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EFFECT OF EXOGENOUS APPLICATION OF GIBBERELLIC ACID AND SALICYLIC ACID ON ANTIOXIDATIVE ENZYMES IN INDIAN MUSTARD IRRIGATED WITH SALINE WATER

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ABSTRACT

Salinity stress is a major constraint to the growth and productivity of Indian mustard (*Brassica juncea* L.), a vital oilseed crop in India, primarily due to oxidative damage caused by reactive oxygen species. This study evaluated the effects of exogenous application of gibberellic acid (GA_3) and salicylic acid (SA) on antioxidant enzyme activity in mustard under saline irrigation conditions. A greenhouse pot experiment was conducted using a factorial completely randomized design with three salinity levels (tap water, 4 dS/m, and 8 dS/m) and six plant growth regulator (PGR) treatments: control, water spray, GA_3 at 50 and 100 ppm, and SA at 0.2 and 0.4 µM. Enzyme activities polyphenol oxidase (PPO), peroxidase (POX), catalase (CAT), and ascorbate peroxidase (APX) were assessed at 20 and 30 days after sowing (DAS). Salinity significantly increased all enzyme activities, with the highest values observed at 8 dS/m. PGR treatments further modulated these responses, with 0.4 µM SA and 100 ppm GA_3 notably enhancing POX and APX activities. PPO and CAT responses varied across treatments. Significant interactions between salinity and PGRs were observed, with maximum enzyme activities often recorded in saline-stressed plants treated with SA. Enzyme activity also increased with plant age, showing higher levels at 30 DAS compared to 20 DAS. These results suggest that exogenous application of SA, and to a lesser extent GA_3, can effectively enhance antioxidant defense mechanisms in mustard under salt stress. The findings support the use of PGRs, particularly SA, as a potential strategy for improving salinity tolerance in mustard, contributing to more resilient and sustainable crop production in salt-affected regions.

***Keywords*:** salinity stress, gibberellic acid, salicylic acid, antioxidant enzymes, oxidative stress.

Introduction

Indian mustard (*Brassica juncea* L.) is one of India's most important oilseed crops, playing a crucial role in edible oil production. However, its productivity is frequently challenged by soil salinity, a growing abiotic stress in arid and semi-arid regions. Salinity adversely affects plant physiology and metabolism, leading to impaired photosynthesis, ionic imbalance, and the overproduction of reactive oxygen species (ROS), which in turn cause oxidative damage to lipids, proteins, and nucleic acids (Anjum *et al.,* 2022; Chovatia *et al.,* 2024; Joshi *et al.,* 2024). To mitigate such stress, plants activate a complex antioxidant defense system that includes enzymes like superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT), which detoxify ROS and maintain cellular redox balance (Esfandiari *et al*., 2007; Purohit *et al.,* 2020). Exogenous application of plant growth regulators (PGRs) such as gibberellic acid (GA_3) and

salicylic acid (SA) has shown promise in enhancing stress resilience in various crops. GA_3 promotes plant growth under stress by stimulating cell elongation, enzyme activity, and nutrient uptake (Kapgate *et al.,* 1989), whereas SA acts as a signaling molecule involved in modulating stress responses, particularly through enhancing antioxidant enzyme activity and osmotic adjustment (Noreen *et al.,* 2009). Several studies have reported that external application of GA_3 and SA can alleviate salinity-induced damage in crops like mustard by improving chlorophyll content, proline accumulation, and antioxidative enzyme efficiency (Garg *et al.,* 1993; Abdelaal, 2015).

Although both PGRs independently contribute to stress mitigation, research on their combined or interactive effects on the antioxidative response of mustard under saline conditions remains limited. Therefore, the present investigation was undertaken to assess the influence of exogenous GA_3 and SA on the activity of key antioxidative enzymes in *Brassica juncea* genotypes irrigated with saline water. The results will help advance understanding of PGR-mediated stress alleviation mechanisms and support the development of effective strategies for improving salt tolerance and sustaining productivity in mustard grown in salinity-affected regions.

