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Amelioration of Zinc Stress by Salicylic Acid in Soybean

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ABSTRACT
Salicylic Acid (SA) is a key signaling molecule among naturally occurring plant hormones, known for its role in regulating various physiological processes. This study sought to investigate whether the application of SA could improve growth parameters including root length, shoot length, and dry weight of roots and shoots and alleviate zinc-induced stress in soybean seedlings during pre-, peak-, and post-flowering stages of plant growth. Zinc concentrations of 250, 500, 750, 1000, and 1250 mg/kg of soil were applied to the plants, along with 0.5 mM SA treatment, and compared with control groups. Soybean plants subjected to zinc stress and treated with SA exhibited improved growth and enhanced morphological parameters compared to those without treatment. Notably, the most pronounced positive effect on soybean biomass was observed at a zinc concentration of 500 mg/kg of soil. 0.5 mM SA treatment not only promoted growth but also mitigated the adverse effects of elevated Zinc levels (750-1250 mg/kg) although the reduction was less pronounced as compared to plants treated with Zinc alone, enhancing plant resilience. These findings suggest that SA may act as a protective agent against heavy metal toxicity, potentially enhancing the internal immunity of soybean plants and improving their resilience to environmental stressors, thereby offering valuable implications for agricultural practices.

Key words: Soybean, Salicylic acid (SA), Zinc (Zn), Plant height, Biomass

INTRODUCTION

Soybean [*Glycine max* (Linn.) Merr.] is a globally significant crop known for producing the most commonly used vegetable oil and high-quality concentrated protein. Its seeds have been utilized for centuries worldwide in various fresh, fermented, and dried foods, while soybean seedlings are particularly popular as fresh vegetables in many Asian countries (Salazar et al. 2012). However, one pressing issue currently gaining attention is the accumulation of harmful elements, such as heavy metals, in soil and the potential for these to be absorbed by crops, ultimately entering the food chain. This contamination has become a serious environmental concern, with heavy metal pollution in crops posing significant risks. Heavy metals, such as Zinc, copper, manganese, cadmium, mercury, and lead, are introduced into the environment through natural and human activities, particularly mining and industrial operations. These metals negatively impact plant growth, especially during the early stages of seedling development. Among these, Zinc (Zn) is a crucial micronutrient, playing a vital role as a catalytic component in over 300 enzymes essential for various biochemical processes within plants (Stanislawska-Glubiak and Korzeniowska 2022). Although Zinc is indispensable for plant growth, excessive concentrations can lead to toxicity, disrupting vital physiological and biochemical functions. This includes inhibited growth, reduced photosynthesis and respiration, impaired biosynthesis of chlorophyll and carotenoids, and decreased phosphorylation, which can even result in the death of the plant (Gupta and Meena 2024). Given the detrimental effects of Zinc toxicity on plant health, addressing its impact is paramount. One promising avenue is Salicylic Acid (SA), a natural signalling molecule that regulates numerous physiological processes and enhances plant resistance to biotic and abiotic stresses (Gupta et al. 2017). Research has demonstrated that SA can mitigate the harmful effects of heavy metal exposure in crops (Shi and Zhu 2008). Building on these findings, we hypothesize that SA could play a key role in alleviating the toxic effects of excessive Zinc on soybean plants. This study aims to explore salicylic acid's potential in countering Zinc-induced growth changes in soybean seedlings, offering insights into a potential solution to Zinc toxicity in crops.

MATERIALS AND METHODS

The experiment took place in April at the Department of Botany, University of Rajasthan, Jaipur, using greenhouse facilities. Conditions included a 12 hr photoperiod and 30°C temperature. Each pot, measuring 30 cm tall and 25 cm in diameter, contained 4 kg of garden soil. A randomized pot placement was conducted to minimize environmental biases of effects from sunlight, temperature variations, and soil composition differences. Zinc sulfate was applied in concentrations of 250, 500, 750, 1000, and 1250 mg/kg of soil, along with 0.5 mM SA, without additional nutrients. Control pots lacked Zinc and SA. Soybean seeds were surface sterilized with 0.1% $HgCl_2$ and sown evenly at 2 cm depth with ten seeds per pot. Plant count uniformity was ensured throughout the experiment, with watering every alternate day. Each treatment included three replicates per stage (pre-flowering at 30 days, peak-flowering at 45 days, and post-flowering at 60 days), facilitating robust data collection across the growth cycle. Harvesting at specified intervals enabled a comprehensive assessment of growth parameters.

