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EFFECT OF FOLIAR APPLICATION OF GLYCINE BETAINE AND SALICYLIC ACID ON BIO-CHEMICAL INDICES OF LATE SOWN WHEAT (*TRITICUM AESTIVUM* L.)

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ABSTRACT

This study investigated the impact of foliar application of salicylic acid and glycine betaine on stress tolerance and antioxidant defense mechanisms in two wheat varieties, PBW-343 and Halna, during the *rabi* season of 2021-22 in Faizabad, Uttar Pradesh, India. Significant enhancements were observed in various biochemical parameters in response to the treatments. Glycine betaine and salicylic acid applications led to increases in chlorophyll content, total soluble sugars, proline content, peroxidase activity and catalase activity in both wheat varieties. Notably, glycine betaine treatments at 100mM concentration exhibited the most pronounced effects on chlorophyll content and proline accumulation, while salicylic acid treatments, especially at higher concentrations, significantly enhanced total soluble sugars content, peroxidase activity, and catalase activity. These findings suggest that foliar application of glycine betaine and salicylic acid effectively improves stress tolerance and antioxidant defense mechanisms in wheat plants. The results highlight the importance of treatment selection in mitigating stress-related damage and enhancing wheat crop productivity, particularly under challenging environmental conditions. This study contributes valuable insights into the physiological responses of wheat to exogenous applications of stress-alleviating compounds, offering potential strategies for crop improvement and sustainable agricultural practices.

Key words : Foliar application, Catalase activity, Peroxidase activity, Glycine betaine.

Introduction

Wheat is grown in many countries throughout the world including India. The production of wheat in world for year 2021/22 is 778.6 mmt (Statistica, 2022). The top wheat producing countries in the world are European Union (1,38,900m/t), China (1,36,946m/t), India (1,09,520m/t), Russia (75,500m/t), United States (44,790m/t) with their production in thousand metric tones' for year 2021-22 (Statistica, 2021/22). Wheat is the second important crop cultivated after rice in India. India ranks second in terms of area and production of wheat in year 2020-21. The area, yield and production of wheat in India was 31,357 thousand hectares, 3.4mt/hectare, 107,860 thousand metric tons respectively in year 2020-21 (USDA). In India wheat is mainly cultivated in states of Uttar Pradesh, Madhya Pradesh, Punjab, Rajasthan, Haryana. Wheat production in Uttar Pradesh for year 2020-21 is 35.77 lakh metric tons and for 2021-22 it is estimated about 53.80 lakh metric tons. Wheat has been grown since prehistoric- time, all available evidences suggest that origin of Wheat from North-East where wild form of wheat is cultivated. Wheat is annual plant of Poaceae (Gramineae) family it belongs to genus *Triticum*. The economically important species of wheat cultivated are, *Triticum aestivum*, *Triticum durum*, *Triticum dicoccum* in India. The chromosome number of wheat is 2n= 14, 28, 42 depending upon ploidy level of species. Fruit type of wheat is Caryopsis and inflorescence is called Spikelet. Wheat is a long day plant. The ideal wheat climate has winter temperature 10 to 15°C and summer temperature varying from 21 to 26°C. Wheat contains energy 1368kj (327Kcal), carbohydrates 71.18gm/100g, sugar 0.41gm, dietary fiber's 12.29gm, fat 1.54 gm, protein 12.61gm, water 13.1% and also contains various vitamins and minerals in 100gm whole Wheat.

Salicylic acid is a critical plant hormone that regulates numerous aspects of plant growth and development as well as activation of defense against biotic and abiotic stresses (Klessig *et al.*, 2018). Exogenously applied salicylic acid to stressed plant by various approaches was reported to induce major abiotic stress tolerance-mechanisms (Horvath *et al.*, 2007; Khan *et al.*, 2014; Anwar *et al.*, 2013). Exogenous application of glycine betaine improves thermotolerance in many plants by enhancing their growth and yield via counteracting metabolic maladjustments caused by HS. For example, while appraising the role of exogenous glycine betaine application on heat-stressed tomato plants (Li *et al.*, 2011) reported enhanced seed germination, expression of heat shock genes and accumulation of heat-shock protein 70 (HSP70). Likewise, exogenous glycine betaine supplementation likely controls many other key metabolic processes in heat-stressed plants. Furthermore, glycine betaine supplied to sprouting sugarcane nodal buds under HS markedly reduced H_2O_2 generation, increased K^+ and Ca^{2+} contents, and increased the levels of endogenous glycine betaine, free proline, and soluble sugars, enhancing the overall growth (Rasheed *et al.*, 2011). The high-temperature-induced oxidative stress in marigold was mitigated due to reduced levels of H_2O_2, peroxide, superoxide, and lipid peroxidation (Sorwong *et al.*, 2015). In apple, the application of glycine betaine enhanced photosynthesis under individual HS or drought stress and combined stresses (Wang *et al.*, 2020).