Material and Methods

A greenhouse pot experiment was conducted during the Rabi season of 2022–2023 at Junagadh Agricultural University, Gujarat, India, to investigate the impact of salinity stress and exogenous application of plant growth regulators on antioxidative enzyme activity in Indian mustard (*Brassica juncea* L. var. GDM-4).

The experimental soil was calcareous and slightly alkaline in nature, with low available nitrogen, medium organic carbon and phosphorus, and high potassium content, typical of the soils found in the Saurashtra region. The experiment was arranged in a factorial completely randomized design (FCRD) with three replications. The treatment structure comprised three levels of salinity control (tap water), 4 dS/m, and 8 dS/m prepared by diluting coastal saline water to the desired electrical conductivity levels. Plant growth regulator (PGR) treatments included a no-spray control, water spray, gibberellic acid (GA_3) at 50 ppm and 100 ppm, and salicylic acid (SA) at 0.2 µM and 0.4 µM concentrations. Foliar sprays of the respective PGRs were applied twice, at 15 and 25 days after sowing (DAS). Saline irrigation was maintained consistently as per the salinity treatment schedule. Leaf samples were collected five days after the second foliar spray (i.e., at 30 DAS) for biochemical analysis of antioxidative enzymes.

Catalase (CAT) activity was estimated using the method described by Aebi (1984), based on the decomposition rate of hydrogen peroxide (H_2O_2) measured spectrophotometrically at 240 nm. Peroxidase (POD) activity was determined following the procedure outlined by Malik and Singh (1980), using pyrogallol as the hydrogen donor and measuring the increase in absorbance at 420 nm. These enzyme assays were performed to evaluate the role of antioxidant defense mechanisms in response to salinity stress and the mitigating effects of GA_3 and SA. The data obtained were subjected to analysis of variance (ANOVA) to test the significance of treatment effects. Mean comparisons were performed using the least significant difference (LSD) test at a 5% level of significance. This methodological framework enabled a comprehensive assessment of the physiological responses of Indian mustard to salinity stress and plant growth regulator application under controlled conditions.

Result and Discussion

The present study evaluated the impact of salinity stress and exogenous application of plant growth regulators (GA_3 and SA) on antioxidant enzyme activities in Indian mustard (*Brassica juncea* L. *var.* GDM-4). Enzymes such as polyphenol oxidase (PPO), peroxidase (POX), catalase (CAT), and ascorbate peroxidase (APX) were analyzed to understand the oxidative stress response under varying salinity levels, growth stages, and treatment combinations.

Polyphenol oxidase (PPO)

Polyphenol oxidase (PPO) activity (ΔO.D./min./g, fresh weight basis) was assessed from mustard leaves treated with different concentrations of plant growth regulators (T_1 to T_6) at 15 and 25 DAS, under three salinity levels tap water (I_0), 4 dS/m (I_1), and 8 dS/m (I_2) and at two growth stages: 20 DAS (G_1) and 30 DAS (G_2). The results are presented in Table 1 and Figures 1 and 2.

Salinity had a significant effect on PPO activity. The highest mean value (9.57 ΔO.D./min./g) was observed under 8 dS/m salinity (I_2), while the lowest (7.73 ΔO.D./min./g) was recorded in control (I_0), consistent with findings by Kumar *et al.* (2023). PPO activity also increased with plant age, from 8.05 ΔO.D./min./g at G_1 to 9.25 ΔO.D./min./g at G_2. Among growth regulator treatments, the highest PPO activity was found in the untreated control (T_6: 9.38

ΔO.D./min./g), followed by T_5 and T_4. These results indicate that oxidative stress was more pronounced in untreated plants, while PGRs may have moderated the enzyme response. Significant interaction effects were noted. The I × T interaction showed the lowest PPO in I_0T_1 (7.31) and the highest in I_2T_6 (10.48). For G × T, activity ranged from 7.27 (G_1T_1) to 10.08 (G_2T_6), while the I × G interaction showed a minimum of 7.11 (I_0G_1) and a maximum of 10.15 (I_2G_2). These findings support earlier reports by Trivedi *et al.* (2018) and Solanki *et al.* (2018), highlighting that salinity elevates PPO activity, although high doses of salicylic acid may reduce it.