Plant height

Plants from each concentration were gently removed from pots to measure plant height, ensuring the root and shoot systems remained intact. While shoot length was defined as the distance from the soil surface to the tip of the tallest leaf or flowering axis, root length was measured from the root tip to the soil surface and expressed as mean of replicates.

Root and shoot dry-weight

Plants were delicately removed from pots, preserving the integrity of their root and shoot systems to determine root and shoot dry weights. The roots were thoroughly washed to eliminate soil particles. Subsequently, roots and shoots were separated and dried in an oven at 80°C for 48 hrs until a constant dry weight was achieved. Six plants from each concentration were sampled, and the average values (g/plant) were computed.

Statistical analysis

Statistical analysis was conducted using SPSS version 25.0 and Microsoft Office Excel 2016. All parameters were presented as mean ± standard error (S.E.). To assess variations in growth parameters among plants treated with different concentrations of Zinc with Salicylic Acid, analysis of variance (ANOVA) was employed. Significant differences between treatment means were determined at the 0.05 significance level.

RESULTS

The growth analysis of soybean seedlings under Zn^{2+} stress demonstrated the beneficial effects of a 0.5 mM Salicylic Acid (SA) treatment (Tables 1, 2). Significant improvements were observed in root and shoot length, and dry biomass in the SA-treated seedlings, compared to the non-treated seedlings under Zinc stress ($p \leq 0.05$). The application of SA consistently improved growth parameters at all Zinc concentrations, suggesting that SA alleviates the adverse effects of Zinc stress by enhancing the plant's

Table 1. Impact of Zinc with Salicylic Acid (SA) on root and shoot length (cm) in Soybean

Treatment	Level	Pre-flowering stage		Peak-flowering stage		Post-flowering stage	
		Root length	Shoot length	Root length	Shoot length	Root length	Shoot length
Control	0	28.58±0.43	47.46±0.54	36.02±0.44	55.94±0.82	48.18±0.78	67.02±0.62
Zinc	250	36.67±0.22^c	54.06±0.26^C	45.62±0.38^c	62.42±0.45^b	59.19±0.24^c	72.71±0.55^c
(mg/kg)	500	39.48±0.11^c	62.35±0.58^b	51.19±0.18^c	67.22±0.65^c	64.44±0.13^c	79.05±0.69^b
+SA	750	30.02±0.59^b	48.08±0.60^a	39.56±0.25^a	61.47±0.64^c	51.52±0.26^b	65.84±0.54^a
(0.5mM)	1000	24.36±0.65^b	41.76±0.17^b	28.12±0.31^b	52.02±0.52^b	43.47±0.23^b	58.23±0.79^c
	1250	19.42±0.22^c	36.94±0.45^c	21.33±0.44^c	43.30±0.58^c	36.80±0.42^c	49.86±0.49^c

Values were expressed as mean ± SE, Significance level p= $^a \leq 0.1$, $^b \leq 0.05$, $^c \leq 0.01$

Table 2. Impact of Zinc with Salicylic Acid (SA) on root and shoot weight (g) in Soybean

Treatment	Level	Pre-flowering stage		Peak-flowering stage		Post-flowering stage	
		Root weight	Shoot weight	Root weight	Shoot weight	Root weight	Shoot weight
Control	0	0.462±0.023	2.695±0.086	0.724±0.032	4.476±0.085	0.902±0.025	4.741±0.046
Zinc	250	0.541±0.01 [c]	3.210±0.05 [c]	0.793±0.03 [c]	4.919±0.09 [a]	0.991±0.03	4.991±0.09 [a]
(mg/kg)	500	0.623±0.02 [c]	3.523±0.12 [c]	0.856±0.01 [c]	5.756±0.10 [c]	1.541±0.08 [c]	5.414±0.06 [b]
+SA	750	0.443±0.018 [b]	2.469±0.14 [a]	0.695±0.05 [a]	4.117±0.06 [a]	0.861±0.01 [c]	4.512±0.08 [b]
(0.5mM)	1000	0.384±0.03 [a]	1.992±0.05 [a]	0.616±0.04 [c]	3.723±0.17 [c]	0.714±0.06 [b]	3.876±0.23 [c]
	1250	0.319±0.06 [c]	1.713±0.02 [b]	0.543±0.09 [c]	3.104±0.06 [c]	0.663±0.10 [b]	3.116±0.19 [c]