Materials and Methods

Experimental site

A field experiment was conducted during *rabi* season of 2021-22 at College of Research, Acharya Narendra Deva University of Agriculture and Technology, Kumarganj, Ayodhya University in Faizabad, Uttar Pradesh, India (Fig. 1). The experimental plot is situated at latitude of 24.4^0 to 26.56^0 N and longitude of 82.12^0 to 83.98^0E on an elevation of 113 meters in the gangetic alluvium of eastern Uttar Pradesh.

Weather conditions during the crop season

The meteorological data on weather conditions prevailing during *rabi* season of year 2021-22 have been illustrated in Fig. 2.

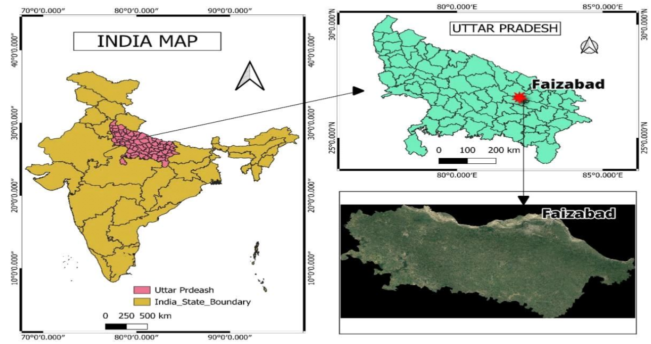

Fig. 1 : Map of the experimental area, Faizabad, Uttar Pradesh, India.

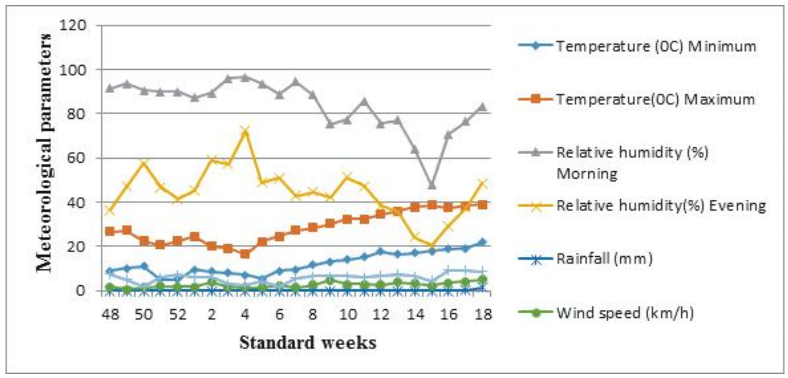

Fig. 2 : Meteorological data during the crop season (2021-22).

Experimental Procedure

Experimental details

Number of treatments: 7:

T_1 (Control)

T_2 (glycine betaine 50milli molar)

T_3 (glycine betaine 100milli molar)

T_4 (glycine betaine 150milli molar)

T_5 (salicylic acid 150ppm)

T_6 (salicylic acid 250ppm)

T_7 (salicylic acid 350ppm)

Variety : V_1 (PBW-343), V_2 (Halna)

Preparations of solutions of salicylic acid and glycine betaine for foliar application treatments

The different concentrations of salicylic acid were prepared in desired volume of water to make 150ppm, 250ppm, 350ppm respectively. In order to improve spray retention sticky agent teepol was mixed into the spray solution @ 0.5ml/litre. Similarly different concentration of glycine betaine was prepared in one litre of water to make 50mM, 100mM, 150mM solution. Foliar application was done with the help of knapsack sprayer at 50 days after sowing.

Biochemical parameters

All the biochemical parameters were done in leaves at 60 days and 90 days after sowing.

Total chlorophyll content

The total chlorophyll content was estimated using the method described by Arnon (1949) and expressed as milligrams per gram of fresh weight.

Total soluble sugars

Total soluble sugar was determined following the method described by Yemm and Wills (1954) and expressed as milligrams per gram of fresh weight.

Free Proline content in leaves

The free proline content in leaves was estimated spectrophotometrically using the method outlined by Bates *et al*. (1973).

