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Exogenous spermine mediated response of glutathione reductase and glutathione peroxidase under salinity induced stress in wheat (*Triticum aestivum em Thell.*)

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Abstract
Wheat production is adversely affected by soil salinity due to limited access to irrigation facilities. Effect of soil salinity on four wheat varieties *viz.* DBW 88, HD 3086, Kharchia 65 and KRL 210 differing in tolerance to salinity have been investigated in the present study. Antioxidant enzymes are crucial in defusing reactive oxygen species (ROS) produced during salinity stress condition. Glutathione reductase (GR) and Glutathione peroxidase (GPx) constitute effective enzymatic anti-oxidant system whose responses are detrimental in imparting stress tolerance. GR activity was linearly increased against increasing salinity in all four wheat varieties studied and more basal increase was observed in tolerant varieties.
Higher GPx activities were observed in tolerant varieties and showed slight decreased GPx activities in susceptible wheat varieties under salinity stress. Exogenous application of spermine (Spm) increased the responses of GR and GPx. From our study it was noticed that exogenous spermine application enhances the activities of GR and GPx and provides stress tolerance to wheat crop against salinity induced oxidative stress.

Keywords: Wheat, salinity spermine, glutathione reductase and glutathione peroxidase

Introduction

Soil salinity is one of the major impediments in wheat production, particularly in arid and semi-arid regions where availability to limited irrigation affects crop growth and development. Accumulation of salts at topsoil as a result of continuous dry spells imparts stress on crops at various developmental stages. Further, high salinity in the soil inhibits water uptake by plant roots and it also affects the metabolism of cell growth upon reaching to toxic concentrations (Wani *et al.,* 2021) [20]. Prolonged salinity leads to the development of adaptive strategies in plants including regulation of osmotic adjustment, ion homeostasis for minimal cell damage, regaining antioxidant defense and other tolerance mechanisms (Kumar *et al.,* 2021) [8]. Plant salt tolerance ability is not uniform that differs greatly from crop to crop where levels of crop loss depend on the respective levels of tolerance mechanisms stimulated. (Kumar *et al.,* 2021) [8]. There are several strategies to improve plant growth in salinity-affected environments (Faride *et al.,* 2020) [5].

Antioxidant defense systems protect plants against Reactive Oxygen Species (ROS) generation like superoxide ions ($^{\cdot}O_2^{-}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($^{\cdot}OH^{-}$) which create oxidative stress. Mitigation of stress through application of exogenous protectants such as osmoregulators (proline, trehalose, etc.), plant hormones (Gibberline, Salicylic acid, etc.), antioxidants (ascorbic acid, tocopherol etc.), chitin, and polyamines are well known in the elimination of ROS. The exogenous application of polyamine effectively alleviates the damage caused by salt stress and also enhances the activities of different antioxidant enzymes. (Qian *et al.,* 2021) [13]. Enzymatic antioxidant defense components such as superoxide dismutase (SOD), glutathione reductase (GR), glutathione dehydrogenase (GDH), glutathione peroxidase (GPx), glutathione-S transferase (GST) play a vital role in defusing ROS (Latique *et al.,* 2021) [9]. The main objective of this study is to understand the response of glutathione reductase (GR) and glutathione peroxidase (GPx) and the effects of exogenously applied spermine under salinity-induced stress conditions.

Materials and Methods

Plant material

Seeds of the four varieties *viz.* DBW 88, HD 3086, Kharchia 65 and KRL 210 differing in tolerance to salinity were obtained from Wheat and Barley Section, Department of Genetics and Plant Breeding, CCS HAU, Hisar. Kharchia 65 and KRL 210 are tolerant varieties and other varieties are to be identified for tolerance to salinity.

Raising of the crop

Seeds of wheat varieties were surface sterilized by soaking them for 5 min with 0.2 per cent (w/v) solution of mercuric chloride. These surface sterilized seeds were grown in earthen pots lined with polyethylene bags filled with 6 kg dune sand in a screen house under naturally lit conditions. The nutrient solution was used to irrigate the soil supplemented with nutrients in the form of N, P and K in the ratio of 10:3:3.

Artificial saline treatment

Saline solutions containing 8 and 12 dSm^{-1} were prepared separately for irrigating wheat plants grown in pots. The salinity of the solution was maintained by dissolving various quantities of chloride and sulfate of calcium, magnesium and sodium in distilled water in which Na: Ca+Mg ratio was 1:1; Ca: Mg was 1:3 and that of Cl: SO_4 was 7:3. Plants were regularly watered with distilled water after giving salinity treatment.

Spermine treatment

Spermine at 0.5 and 1.0 mM concentration was sprayed over the plants at 21 and 90 days after sowing (DAS) and superoxide dismutase and ascorbate peroxidase activities were studied at 5, 10 and 15 days after treatment (DAT).