Values were expressed as mean ± SE, Significance level p= $^a \leq 0.1$, $^b \leq 0.05$, $^c \leq 0.01$

internal defense mechanisms. Growth parameters were significantly greater with SA treatment at moderate Zinc concentrations (250 and 500 mg/kg) compared to plants treated with Zinc alone, indicating the protective role of SA under Zinc stress. Although these parameters still decreased at higher Zinc concentrations (750-1250 mg/kg) even with SA treatment, the reduction was less severe than the plants treated with Zinc alone.

Root length

At the pre-flowering stage, while the control plants exhibited a root length of 28.58 cm, SA treatment resulted in root lengths of 36.67 and 39.48 cm at 250 and 500 mg/kg Zinc, respectively, compared to the control. At the highest Zinc concentration (1250 mg/kg), the root length in SA-treated plants was 19.42 cm, significantly improving over the 11.72 cm observed in plants treated with Zinc alone.

At the peak-flowering stage, while the control plants exhibited a root length of 36.02 cm, SA treatment resulted in root lengths of 45.62 and 51.19 cm at 250 and 500 mg/kg Zinc, respectively, compared to the control. At the highest Zinc concentration (1250 mg/kg), the root length in SA-treated plants was 28.12 cm, significantly improving over the 21.78 cm observed in plants treated with Zinc alone.

At the post-flowering stage, while the control plants exhibited a root length of 48.18 cm, SA treatment resulted in root lengths of 59.19 and 64.44 cm at 250 and 500 mg/kg Zinc, respectively, compared to the control. At the highest Zinc concentration (1250 mg/kg), the root length in SA-treated plants was 36.80 cm, significantly improving over the 31.10 cm observed in plants treated with Zinc alone (Table 1).

Shoot length

At the pre-flowering stage, while the control plants exhibited a shoot length of 47.46 cm, SA treatment resulted in shoot lengths of 54.06 and 62.35 cm at 250 and 500 mg/kg Zinc, respectively, compared to the control. At the highest Zinc concentration (1250 mg/kg), the shoot length in SA-treated plants was 36.94 cm, significantly improving over the 30.17 cm observed in plants treated with Zinc alone.

At the peak-flowering stage, while the control plants exhibited a shoot length of 55.94 cm, SA treatment resulted in shoot lengths of 62.42 and 67.22 cm at 250 and 500 mg/kg Zinc, respectively, compared to the control. At the highest Zinc concentration (1250 mg/kg), the shoot length in SA-treated plants was 43.30 cm, significantly improving over the 35.56 cm observed in plants treated with Zinc alone.

At the post-flowering stage, while the control plants exhibited a shoot length of 67.02 cm, SA treatment resulted in shoot lengths of 72.71 and 79.05 cm at 250 and 500 mg/kg Zinc, respectively, compared to the control. At the highest Zinc concentration (1250 mg/kg), the shoot length in SA-treated plants was 49.86 cm, significantly improving over the 46.22 cm observed in plants treated with Zinc alone (Table 1).

Root weight

At the pre-flowering stage, while the control plants exhibited a root weight of 0.462 g, SA treatment resulted in root weight of 0.541 and 0.623 g at 250 and 500 mg/kg Zinc, respectively, compared to the control. At the highest Zinc concentration (1250 mg/kg), the root weight in SA-treated plants was 0.319gm, significantly improving over the 0.296 g observed in plants treated with Zinc alone.

At the peak-flowering stage, while the control plants exhibited a root weight of 0.724 g, SA treatment resulted in root weights of 0.793 and 0.856 g at 250 and 500 mg/kg Zinc, respectively, compared to the control. At the highest Zinc concentration (1250 mg/kg), the root weight in SA-treated plants was 0.543gm, significantly improving over the 0.448 g observed in plants treated with Zinc alone.

At the post-flowering stage, while the control plants exhibited a root weight of 0.902 g, SA treatment resulted in root weights of 0.991 and 1.541 g at 250 and 500 mg/kg Zinc, respectively, compared to the control. At the highest Zinc concentration (1250 mg/kg), the root weight in SA-treated plants was 0.663 g, significantly improving over the 0.519 g observed in plants treated with Zinc alone (Table 2).