Peroxidase activity estimation

The activity of peroxidase was determined using the method described by Curne and Galston (1959).

Catalase activity estimation

Catalase activity was assayed calorimetrically according to the method provided by Sinha (1972).

Statistical analysis

All the data generated in this research were, at least in four replicates for statistical reliability. Using SPSS version 20.0 data were subjected to one-way ANOVA with CD at 1% and 5% level of significance and DMRT (Duncan's multiple range test) method used for statistical analysis using R Studio software.

Results and Discussion

Impact of Glycine Betaine and Salicylic Acid Treatments on Chlorophyll Content in PBW-343 and Halna Wheat varieties

The leaf chlorophyll contents (mg g^{-1} fresh weight) were assessed in PBW-343 (V_1) and Halna (V_2) wheat varieties across various treatments at 60 and 90 days after sowing. Comparing the remaining treatments with T_1 (Control), significant differences were observed. For PBW-343, T_3 (Glycine Betaine 100mM) showed the highest chlorophyll content at both 60 and 90 days after sowing (3.28 and 2.98, respectively), followed by T_6 (Salicylic Acid 250ppm) (3.21 and 2.92) and T_4 (Glycine Betaine 150mM) (2.86 and 2.58). Similarly, for Halna, T_3 also resulted in the highest chlorophyll content (3.18 and 3.08), followed by T_6 (3.12 and 3.03) and T_4 (2.78 and 2.70) (Table 1). These results indicate that glycine betaine treatments at 100mM concentration significantly increased chlorophyll content in both PBW-343 and Halna varieties, suggesting a positive effect on photosynthesis. Additionally, salicylic acid treatments also showed positive effects on chlorophyll content, with higher concentrations generally leading to increased content. These findings highlight the importance of treatment selection in regulating chlorophyll content in wheat plants, with differences observed between varieties. Similar findings were found by Yildirim *et al*. (2008) and Zhao *et al*. (2007) concluded that foliar application of salicylic acid and glycine betaine increased total chlorophyll content under stress condition. The increase in chlorophyll content may be due to role played in protecting the photosynthetic machinery from oxidative damage.

Effect of Glycine Betaine and Salicylic Acid Treatments on Total Soluble Sugars Content in PBW-343 and Halna Wheat varieties

Total soluble sugars content (mg g^{-1} fresh weight) was assessed in PBW-343 (V_1) and Halna (V_2) wheat varieties under various treatments at 60 and 90 days after sowing. Significant differences were observed when comparing other treatments with T_1. For PBW-343, T_6 (Salicylic Acid 250ppm) showed the highest total soluble sugars content at both 60 and 90 days after sowing (98.30 and 101.92, respectively), followed by T_3 (Glycine Betaine 100mM) (98.70 and 101.62) and T_4 (Glycine Betaine 150mM) (97.40 and 100.88). Similarly, for Halna, T_6 also resulted in the highest total soluble sugars content (95.06 and 98.86), followed by T_3 (95.06 and 98.86) and T_4 (94.09 and 97.85) (Table 2). These results suggest that treatments with glycine betaine and salicylic acid significantly increased total soluble sugars content in both PBW-343 and Halna varieties, indicating their potential roles in enhancing sugar accumulation. The variation observed underscores the importance of treatment selection in regulating sugar metabolism in wheat, with differences noted between varieties. The results are in confirmity with Mathur and Vyas (2007).

Impact of Glycine Betaine and Salicylic Acid Treatments on Proline Content in PBW-343 and Halna Wheat Varieties

Proline content (mg g^{-1} fresh weight) was measured in PBW-343 (V_1) and Halna (V_2) wheat varieties under various treatments at 60 and 90 days after sowing. Comparing the other treatments with T_1 (Control), significant differences were observed. For PBW-343, T_3 (Glycine Betaine 100mM) exhibited the highest proline content at both 60 and 90 days after sowing (195.65 and 240.79, respectively), followed by T_6 (Salicylic Acid 250ppm) (175.12 and 249.23) and T_5 (Salicylic Acid 150ppm) (167.95 and 178.03). Similarly, for Halna, T_3

Table 1 : Effect of foliar application of Glycine betaine and Salicylic acid on Chlorophyll content (mg g^{-1} fresh weight) in PBW-343 (V_1) and Halna (V_2) wheat varieties under various treatments at 60 and 90 days after sowing. Different letters represent significant differences between treatments within each sampling time for each variety, based on DMRT statistical analysis.