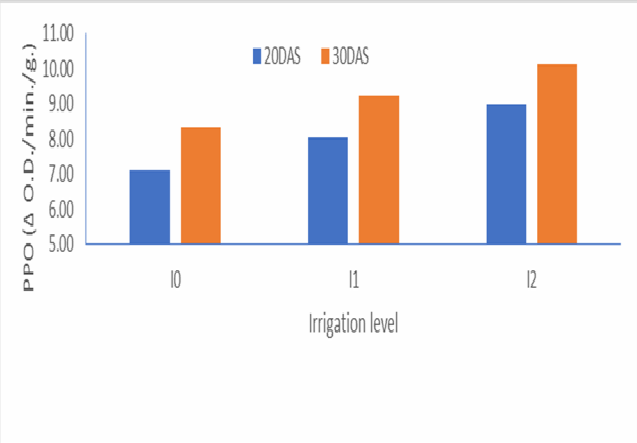

Fig. 1: Mean effect of plant growth regulators on leaf poly phenol oxidase (PPO) (Δ O.D./min./g.) of Indian mustard under irrigation of Tap and saline water at different growth stages.

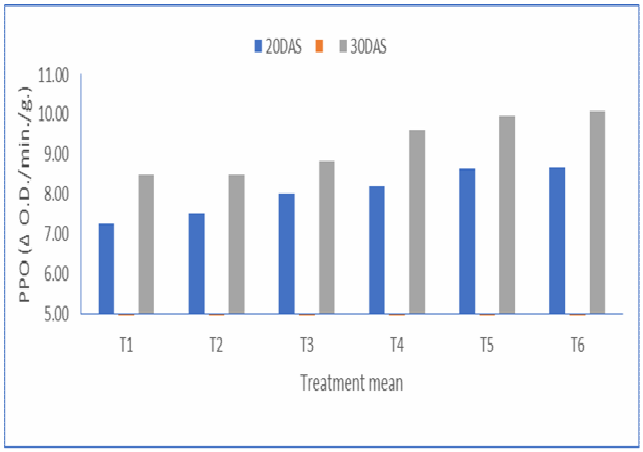

Fig. 2 : Mean effect of plant growth regulators on leaf poly phenol oxidase (PPO) (Δ O.D./min./g.) of Indian mustard under irrigation of saline water

Table 1: Effect of plant growth regulators on leaf poly phenol oxidase (PPO) (Δ O.D./min./g.) of Indian mustard under irrigation of saline water.

Treatment (T)	20 DAS (G_1)			Mean (I)	30 DAS (G_2)			Mean (I)
	I_0 (Tap water)	I_1 (4 EC)	I_2 (8 EC)		I_0 (Tap water)	I_1 (4 EC)	I_2 (8 EC)	
T_1 (Control)	6.31	7.19	8.32	7.27	8.30	8.14	9.01	8.48
T_2 (Water spray)	6.61	7.48	8.44	7.51	8.11	8.36	9.01	8.49
T_3(GA_3@50ppm)	6.88	8.15	9.02	8.02	7.01	9.30	10.22	8.84
T_4(GA_3@100ppm)	7.10	8.25	9.28	8.21	8.66	9.78	10.47	9.64
T_5 (0.2 µM SA)	7.54	9.08	9.30	8.64	8.69	10.40	10.77	9.95
T_6 (0.4 µM SA)	8.20	8.27	9.55	8.67	9.30	9.54	11.40	10.08
Mean	7.11	8.07	8.99	8.05	8.35	9.25	10.15	9.25
	I	**T**	**I x T**		**I**	**T**	**I x T**	
S.Em±	0.09	0.14	0.24		0.11	0.16	0.27	
CD at 5 %	0.28	0.40	NS		0.32	0.45	0.79	
CV%	5.10				5.20			

