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Interactive effects of salinity and plant growth regulators on antioxidative enzymes in soybean (*Glycine max* L.)

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Abstract
Salinity is a major abiotic stress that disrupts plant growth by inducing oxidative damage through the overproduction of reactive oxygen species (ROS). This study aimed to evaluate the impact of exogenous application of plant growth regulators (PGRs) on the antioxidant enzyme responses in soybean (*Glycine max* L.) under saline irrigation. A pot experiment was conducted using variety GS-4, with two irrigation treatments (tap water and saline water at 6 dS/m) and seven foliar treatments: control, GA_3 (100 ppm), SA (100 ppm), IAA (500 ppm), and combinations thereof. Leaf samples were collected at 25 and 35 days after sowing (DAS) and analyzed for the activities of peroxidase (POX), catalase (CAT), polyphenol oxidase (PPO), and ascorbate peroxidase (APX). Results revealed that salinity significantly enhanced the activity of all four antioxidant enzymes, indicating the plant's defensive response to oxidative stress. Among the treatments, the combination of SA + IAA (T_6) consistently resulted in the highest activities of POX and APX, while CAT and PPO activities were generally reduced in treatments with combined PGRs. The highest enzyme activities were observed at 35 DAS under saline conditions. Interaction effects among irrigation, growth regulator treatments, and growth stages were statistically significant for all enzymes except APX. Overall, the findings suggest that foliar application of SA and IAA effectively boosts antioxidant enzyme activity in soybean, offering a potential strategy to mitigate salinity-induced oxidative stress and improve crop resilience under adverse environmental conditions.

Keywords: APX, Soybean, IAA, *Glycine max* L., Salinity, Gibberellic acid, Salicylic acid, antioxidant enzymes, PPO.

Introduction

Salinity is a major abiotic stress that adversely affects crop growth and productivity by inducing osmotic stress, ionic toxicity, and oxidative damage. Under saline conditions, plants generate excessive reactive oxygen species (ROS), including hydrogen peroxide and superoxide radicals, which can disrupt cellular membranes, proteins, and DNA. To mitigate these effects, plants rely on an antioxidant defense system involving key enzymes such as peroxidase (POX), catalase (CAT), polyphenol oxidase (PPO), and ascorbate peroxidase (APX) (Shaikh *et al*., 2021; Solanki *et al*., 2018a and Solanki *et al*., 2018b) [15, 16, 17]. Exogenous application of plant growth regulators (PGRs) like gibberellic acid (GA_3), salicylic acid (SA), and indole-3-acetic acid (IAA) has shown potential in enhancing stress tolerance by modulating physiological and biochemical responses (Chovatia *et al.*, 2024; Joshi *et al.*, 2024) [7]. These PGRs are known to influence growth, antioxidant enzyme activity, and stress signaling pathways. Several studies have demonstrated that PGRs can improve oxidative stress resistance by boosting enzymatic activity under saline conditions (Sairam *et al.*, 2005; Purohit *et al.*, 2020) [14, 13].

Soybean (*Glycine max* L.), a vital oilseed and protein crop, is moderately sensitive to salinity, especially during early growth stages. Enhancing its stress resilience through the use of PGRs could be a promising approach to improve productivity in saline environments. The present study was undertaken to investigate the impact of GA_3, SA, IAA, and their combinations on antioxidant enzyme activity in soybean under normal and saline irrigation. Enzymatic responses were evaluated at two growth stages—25 and 35 days after sowing—to understand the temporal effect of treatments. The results aim to identify effective strategies for mitigating oxidative stress and improving salinity tolerance in soybean.

Materials and Methods

The present investigation was carried out during the *Kharif* season of 2024-25 at the Department of Biochemistry, College of Agriculture, Junagadh Agricultural University, Junagadh. The laboratory analytical work was conducted using standard protocols for antioxidant enzyme assays. Soybean [*Glycine max* L.] variety GS-4 was selected as the experimental material. The experiment was laid out in a factorial completely randomized design (FCRD) in pots using calcareous, slightly alkaline soil collected from the Agronomy farm of JAU. Irrigation treatments consisted of two levels: I_1 (tap water) and I_2 (saline water of 6 dS/m EC), with the latter prepared by diluting natural saline water collected from the coastal region of Mangrol using distilled water.