Treatments	Leaf chlorophyll contents (mg g^{-1} fresh weight)					
	60 Days after sowing		90 Days after sowing		Pooled mean	
	V_1	V_2	V_1	V_2	V_1	V_2
T_1 (Control)	1.36^f	1.32^d	1.12^d	1.28^f	1.24^f	1.30^d
T_2 (glycine betaine 50mM)	2.64^{de}	2.56^{bc}	2.36^c	2.48^{de}	2.50^{de}	2.52^{bc}
T_3 (glycine betaine 100mM)	3.28^a	3.18^a	2.98^a	3.08^a	3.13^a	3.13^a
T_4 (glycine betaine 150mMr)	2.86^{cd}	2.78^b	2.58^b	2.70^{cd}	2.72^{cd}	2.74^b
T_5 (salicylic acid 150ppm)	2.52^e	2.45^c	2.25^c	2.38^e	2.38^e	2.38^c
T_6 (salicylic acid 250ppm)	3.21^{ab}	3.12^a	2.92^a	3.03^{ab}	3.07^{ab}	3.08^a
T_7 (salicylic acid 350ppm)	2.96^{bc}	2.87^{ab}	2.67^b	2.78^{bc}	2.82^{bc}	2.83^{ab}
SEm	**0.04**	**0.02**	**0.01**	**0.03**	**0.03**	**0.04**
CD at 5 %	**0.12**	**0.06**	**0.04**	**0.09**	**0.07**	**0.10**
CV %	**2.90**	**1.51**	**1.11**	**2.49**	**1.97**	**2.79**

Table 2 : Effect of foliar application of Glycine betaine and Salicylic acid on Total soluble sugars (mg g^{-1} fresh weight) in PBW-343 (V_1) and Halna (V_2) wheat varieties under various treatments at 60 and 90 days after sowing. Different letters represent significant differences between treatments within each sampling time for each variety, based on DMRT statistical analysis.

Treatments	Total soluble sugars (mg g^{-1} fresh weight)					
	60 Days after sowing		90 Days after sowing		Pooled mean	
	V_1	V_2	V_1	V_2	V_1	V_2
T_1 (Control)	85.10^e	82.45^e	88.40^e	85.75^e	86.75^e	84.10^e
T_2 (glycine betaine 50mM)	88.35^d	85.36^d	91.52^d	88.77^d	89.94^d	87.07^d
T_3 (glycine betaine 100mM)	98.70^a	95.06^a	101.62^a	98.86^a	100.31^a	96.96^a
T_4 (glycine betaine 150mMr)	97.4^{ab}	94.09^{ab}	100.88^{ab}	97.85^{ab}	99.14^{ab}	95.97^{ab}
T_5 (salicylic acid 150ppm)	96.35^b	93.12^b	99.84^b	96.84^b	98.10^b	94.98^b
T_6 (salicylic acid 250ppm)	98.30^a	95.06^a	101.92^a	98.86^a	100.11^a	96.96^a
T_7 (salicylic acid 350ppm)	91.25^c	88.27^c	94.64^c	91.80^c	92.95^c	90.04^c
SEm	**0.91**	**1.07**	**0.97**	**1.19**	**0.82**	**0.69**
CD at 5 %	**2.17**	**3.19**	**2.90**	**3.52**	**2.44**	**2.05**
CV %	**1.95**	**2.37**	**2.01**	**2.52**	**1.76**	**1.52**

also showed the highest proline content (190.40 and 242.30), followed by T_6 (170.50 and 241.75) and T_5 (162.91 and 172.69) (Table 3). These results indicate that glycine betaine treatments at 100mM concentration significantly increased proline content in both PBW-343 and Halna varieties, followed by salicylic acid treatments. The variation observed underscores the importance of treatment selection in regulating proline accumulation in wheat plants, with differences noted between varieties. The results are in agreement with Ding *et al*. (2010) and Gupta and Agarwal (2013). Proline has been assigned the work of cyst solute a storage compound or a protective agent for cytoplasmic enzymes and cellular structure, increased proline content may be utilized during recovery and thereby help to reduce damage to plant cell and maintain membrane integrity.