Peroxidase (POX)

Peroxidase (POX) activity (ΔO.D./min./g, fresh weight basis) was measured from leaf samples of mustard plants subjected to different growth regulator treatments (T_1 to T_6) at 15 and 25 DAS, irrigated with three salinity levels tap water (I_0), 4 dS/m (I_1), and 8 dS/m (I_2) and sampled at two stages: 20 DAS (G_1) and 30 DAS (G_2). The data are presented in Table 2 and illustrated in Figures 3 and 4.

Salinity had a significant influence on POX activity, with the highest mean activity (30.02 ΔO.D./min./g) observed under I_2 (8 dS/m) and the lowest (17.09 ΔO.D./min./g) under I_0 (tap water). These results are in agreement with Patel *et al.* (2019), who reported elevated POX activity in response to increasing salinity. POX activity also increased with plant age, rising from 22.04 at 20 DAS to 23.74 ΔO.D./min./g at 30 DAS. Plant growth regulators significantly impacted POX activity. Among treatments, the highest

mean value was recorded in T_6 (0.4 µM SA, 30.86 ΔO.D./min./g), followed by T_5 (0.2 µM SA) and T_4 (100 ppm GA_3), while the control treatment (T_1) showed the lowest activity (16.09 ΔO.D./min./g), indicating the effectiveness of SA and GA_3 in enhancing antioxidant defense.

Significant interaction effects were observed between salinity and growth regulators (I × T), with POX activity ranging from 10.03 ΔO.D./min./g in I_0T_1 to 39.20 ΔO.D./min./g in I_2T_6. Similarly, growth stage and treatment interaction (G × T) showed a maximum POX activity of 31.67 ΔO.D./min./g in G_2T_6 and a minimum of 15.62ΔO.D./min./g in G_1T_1. The interaction between salinity and growth stage (I × G) revealed that I_2G_2 resulted in the highest POX activity (30.96 ΔO.D./min./g), while I_0G_1 recorded the lowest (16.54 ΔO.D./min./g). These findings support earlier reports by Simaei *et al.* (2012), Sofy (2016), and Mayank *et al.* (2018), who demonstrated that SA and GA_3 enhance POX activity under salinity stress, although effects may vary by species and dosage. While GA_3 has shown both inhibitory and stimulatory responses across studies (Tuna *et al.,* 2008; Ahmad, 2009), the current study indicates that moderate concentrations of SA and GA_3 can significantly enhance POX activity, aiding in salinity stress mitigation in Indian mustard.

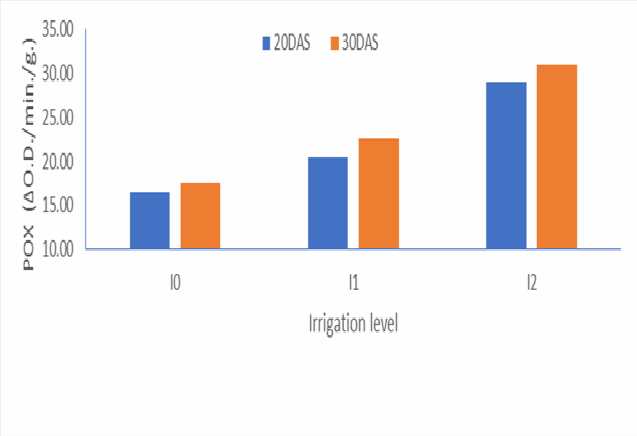

Fig. 3: Mean effect of plant growth regulators on leaf peroxidase (POX) (Δ O.D./min./g.) of Indian mustard under irrigation of tap and saline water at different growth stages.

