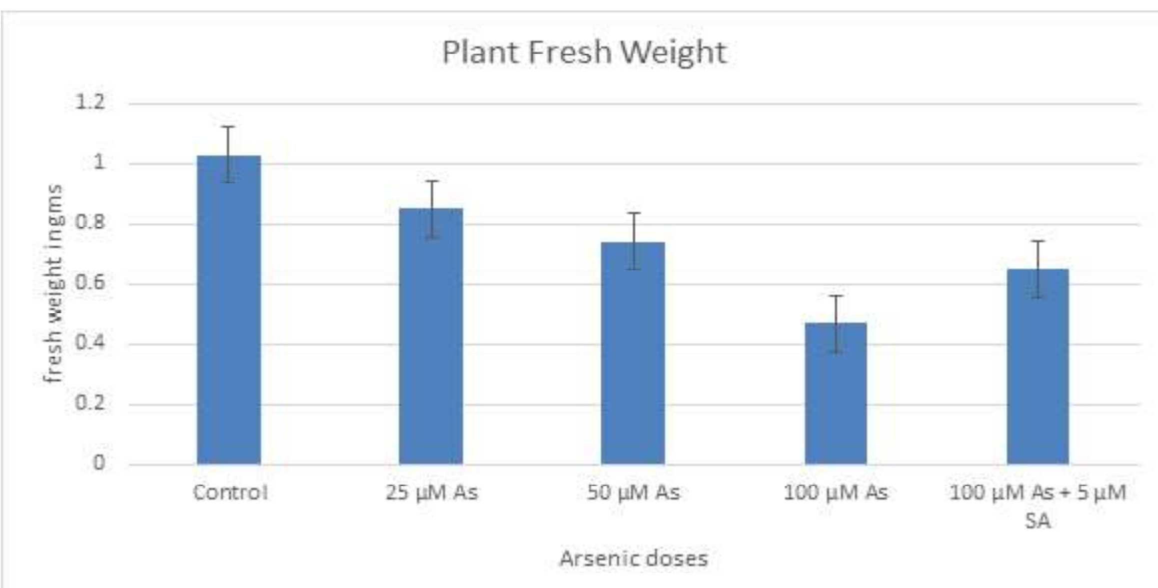

Fig. 3: Effect of Arsenite and SA on plant fresh weight (gms)

in table: 1. SA showed positive effect with respect to both shoot length and fresh weight. Seedling Vigour Index I also showed similar trend with the highest value recorded in control plants and lowest at the 100 µM As. Yadav *et al.,* (2014) found that As toxicity caused a decline in all growth parameters, including fresh mass, shoot length, and root length, in *Helianthus annus* L. var. DRSF-113 seedlings. It can be interpreted that since the roots of plants are the initial point of interaction with the arsenic species, a notable decrease in root length was noted in comparison to shoot length (Zengin F. , 2015). Zengin F. (2015) reported significant reduction in root length in a dose dependent manner which showed improvement with 1mM SA pretreatment. Hormesis is a positive response to minimal exposure to a chemical or stress condition. This phenomena could be exploited for increased crop output (Jalal, *et al.,* 2021). Piršelová, *et al.,* (2022) also reported stimulated growth at lower dose of As (1-5 mg/kg) which supports the data obtained in this study regarding the increment of shoot length at 25 µM As. Various studies have reported the restoration of plant growth on SA supplementation for mitigating arsenic toxicity. Therefore, it can be suggested that the appropriate level of SA administration can improve plant development under stress conditions while limiting As' effects on growth and development.

Effect of arsenite on Physiological attributes:

Photosynthetic pigments and protein content declined considerably with a rise in arsenic levels as depicted in Fig.: 4 and 5. A significant rise in the total chlorophyll and protein content was observed in the SA treatment. Miteva *et al.,*(2005) found that arsenic treatment reduced pigment concentration in tomato plants, specifically chlorophyll a, b, and carotenoids. Choudhary *et al.,* (2011) similarly showed decreased pigment levels in rice seedlings subjected to increasing amounts of arsenic. Other plant species, such as red clover (Mascher, *et al.,* 2002) and bean (Stoeva, *et al.,* 2005), also showed a decrease in pigment concentration when exposed to arsenic. Through its interactions with enzymes, arsenic interferes with the manufacture of photosynthetic pigments or speeds up their breakdown, thereby disrupting the process of photosynthesis (Farooq, *et al.,* 2016). Arsenic mitigating effect of SA has also been reported by Bano, *et al.,* (2022), Zengin, (2015) and Naeem, *et al.,* (2020). The distribution of N, C, and S for the manufacture of proteins, pigments, and enzymes may be the cause for the rise in photosynthetic capability, chlorophyll concentration, and Rubisco enzyme activity. The ATP-S enzyme's ability to allocate N and S to the leaf was enhanced in plants treated with SA that had high photosynthetic NUE and -SUE (Ahanger, *et al.,* 2019; Nazar, *et al.,* 2015).