Effect of Salicylic Acid and Glycine Betaine Treatments on Peroxidase Activity in PBW-343 and Halna Wheat varieties

Peroxidase activity (mg g^{-1} fresh weight min^{-1}) was examined in PBW-343 (V_1) and Halna (V_2) wheat varieties under various treatments at 60 and 90 days after sowing. When comparing the other treatments with T1 (Control), significant differences were observed. For PBW-343, T_6 (Salicylic Acid 250ppm) exhibited the highest peroxidase activity at both 60 and 90 days after sowing (235.05 and 228.20, respectively), followed by

Table 3 : Effect of foliar application of Glycine betaine and Salicylic acid on Proline content (mg g^{-1} fresh weight) in PBW-343 (V_1) and Halna (V_2) wheat varieties under various treatments at 60 and 90 days after sowing. Different letters represent significant differences between treatments within each sampling time for each variety, based on DMRT statistical analysis.

Treatments	Proline content (mg g^{-1} fresh weight)					
	60 Days after sowing		90 Days after sowing		Pooled mean	
	V_1	V_2	V_1	V_2	V_1	V_2
T_1 (Control)	132.23^g	128.26^g	140.16^f	135.96^f	136.20^g	132.11^g
T_2 (glycine betaine 50mM)	145.65^f	141.28^f	154.39^e	149.76^e	150.02^f	145.52^f
T_3 (glycine betaine 100mM)	195.65^a	190.40^a	249.79^a	242.30^a	222.72^a	216.35^a
T_4 (glycine betaine 150mMr)	158.95^e	154.18^e	168.49^d	163.43^d	163.72^e	158.81^e
T_5 (salicylic acid 150ppm)	167.95^c	162.91^c	178.03^c	172.69^c	172.99^d	167.80^d
T_6 (salicylic acid 250ppm)	175.12^b	170.50^b	249.23^a	241.75^a	212.18^b	206.13^b
T_7 (salicylic acid 350ppm)	165.20^d	160.67^d	223.66^b	216.95^b	194.43^c	188.81^c
SEm	**1.70**	**1.68**	**1.85**	**2.21**	**1.99**	**2.16**
CD at 5 %	**5.05**	**5.01**	**5.51**	**6.58**	**5.92**	**6.41**
CV %	**2.09**	**2.13**	**1.91**	**2.34**	**2.23**	**2.48**

Table 4 : Effect of foliar application of Glycine betaine and Salicylic acid on Peroxidase activity (mg g^{-1} fresh weight min^{-1}) in PBW-343 (V_1) and Halna (V_2) wheat varieties under various treatments at 60 and 90 days after sowing. Different letters represent significant differences between treatments within each sampling time for each variety, based on DMRT statistical analysis.

Treatments	Peroxidase activity (mg g^{-1} fresh weight min^{-1})					
	60 Days after sowing		90 Days after sowing		Pooled mean	
	V_1	V_2	V_1	V_2	V_1	V_2
T_1 (Control)	190.45^e	195.76^e	190.09^d	200.79^d	190.27^e	198.28^e
T_2 (glycine betaine 50mM)	222.12^d	213.23^d	215.55^c	209.18^c	218.84^d	211.21^d
T_3 (glycine betaine 100mM)	235.1^a	225.69^a	226.95^a	221.22^a	231.03^a	223.46^a
T_4 (glycine betaine 150mMr)	225.23^c	216.22^c	218.67^b	211.91^b	221.95^c	214.07^c
T_5 (salicylic acid 150ppm)	228.42^b	216.99^{bc}	219.45^b	213.46^b	223.94^{bc}	215.23^c
T_6 (salicylic acid 250ppm)	235.05^a	218.63^b	228.20^a	221.25^a	231.63^a	219.94^b
T_7 (salicylic acid 350ppm)	227.74^b	215.42^c	221.11^b	214.17^b	224.43^b	214.80^c
SEm	2.82	1.87	2.89	2.39	1.85	1.93
CD at 5%	8.38	5.57	8.59	7.11	5.51	5.74
CV %	2.53	1.75	2.66	2.25	1.68	1.81

T_3 (Glycine Betaine 100mM) (235.1 and 226.95) and T_7 (Salicylic Acid 350ppm) (227.74 and 221.11). Similarly, for Halna, T_6 also showed the highest peroxidase activity (218.63 and 221.25), followed by T_3 (225.69 and 221.22) and T_7 (215.42 and 214.17) (Table 4). These results indicate that treatments with salicylic acid, particularly at higher concentrations, significantly increased peroxidase activity in both PBW-343 and Halna varieties, suggesting a potential role in enhancing plant defense mechanisms. The variation observed underscores the importance of treatment selection in regulating peroxidase activity in wheat plants, with differences noted between varieties. The results are in obedience to (2010), Kabiri *et al*. (2012), Hasanuzzaman *et al*. (2012). Reactive oxygen species are formed due to stress in plants which react proteins, lipids and DNA impairing the normal activities of plants these reactive oxygen species are scavenged by antioxidative enzymes such as catalase, peroxidase and superoxide dismutase. There is positive coorelation between antioxidant enzyme and grain yield.