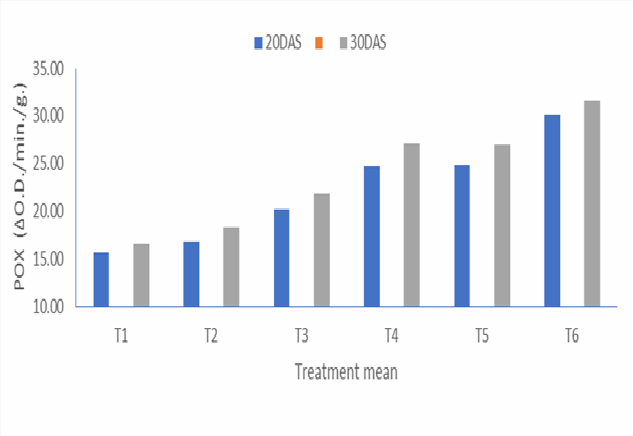

Fig. 4: Mean effect of plant growth regulators on leaf peroxidase (POX) (ΔO.D./min./g.) of Indian mustard under irrigation of saline water.

Table 2: Effect of plant growth regulators on leaf peroxidase (POX) O.D./min./g) of Indian mustard under irrigation of saline water.

Treatment (T)	20 DAS (G_1)			Mean (I)	30 DAS (G_2)			Mean (I)
	I_0 (Tap water)	I_1 (4 EC)	I_2 (8 EC)		I_0 (Tap water)	I_1 (4 EC)	I_2 (8 EC)	
T_1 (Control)	9.85	15.68	21.32	15.62	10.20	16.30	23.20	16.57
T_2 (Water spray)	10.74	16.11	23.42	16.76	11.44	18.42	25.01	18.29
T_3(GA_3@50ppm)	14.89	19.63	26.12	20.21	16.20	21.40	28.00	21.87
T_4(GA_3@100ppm)	18.87	24.26	31.00	24.71	20.10	27.30	33.73	27.04
T_5 (0.2 µM SA)	19.91	21.01	33.67	24.86	20.55	23.95	36.43	26.98
T_6 (0.4 µM SA)	24.97	26.21	38.99	30.06	27.40	28.20	39.40	31.67
Mean	16.54	20.48	29.09	22.04	17.65	22.60	30.69	23.74
	I	**T**	**I x T**		**I**	**T**	**I x T**	
S.Em±	0.28	0.40	0.69		0.30	0.43	0.74	
CD at 5 %	0.81	1.15	2.00		0.87	1.24	2.15	
CV%	5.50				5.10			

Catalase (CAT)

Catalase (CAT) activity (ΔO.D./min./g, fresh weight basis) was measured from mustard leaves collected at 20 and 30 days after sowing (DAS), following treatment with different plant growth regulators (T_1 to T_6) and irrigation with tap water (I_0),

4 dS/m saline water (I_1), and 8 dS/m saline water (I_2). The data are presented in Table 3 and Figures 5 and 6. Salinity had a statistically significant effect on CAT activity. The highest activity (24.82 ΔO.D./min./g) was recorded under 8 dS/m salinity (I_2), while the lowest (15.75 ΔO.D./min./g) occurred under control conditions (I_0). These results are in agreement with Tabatabaei (2013), who reported enhanced CAT activity under salt stress. However, contrasting findings have been noted by Trivedi *et al.* (2018), who observed a decline in greengram.