The experiment comprised seven foliar spray treatments of plant growth regulators (PGRs) applied at 15 days after sowing (DAS) as follows: T_1 – Control (no spray), T_2 – GA_3 @ 100 ppm, T_3 – SA @ 100 ppm, T_4 – IAA @ 500 ppm, T_5 – GA_3 @ 100 ppm + SA @ 100 ppm, T_6 – SA @ 100 ppm + IAA @ 500 ppm, and T_7 – GA_3 @ 100 ppm + SA @ 100 ppm + IAA @ 500 ppm. All plants received recommended doses of fertilizers. Each treatment was replicated three times. Sampling of leaf tissues was carried out at two growth stages, 25 DAS (G_1) and 35 DAS (G_2), corresponding to 10 and 20 days after foliar spray, respectively.

Leaf samples were collected in the morning, immediately placed in ice, and brought to the laboratory for antioxidant enzyme analysis. Fresh tissues were weighed and processed

promptly to minimize enzymatic degradation. For each assay, three biological replicates were maintained.

Peroxidase (POX) activity was assayed following Malik and Singh (1980) [8]. Fresh leaf tissue (100 mg) was homogenized in ice-cold 50 mM sodium phosphate buffer (pH 7.0) and centrifuged at 10,000 rpm for 15 min. The reaction mixture contained 2.99 ml of 0.03% hydrogen peroxide in 0.1 M phosphate buffer (pH 6.0) with 0.01% orthodianisidine dye, and the reaction was initiated by adding 10 µl of the enzyme extract. The change in absorbance was measured at 460 nm for one minute at 15-second intervals. Activity was expressed as ΔOD min^{-1} g^{-1} fresh weight.

Catalase (CAT) activity was determined according to the method of Aebi (1984) [3]. The enzyme extract was prepared as for POX. The assay mixture (3 ml) included 50 mM sodium phosphate buffer (pH 7.0), 18 mM H_2O_2, and 50 µl enzyme extract. The decomposition of H_2O_2 was monitored by measuring the decline in absorbance at 240 nm. Results were expressed in ΔOD min^{-1} g^{-1} fresh weight.

Polyphenol Oxidase (PPO) was estimated following the method described by Esterbauer *et al.* (1977) [6]. A 0.1 g leaf sample was homogenized in 5 ml of 100 mM phosphate buffer (pH 6.5), centrifuged at 10,000 rpm for 15 min at 4°C, and the supernatant was used. The reaction mixture contained 2.9 ml of 10 mM catechol in buffer and 100 µl enzyme extract. The increase in absorbance at 490 nm was recorded for one minute at 15-second intervals, and the specific activity was expressed as ΔOD min^{-1} g^{-1} fresh weight.

Ascorbate Peroxidase (APX) activity was assayed as per Mehr and Bahabadi (2013) [9]. The assay mixture (3 ml) contained 50 mM sodium phosphate buffer (pH 7.0), 0.1 mM EDTA, 0.1 mM H_2O_2, 0.5 mM ascorbic acid, and 100 µl enzyme extract. The decrease in absorbance at 290 nm due to ascorbate oxidation was monitored, and activity was expressed as ΔOD min^{-1} g^{-1} fresh weight.

The collected data were subjected to statistical analysis using the 'F' test as outlined by Panse and Sukhatme (1985) [11]. Treatment means were compared at appropriate levels of significance to interpret the effect of salinity, growth regulator treatments, and their interactions on enzymatic antioxidant activity in soybean.

Results and Discussion

Abiotic stresses such as salinity lead to the excessive generation of reactive oxygen species (ROS) in plant cells, causing oxidative damage to cellular membranes, proteins, and DNA. Plants counteract this oxidative stress through a robust antioxidant defense system involving key enzymes like Peroxidase (POX), Catalase (CAT), Polyphenol Oxidase (PPO), and Ascorbate Peroxidase (APX). In the present study, soybean plants were treated with various combinations of plant growth regulators (PGRs) at 15 DAS and irrigated with either tap water (I_1) or saline water of 6 dS/m (I2). Enzyme activity was assessed at two growth stages, 25 DAS (G1) and 35 DAS (G2), and the results are summarized below