Shoot weight

At the pre-flowering stage, while the control plants exhibited a shoot weight of 2.695 g, SA treatment resulted in a shoot weight of 3.210 and 3.523 g at 250 and 500 mg/kg Zinc, respectively, compared to the control. At the highest Zinc concentration (1250 mg/kg), the shoot weight in SA-treated plants was 1.713 g, significantly improving over the 1.906 g observed in plants treated with Zinc alone.

At the peak-flowering stage, while the control plants exhibited a shoot weight of 4.476 g, SA treatment resulted in a shoot weight of 4.919 and 5.756 g at 250 and 500 mg/kg Zinc, respectively, compared to the control. At the highest Zinc concentration (1250 mg/kg), the shoot weight in SA-treated plants was 3.104 g, significantly improving over the 2.419 g observed in plants treated with Zinc alone.

At the post-flowering stage, while the control plants exhibited a shoot weight of 4.741 g, SA treatment resulted in a shoot weight of 4.991 and 5.414 g at 250 and 500 mg/kg Zinc, respectively, compared to the control. At the highest Zinc concentration (1250 mg/kg), the shoot weight in SA-treated plants was 3.116 g, significantly improving over the 2.923 g observed in plants treated with Zinc alone (Table 2).

DISCUSSION

In the present study, salicylic acid (SA), a novel endogenous plant hormone, was applied to evaluate its role in alleviating Zinc-induced stress in soybeans. The results revealed significant improvements in root and shoot length and root and shoot dry biomass in seedlings treated with 0.5 mM SA compared to untreated controls under Zinc stress ($p \leq 0.05$). SA application consistently enhanced growth parameters across all Zinc concentrations, suggesting that SA mitigates the adverse effects of Zinc stress by bolstering the plant's internal defense mechanisms. Growth parameters were significantly improved with SA treatment at moderate Zinc concentrations (250 and 500 mg/kg) compared to Zinc-only treatments, indicating SA's protective role under these conditions. Previous research (Gupta and Meena 2024) concluded that moderate Zinc concentrations (250 and 500 mg/kg) promote plant growth, but adding 0.5 mM SA further enhanced plant growth. Although growth parameters declined at higher Zinc concentrations (750-1250 mg/kg) even with SA treatment, the reduction was less severe than in plants treated with Zinc alone. These findings align with existing scientific knowledge on the protective effects of SA under heavy metal stress. Previous studies have shown that SA improves plant growth and yield in response to environmental stress (Pal et al. 2002). While Jazi et al. (2011) demonstrated the positive effects of SA on *Brassica napus*, Shakirova et al. (2003) observed increased cell division in the apical meristem of wheat seedlings treated with 0.5 mM SA. Moreover, SA has been shown to promote growth under stress from various heavy metals in different plant species (Metwally et al. 2003, Wang et al. 2004, Belkadhi et al. 2014). Although the beneficial effects of SA were most pronounced at moderate Zinc concentrations, even at high Zinc concentrations (1250 mg/kg), 0.5 mM SA-treated plants exhibited improved growth compared to untreated plants, although the improvement was less substantial. This suggests that while 0.5 mM SA can mitigate Zinc toxicity, its protective efficacy decreases under extreme stress conditions, which is consistent with previous research indicating that the effectiveness of SA diminishes as metal stress intensifies (Safari et al. 2019).

CONCLUSION

The results from this study provide strong evidence that Salicylic Acid can alleviate the toxic effects of Zinc on soybean seedlings, particularly at moderate Zinc concentrations in soil. The improvements in root and shoot length and biomass under 0.5mM SA treatment suggest that SA is a potent mitigator of Zinc-induced stress, likely through enhanced antioxidant activity and better regulation of plant physiological processes. While SA treatment was less effective under high Zinc concentrations (750-1250 mg/kg of Zinc), its application still resulted in substantial improvements in plant growth compared to Zinc-only treatment, highlighting the potential of SA as an effective strategy for enhancing crop resilience in heavy metal-contaminated environments. These findings contribute to the growing literature supporting using plant growth regulators like SA to improve agricultural productivity under abiotic stress conditions. Future studies should explore the molecular mechanisms underlying SA's protective effects, particularly focusing on its role in regulating stress-responsive genes and antioxidant defense systems.

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Conflict of interest: Authors declare no conflict of interest

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