Across growth stages, CAT activity showed a marginal increase from 20.64 at 20 DAS (G_1) to 20.87 ΔO.D./min./g at 30 DAS (G_2). Growth regulator treatments also had a significant influence, with the highest mean CAT activity found in T_6 (22.14 ΔO.D./min./g), and the lowest in T_1 (19.40 ΔO.D./min./g), indicating that salicylic acid may enhance CAT activity under stress conditions. Interaction effects between salinity and treatment (I × T) and between growth stage and treatment (G × T) were statistically non-significant. Nevertheless, the lowest CAT activity (15.15 ΔO.D./min./g) was observed in I_0T_1, while the highest (26.66 ΔO.D./min./g) occurred in I_2T_6. Similarly, the lowest and highest activities in G × T were found in G_1T_1 (19.23) and G_2T_6 (22.25), respectively. The interaction between salinity and growth stage (I × G) was significant, with the minimum CAT activity in I_0G_1 (15.66 ΔO.D./min./g) and maximum in I_2G_2 (24.97 ΔO.D./min./g). These findings suggest that both salinity and PGRs influence CAT activity in mustard, consistent with Ghosh *et al.* (2015), who reported variable responses of antioxidant enzymes under combined salt and PGR treatments. Jini and Joseph (2017), along with Solanki *et al.* (2018), noted that high doses of salicylic acid can suppress CAT activity, indicating dose-dependent and species-specific responses. In the present study, moderate SA application appeared to support CAT activity under salt stress, contributing to enhanced oxidative stress tolerance.

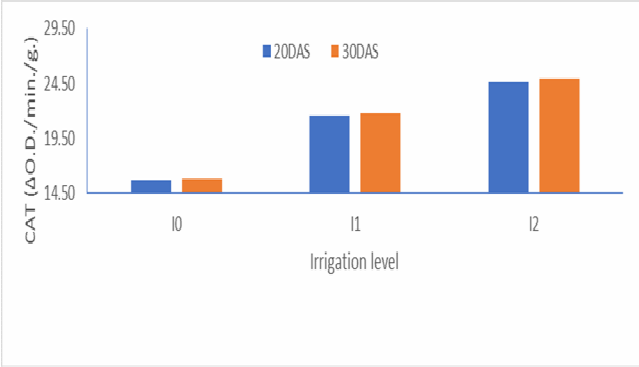

Fig. 5: Mean effect of plant growth regulators on leaf catalase (CAT) (ΔO.D./min./g) of Indian mustard under irrigation of Tap and saline water at different growth stages.

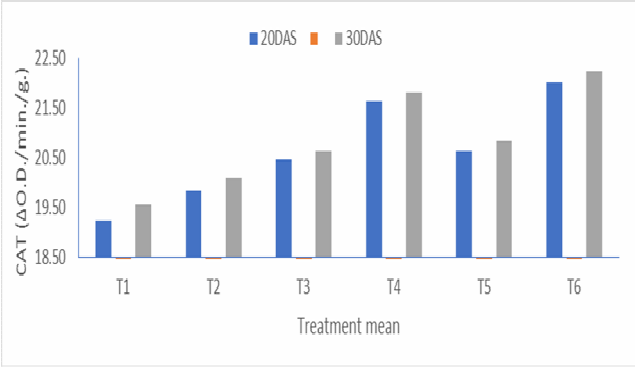

Fig. 6: Mean effect of plant growth regulators on leaf catalase (CAT) (ΔO.D./min./g) of Indian mustard under irrigation of saline water.

Table 3: Effect of plant growth regulators on leaf catalase (CAT) (ΔO.D./min./g) of Indian mustard under irrigation of saline water.

Treatment (T)	20 DAS (G_1)			Mean (I)	30 DAS (G_2)			Mean (I)
	I_0 (Tap water)	I_1 (4 EC)	I_2 (8 EC)		I_0 (Tap water)	I_1 (4 EC)	I_2 (8 EC)	
T_1 (Control)	15.00	19.80	22.90	19.23	15.30	20.01	23.40	19.57
T_2 (Water spray)	15.90	20.10	23.50	19.83	16.10	20.32	23.90	20.10
T_3 (GA_3@50ppm)	15.42	21.50	24.50	20.47	15.56	21.70	24.70	20.65
T_4(GA_3@100ppm)	15.99	23.30	25.62	21.63	16.12	23.60	25.71	21.81
T_5 (0.2 µM SA)	15.56	21.30	25.04	20.63	15.72	21.50	25.31	20.84
T_6 (0.4 µM SA)	16.07	23.50	26.51	22.02	16.25	23.70	26.82	22.25
Mean	15.66	21.58	24.67	20.64	15.84	21.80	24.97	20.87
	I	**T**	**I x T**		**I**	**T**	**I x T**	
S.Em±	0.25	0.36	0.63		0.26	0.36	0.63	
CD at 5 %	0.73	1.04	NS		0.74	1.05	NS	
CV%	5.21				5.29			