Peroxidase

The data on enzyme activity of peroxidase activity (ΔO.D.min. $^{-1}$g.$^{-1}$Fr.Wt.) analysed from leaf tissues of soybean are depicted Table 1. Peroxidase activity in soybean leaves was significantly influenced by salinity, plant growth regulator treatments, and growth stage. Overall, plants irrigated with saline water (I_2) showed higher peroxidase activity (8.44 ΔOD min^{-1} g^{-1} fresh weight) compared to those under normal water (I_1, 6.44). This indicates the activation of the antioxidative machinery in response to salt-induced stress. Among the treatments, T_6, which consisted of salicylic acid (100 ppm) combined with IAA (500 ppm), recorded the highest peroxidase activity (8.86), suggesting a synergistic effect of these two regulators in enhancing the stress-responsive enzymatic defense. This was followed by T_5 (GA_3 + SA) and T_4 (IAA alone), which also performed better than the control. The lowest activity was recorded in the untreated control (T_1), confirming the role of PGRs in inducing enzymatic defense. The interaction effects further revealed that the highest peroxidase activity was observed in plants irrigated with saline water and treated with SA + IAA (I_2T_6), while the lowest was in control plants irrigated with tap water (I_1T_1). Plants observed at 35 DAS (G_2T_6) exhibited greater peroxidase activity compared to those at 25 DAS (G_1T_1), highlighting that enzyme activity increased as the plant matured and accumulated more stress. These findings align with Patel *et al.* (2019) [12] and Ahmad (2009) [4], who reported elevated peroxidase activity in response to salinity. However, some studies such as Abdel (2013) [1] have shown that GA_3 can suppress peroxidase activity under saline conditions, suggesting that not all PGRs have similar effects under stress.

Catalase

The data on enzyme activity of catalase activity (ΔO.D.min. $^{-1}$g.$^{-1}$Fr.Wt.) analysed depicted in Table 2. Catalase activity also showed a significant increase under salt stress. Plants irrigated with saline water recorded higher catalase activity (4.97 ΔOD min^{-1} g^{-1} FW) than those irrigated with tap water (3.91). Interestingly, in contrast to the trend seen in peroxidase, the highest catalase activity was recorded in the control treatment (T_1, 5.39), while the lowest was in T_7 (GA_3 + SA + IAA), followed by T_6 and T_5. This suggests that the combination of all three growth regulators might have an antagonistic or suppressive effect on catalase expression under certain conditions. The interaction between irrigation and treatment revealed that the highest catalase activity occurred in I_2T_1 (saline water with control), and the lowest in I_1T_7. Furthermore, early-stage plants (G_1T_1) had higher catalase activity than those observed later (G_2T_7), indicating that catalase may be more actively engaged during early stress responses. The observed decline in catalase under GA_3 + SA + IAA combination mirrors findings by Abdel (2013) [1] and Trivedi *et al.* (2018) [18], who reported that catalase activity can diminish under severe stress or upon certain hormonal interactions.

Polyphenol oxidase

Ascorbate peroxidase, a key enzyme in the ascorbate-glutathione cycle, showed a consistent and significant increase under salinity stress. Plants irrigated with saline water showed the highest mean APX activity (7.97), as compared to plants under tap water. Among the growth regulator treatments, T_6 (SA + IAA) led to the maximum APX activity (7.92), followed closely by T5 (GA_3 + SA, 7.82) and T_7 (GA_3 + SA + IAA, 7.38), whereas the lowest activity was found in the control treatment (T_1, 5.95). Although the interaction between irrigation and treatment

was not statistically significant, the highest APX activity was still found in I2T6, while I1T2 showed the lowest. With respect to growth stages and treatments, G2T6 (35 DAS with SA + IAA) exhibited the highest APX activity, and G_1T_1 the lowest. Similarly, the interaction between irrigation and growth stages showed that plants irrigated with saline water and evaluated at 35 DAS (I_2G_2) had the highest activity, suggesting a prolonged stress response and increased APX synthesis. These findings are in agreement with those of Sairam *et al.* (2005) [14], Abdulaziz *et al.* (2014) [2] and Moumita *et al.* (2019) [10], who all confirmed that APX activity is significantly induced under salt stress, and growth regulators can enhance this response by modulating antioxidant metabolism.

In conclusion, this study reveals that salinity stress significantly stimulates the antioxidant defense system in soybean, as indicated by increased activities of POX, CAT, PPO, and APX enzymes. Among all growth regulator combinations, the dual application of salicylic acid and IAA (T_6) was the most effective in enhancing peroxidase and ascorbate peroxidase activities, suggesting a positive synergistic effect. While some combinations like GA_3 + SA + IAA suppressed CAT and PPO activities, others promoted a more robust enzymatic response. The interaction of stress level, growth regulator type, and growth stage plays a critical role in determining the effectiveness of antioxidative defense. These results underscore the potential use of exogenous PGRs, particularly SA and IAA, as effective tools for enhancing plant tolerance to salinity during critical growth stages.