Catalase Activity in PBW-343 and Halna Varieties under different Treatments

Catalase activity (mg g^{-1} fresh weight min^{-1}) was evaluated in PBW-343 (V_1) and Halna (V_2) wheat varieties across various treatments at 60 and 90 days after sowing. Significant differences were noted when

Table 5 : Effect of foliar application of Glycine betaine and Salicylic acid on Catalase activity (mg g^{-1} fresh weight min^{-1}) in PBW-343 (V_1) and Halna (V_2) wheat varieties under various treatments at 60 and 90 days after sowing. Different letters represent significant differences between treatments within each sampling time for each variety, based on DMRT statistical analysis.

Treatments	Catalase activity (mg g^{-1} fresh weight min^{-1})					
	60 Days after sowing		90 Days after sowing		Pooled mean	
	V_1	V_2	V_1	V_2	V_1	V_2
T_1 (Control)	132.12^e	128.16^e	135.12^e	130.16^f	133.62^e	129.16^f
T_2 (glycine betaine 50mM)	144.65^d	140.31^d	147.65^d	142.31^e	146.15^d	141.31^e
T_3 (glycine betaine 100mM)	182.14^a	176.68^a	187.14^a	181.68^b	184.64^a	179.18^b
T_4 (glycine betaine 150mMr)	155.85^c	151.17^c	160.85^c	156.17^d	158.35^c	153.67^d
T_5 (salicylic acid 150ppm)	165.89^b	160.91^b	172.89^b	169.89^c	169.39^b	165.40^c
T_6 (salicylic acid 250ppm)	182.10^a	176.64^a	189.10^a	186.10^a	185.60^a	181.37^a
T_7 (salicylic acid 350ppm)	156.98^c	152.27^c	159.98^c	157.27^d	158.48^c	154.77^d
SEm	1.96	1.64	1.95	1.87	1.37	1.43
CD at 5%	5.83	4.86	5.79	5.57	4.06	4.25
CV %	2.45	2.11	2.37	2.34	1.68	1.81

comparing the other treatments with T_1. For PBW-343, T_6 (Salicylic Acid 250ppm) showed the highest catalase activity at both 60 and 90 days after sowing (182.10 and 189.10, respectively), followed by T_3 (Glycine Betaine 100mM) (182.14 and 187.14) and T_5 (Salicylic Acid 150ppm) (165.89 and 172.89). Similarly, for Halna, T_6 also exhibited the highest catalase activity (176.64 and 186.10), followed by T_3 (176.68 and 181.68) and T5 (160.91 and 169.89) (Table 5). These results suggest that treatments with salicylic acid, especially at higher concentrations, significantly increased catalase activity in both PBW-343 and Halna varieties, indicating their potential role in enhancing antioxidant defense mechanisms. The results are in line with Gupta and Agarwal (2013). Reactive oxygen species are formed due to stress in plants which react proteins , lipids and DNA impairing the normal activities of plants these reactive oxygen species are scavenged by antioxidative enzymes such as catalase, peroxidase and superoxide dismutase. There is positive coorelation between antioxidant enzyme and grain yield.

Conclusion

In conclusion, the findings of this study demonstrate the efficacy of foliar application of glycine betaine and salicylic acid in enhancing stress tolerance and antioxidant defense mechanisms in wheat varieties PBW-343 and Halna. The treatments led to significant increases in chlorophyll content, total soluble sugars, proline accumulation, and activities of peroxidase and catalase enzymes. Glycine betaine at 100mM concentration notably improved chlorophyll content and proline accumulation, while salicylic acid treatments, particularly at higher concentrations, enhanced total soluble sugars content and activities of peroxidase and catalase enzymes. These results suggest that exogenous application of these compounds can effectively mitigate oxidative stress and improve stress tolerance in wheat plants, highlighting their potential for enhancing crop productivity, especially under adverse environmental conditions. Further research is warranted to explore the long-term effects of these treatments on wheat growth, yield, and quality, as well as their potential application in field conditions to promote sustainable agriculture.

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