Ascorbate Peroxidase (APX)

Ascorbate peroxidase (APX) activity (ΔO.D./min./g, fresh weight basis) was measured from mustard leaves collected at 20 and 30 days after sowing (DAS) from plants treated with different growth regulator combinations (T_1 to T_6), grown under three salinity levels tap water (I_0), 4 dS/m (I_1), and 8 dS/m (I_2). The results are shown in Table 4 and Figures 7 and 8.

Salinity had a statistically significant effect on APX activity, with the highest mean value (12.15 ΔO.D./min./g) recorded under 8 dS/m salinity (I_2), and the lowest (6.60 ΔO.D./min./g) under control conditions (I_0). These findings are in line with earlier reports by Sairam *et al.* (2005) and Abdulaziz *et al.* (2014), who noted that salinity stress enhances APX activity as part of the plant's antioxidative defense response.Across growth stages, APX activity increased from 8.66 ΔO.D./min./g at 20 DAS (G_1) to 10.50 ΔO.D./min./g at 30 DAS (G_2), indicating progressive enhancement with plant maturity. Among growth regulator treatments, the highest activity was observed in the untreated stressed control (T_6: 12.17 ΔO.D./min./g), followed closely by T_4 (100 ppm GA_3) and T_5 (0.2 µM SA), suggesting that stress conditions alone induced a greater APX response compared to regulator-treated plants. Interaction effects were also notable. The salinity × treatment (I × T) interaction showed the lowest APX activity (5.45 ΔO.D./min./g) in I_0T_1 (tap water + untreated) and the highest (15.32 ΔO.D./min./g) in I_2T_6 (saline water + untreated control). Similar trends were observed across both growth stages, reflecting increased stress-induced enzyme activation. For the growth stage × treatment (G × T) interaction, the highest APX activity (13.50 ΔO.D./min./g) was noted in G_2T_6 (30 DAS + untreated), while the lowest (6.26 ΔO.D./min./g) occurred in G_1T_1 (20 DAS + untreated). The salinity × growth stage (I × G) interaction also showed significant differences, with maximum APX activity (13.18 ΔO.D./min./g) under I_2G_2 (8 dS/m + 30 DAS) and minimum (5.81 ΔO.D./min./g) under I_0G_1. These results confirm that both salinity and developmental stage significantly influence APX activity in mustard, consistent with reports by Madan *et al.* (2004), Cheruth *et al.* (2010), and Saeed (2016). The role of plant growth regulators in modulating APX under stress appears complex, with untreated plants showing higher activity likely due to unregulated oxidative stress. This suggests that APX plays a crucial role in the mustard plant's defense against salt-induced oxidative damage.

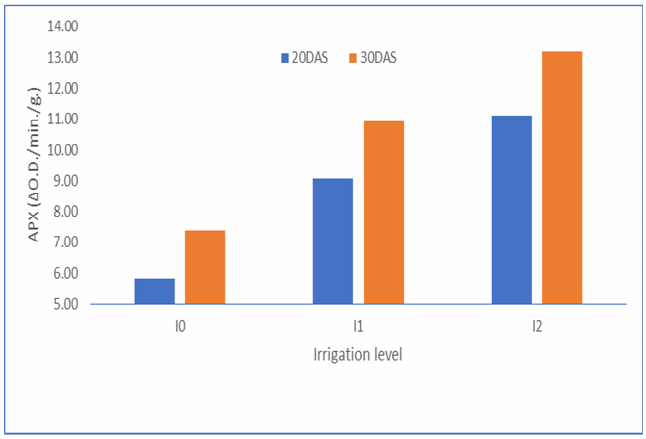

Fig. 7: Mean effect of plant growth regulators on leaf ascorbate peroxidase (APX) (ΔO.D./min./g) of Indian mustard under irrigation of tap and saline water at different growth stages.