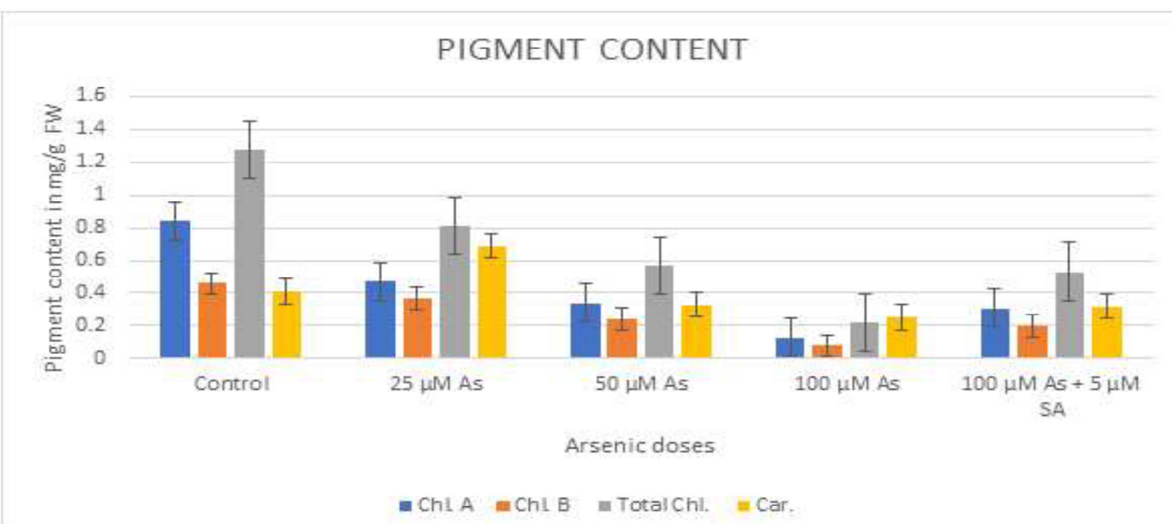

Fig. 4: Effect of arsenite and SA on pigment content in mg/g FW

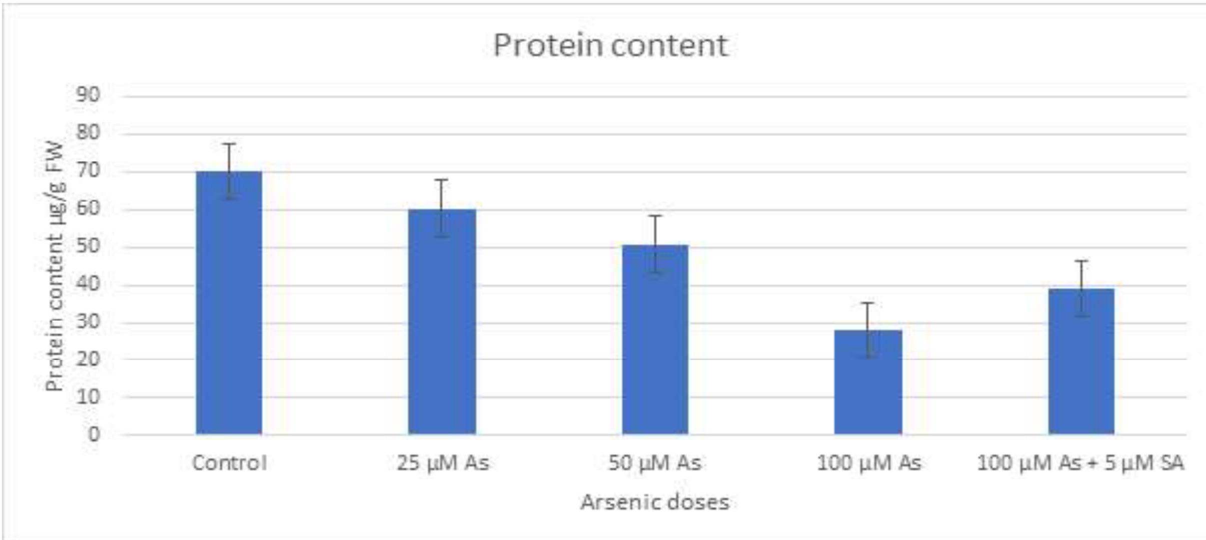

Fig. 5: Effect of arsenite and SA on protein content in µg/g FW

Stress tolerance Indices:

The results of different stress tolerance index are shown in table: 1. GSI was found to be 96.67 for 25 µM As, 70.00 for 50 µM As and 33.33 for 100 µM As. RLSI was found to be 69.59 for 25 µM As, 30.29 for 50 µM As and 11.65 for 100 µM As. SLSI was found to be 100.50 for 25 µM As, 73.17 for 50 µM As and 59.33 for 100 µM As. This clearly shows that at higher doses seedlings were found to be less tolerant to arsenic. For SA treatment at 100 µM As, the values for GSI, RLSI and SLSI were found to be 53.33, 19.19 and 75.78 respectively. Thus, it could be clearly suggested that SA application dramatically improves the tolerance of plant to arsenic stress.

Correlation analysis:

The correlation matrix indicated strong associations between germination, growth, and biochemical traits (Fig: 6). Germination percentage (G%), shoot length (SL), root length (RL), seedling vigor index (SVI), and plant fresh weight (PFW) all showed a strong positive correlation with each other (r = 0.92-0.99). Significant correlations were found between total chlorophyll, chlorophyll a, and chlorophyll b that ranged from 0.94 to 0.99. Protein content was shown to have a significant correlation with both Plant Fresh Weight (r = 0.990) and chlorophyll b (r = 0.994). Carotenoids had moderately positive associations with protein content (r = 0.569), Shoot length (r = 0.813) and

Variable	G%	SL	RL	SVI	PFW	Chl. A	Chl. B	Total Chl.	Car.	Protein	GSI	RLSI	SLSI
G%	1												
SL	0.962	1											
RL	0.928	0.924	1										
SVI	0.962	0.962	0.991	1									
PFW	0.962	0.921	0.95	0.954	1								
Chl. A	0.876	0.861	0.956	0.93	0.966	1							
Chl. B	0.979	0.962	0.967	0.979	0.991	0.945	1						
Total Chl.	0.91	0.899	0.964	0.949	0.982	0.985	0.983	1					
Car.	0.761	0.813	0.613	0.705	0.571	0.388	0.502	0.466	1				
Protein	0.985	0.926	0.946	0.96	0.99	0.955	0.994	0.978	0.569	1			
GSI	0.072	0.054	-0.26	-0.135	-0.169	-0.378	-0.123	-0.249	0.496	-0.13	1		
RLSI	0.337	0.347	0.058	0.182	0.077	-0.122	0.19	0.043	0.709	0.139	0.976	1	
SLSI	-0.226	-0.196	-0.513	-0.398	-0.451	-0.619	-0.413	-0.509	0.235	-0.42	0.947	0.754	1

Fig. 6: Correlation analysis of different growth and physiological parameters. The maximum value range is shown in green colour while the minimum value range is shown in red colour. The midpoint values are shown in yellow colour.

G% (r = 0.761). Significant negative correlations were found between the shoot length tolerance index (SLTI) and both chlorophyll a (r = -0.619) and RL (r = -0.513). Similarly, with the exception of a moderately positive correlation with carotenoids (r = 0.496), the germination stress index (GSI) displayed weak to negative relationships with chlorophyll. On the other hand, the root length tolerance index (RLTI) grouped strongly with other stress indices (r = 0.754–0.976) and had positive relationships with carotenoids (r = 0.709). Overall, the association of attributes illustrated that there is a strong relation between growth traits and chlorophyll and protein accumulation.

CONCLUSION

The present investigation found that the arsenic has an overall negative impact on the plant. It can also be concluded that use of SA protects against As-induced toxicity. Salicylic acid supplementation mitigates arsenic stress by enhancing chlorophyll content as well protein accumulation. Increase in plant fresh weight, germination percentage, shoot and root length on Salicylic acid application also suggests that SA does provide resilience against different stress. As the problem of As contamination in agricultural soils grows more severe, it is therefore, critical to develop methods and techniques to reduce As poisoning in crops. The mechanism of SA-induced As tolerance can be explored further using several biotechnological and genomic studies to develop arsenic tolerant genotypes.

ACKNOWLEDGEMENT

The basic requirements for instrumentation, chemicals and technical support by the Department of Botany, Patna University, Patna, India is gratefully acknowledged. Funding for this work was also provided by DBT, India and minor research project sanctioned by Patna University, Patna (sanction letter no. 03/ R&DC/RP/PU/Sanction, dated 18.10.2023).

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