Table 1: Effect of plant growth regulators Peroxidase (POX) (ΔO.D.min^{-1}g.$^{-1}$) of soybean leaves under irrigation of saline water

Treatment	25 DAS (G_1)			35 DAS (G_2)		
	I_1	I_2	Mean T	I_1	I_2	Mean T
T_1	5.45	6.07	5.76	6.11	7.34	6.72
T_2	6.11	7.23	6.67	6.34	8.27	7.30
T_3	6.20	7.60	6.90	6.37	8.37	7.37
T_4	6.27	7.71	6.99	7.24	8.54	7.89
T_5	7.11	8.28	7.69	8.35	9.19	8.77
T_6	7.17	8.20	7.68	8.47	9.26	8.86
T_7	6.79	7.77	7.28	7.42	8.11	7.76
Mean I	6.44	7.55		7.18	8.44	
	S.Em. ±	C.D. at 5%		S.Em. ±	C.D. at 5%	
I	0.01	0.03		0.01	0.02	
T	0.02	0.06		0.01	0.03	
I×T	0.03	0.09		0.01	0.04	

Table 2: Effect of plant growth regulators Catalase (CAT) (ΔO.D.min^{-1}g.$^{-1}$) of soybean leaves under irrigation of saline water

Treatment	25 DAS (G_1)			35 DAS (G_2)		
	I_1	I_2	Mean T	I_1	I_2	Mean T
T_1	4.97	5.82	5.39	4.87	5.07	4.97
T_2	4.81	5.72	5.26	4.86	4.77	4.81
T_3	4.70	5.37	5.03	3.93	4.27	4.10
T_4	4.57	5.22	4.89	3.79	4.14	3.96
T_5	3.88	4.96	4.42	3.62	3.94	3.78
T_6	3.44	4.50	3.97	3.29	3.23	3.26
T_7	2.88	3.21	3.04	2.99	2.88	2.93
Mean I	4.18	4.97		3.91	4.04	
	S.Em. ±	C.D. at 5%		S.Em. ±	C.D. at 5%	
I	0.01	0.02		0.01	0.02	
T	0.01	0.04		0.01	0.03	
I×T	0.02	0.05		0.01	0.04	

Table 3: Effect of plant growth regulators Polyphenol oxidase (PPO) (ΔO.D.min^{-1}g.$^{-1}$) of soybean leaves under irrigation of saline water

Treatment	25 DAS (G_1)			35 DAS (G_2)		
	I_1	I_2	Mean T	I_1	I_2	Mean T
T_1	6.99	7.18	7.08	6.19	6.04	6.11
T_2	7.09	7.07	7.08	6.07	6.09	6.08
T_3	6.79	6.88	6.83	6.13	5.79	5.96
T_4	6.74	6.83	6.78	6.14	5.58	5.86
T_5	5.84	6.50	6.17	5.24	5.41	5.32
T_6	5.48	6.30	5.89	5.09	5.26	5.17
T_7	5.39	6.38	5.88	4.98	5.35	5.16
Mean I	6.33	6.73		5.69	5.65	
	S.Em. ±	C.D. at 5%		S.Em. ±	C.D. at 5%	
I	0.01	0.02		0.01	0.02	
T	0.01	0.04		0.01	0.03	
I×T	0.02	0.06		0.02	0.05	

Table 4: Effect of plant growth regulators Ascorbate peroxidase (APX) ($\Delta O.D.min^{-1}g.^{-1}$) of soybean leaves under irrigation of saline water

Treatment	25 DAS (G_1)			35 DAS (G_2)		
	I_1	I_2	Mean T	I_1	I_2	Mean T
T_1	5.69	6.22	5.95	6.24	7.17	6.70
T_2	5.56	6.82	6.19	6.66	7.42	7.04
T_3	5.81	6.95	6.38	6.69	7.90	7.29
T_4	5.98	7.53	6.75	7.02	7.88	7.45
T_5	6.71	7.41	7.06	7.19	8.45	7.82
T_6	6.40	7.66	7.03	7.24	8.61	7.92
T_7	7.37	8.11	7.74	6.38	8.39	7.38
Mean I	6.22	7.24		6.78	7.97	
	S.Em. ±	C.D. at 5%		S.Em. ±	C.D. at 5%	
I	0.01	0.02		0.02	0.05	
T	0.01	0.04		0.03	0.09	
I×T	0.02	0.06		0.04	0.12	

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