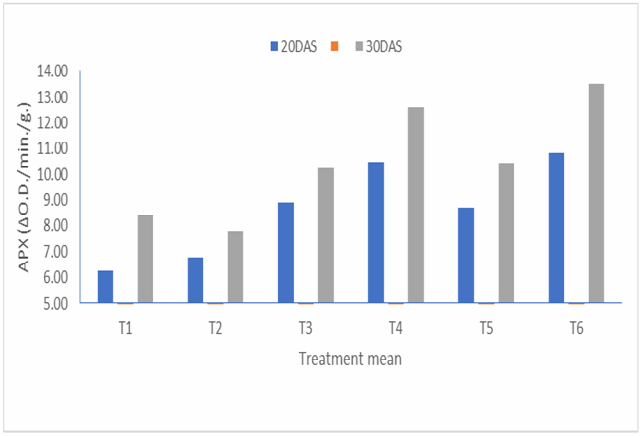

Fig. 8: Mean effect of plant growth regulators on leaf ascorbate peroxidase (APX) (ΔO.D./min./g) of Indian mustard under irrigation of saline water.

Table 4: Effect of plant growth regulators on leaf ascorbate peroxidase (APX) (ΔO.D./min./g) of Indian mustard under irrigation of saline water.

Treatment (T)	20 DAS (G_1)			Mean (I)	30 DAS (G_2)			Mean (I)
	I_0 (Tap water)	I_1 (4 EC)	I_2 (8 EC)		I_0 (Tap water)	I_1 (4 EC)	I_2 (8 EC)	
T_1 (Control)	4.57	6.12	8.10	6.26	6.33	8.20	10.70	8.41
T_2 (Water spray)	4.85	6.94	8.54	6.78	6.20	8.10	9.14	7.81
T_3(GA_3@50ppm)	5.75	9.89	11.10	8.91	6.47	10.74	13.50	10.24
T_4(GA_3@100ppm)	6.93	11.38	13.08	10.46	8.22	14.20	15.40	12.61

T_5 (0.2 µM SA)	5.33	9.48	11.33	8.71	6.88	10.22	14.22	10.44
T_6 (0.4 µM SA)	7.44	10.54	14.53	10.84	10.20	14.20	16.10	13.50
Mean	5.81	9.06	11.11	8.66	7.38	11.11	13.18	10.50
	I	**T**	**I x T**		**I**	**T**	**I x T**	
S.Em±	0.11	0.16	0.27		0.13	0.19	0.33	
CD at 5 %	0.32	0.45	0.78		0.38	0.54	0.94	
CV%	5.30				5.40			

Conclusion

The study demonstrated that salinity stress significantly elevated the activities of key antioxidant enzymes PPO, POX, CAT, and APX in Indian mustard, reflecting the plant's oxidative stress response. Exogenous application of gibberellic acid (GA_3) and salicylic acid (SA) effectively modulated these responses, with 0.4 µM SA showing the greatest enhancement in POX and APX activities, and 100 ppm GA_3 also contributing to stress mitigation. Enzyme activity was generally higher at 30 DAS than at 20 DAS, indicating progressive adaptation to stress. The significant interaction between salinity and PGR treatments, particularly in SA-treated plants, underscores the potential of SA in enhancing salinity tolerance. These findings suggest that PGRs, especially SA, offer promising strategies for improving mustard resilience under saline conditions. Further research under field conditions and at the molecular level is needed to validate and optimize these approaches for practical application.

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