Antioxidant enzymes

Extraction

Two gram of leaf sample was homogenized using pre-chilled pestle and mortar in 5 ml of cold extraction buffer containing 0.1 M phosphate buffer (pH 7.0), 2.5 mM DDT and 1 mM EDTA. Then, the homogenate was centrifuged at 10,000 rpm for 30 min. The whole procedure of preparation of enzyme extract was carried out at 0 - 4 ^{0}C. The supernatant was used for enzymatic assay for determining the activity of glutathione reductase (GR) and glutathione peroxidase (GPx).

Glutathione reductase (EC 1.6.4.2)

Glutathione reductase was determined at 25^0 C by determining the rate of NADPH oxidation- decrease in absorbance at 340 nm (Esterbauer & Grill, 1978). The assay mixture was prepared in cuvette by the addition of 0.25 ml 0.1 M phosphate buffer (pH 7.5), 0.05 ml of 50 mM $MgCl_2$, 0.025 ml of 0.16 M GSSG and 0.05 ml of enzyme extract. The reaction was initiated by adding 25 µl of 8 mM NADPH and monitored the decrease in absorbance at 340 nm. The activity of GR was calculated by using the molar extinction coefficient of NADPH at 340 nm (ε= 6.22 mM cm^{-1}). One unit of enzyme activity is defined as the amount of enzyme required to oxidize one nmol of NADPH per min.

Glutathione peroxidase (EC 1.11.1.9)

The glutathione peroxidase activity was determined by the method based on continuous regeneration of oxidized glutathione produced by the action of glutathione peroxidase (Nagalakshmi & Prasad, 2001) [12]. The reaction mixture consisted of 2.0 ml 0.1 M phosphate buffer (pH 8.0), 0.1 ml of 1 M NaCl, 75 µl of 10 mM EDTA, 0.3 ml of 10 mM GSH, 0.15 ml of 8 mM NADPH, 25 µl of GR (100 U ml^{-1}), 0.25 ml of enzyme extract and 0.1 ml of 25 mM H_2O_2. The kinetic changes in the absorbance were measured by the decrease in the absorbance at 340 nm for 15 min at 30^0 C. One enzyme activity unit was defined as the amount of enzyme required to oxidize one mM NADPH per min. The activity of GPx was calculated by using the molar extinction coefficient of NADPH at 340 nm (ε= 6.22 mM cm^{-1}).

Results

Table 1: Changes in glutathione reductase activity in wheat flag leaf under different levels of salinity and spermine treatment at 21 days after sowing

Glutathione reductase activity (units g^{-1} FW)											
Variety	21 DAS	Saline and spermine treatment									Mean
		Control			8 dSm^{-1}			12 dSm^{-1}			
		Spm^0	Spm0.5mM	Spm1.0mM	Spm^0	Spm0.5mM	Spm1.0mM	Spm^0	Spm0.5mM	Spm1.0mM	
DBW 88	0 DAT	1.78	1.78	1.78	2.31	2.31	2.31	2.88	2.88	2.88	2.32
	5 DAT	1.75	1.94	2.01	2.32	2.60	2.63	2.90	3.07	3.16	2.49
	10 DAT	1.90	2.13	2.10	2.48	2.78	2.68	3.17	3.39	3.35	2.66
	15 DAT	2.06	2.30	2.24	2.66	3.00	2.81	3.40	3.73	3.52	2.86
HD 3086	0 DAT	1.81	1.81	1.81	2.45	2.45	2.45	3.18	3.18	3.18	2.48
	5 DAT	1.84	2.09	2.19	2.54	2.88	2.92	3.31	3.53	3.64	2.77
	10 DAT	1.95	2.27	2.22	2.66	3.04	2.91	3.56	3.84	3.80	2.92
	15 DAT	2.14	2.47	2.37	2.87	3.29	3.06	3.84	4.26	3.99	3.14
Kharchia 65	0 DAT	2.14	2.14	2.14	3.18	3.18	3.18	4.38	4.38	4.38	3.23
	5 DAT	2.10	2.60	2.80	3.23	3.81	3.93	4.46	4.94	5.25	3.68
	10 DAT	2.24	2.86	2.77	3.37	4.02	3.85	4.82	5.40	5.46	3.87
	15 DAT	2.39	3.01	2.83	3.53	4.22	3.89	5.03	5.85	5.53	4.03
KRL 210	0 DAT	2.02	2.02	2.02	2.95	2.95	2.95	4.03	4.03	4.03	3.00
	5 DAT	2.02	2.44	2.60	3.05	3.60	3.69	4.17	4.66	4.89	3.46
	10 DAT	2.15	2.67	2.59	3.18	3.79	3.61	4.50	5.09	5.08	3.63
	15 DAT	2.31	2.83	2.68	3.35	4.00	3.69	4.73	5.54	5.18	3.81
Mean		2.04	2.33	2.32	2.88	3.24	3.16	3.90	4.24	4.21	
CD at 5% level								Where,			
a →	0.02	ab →	0.04	bd → 0.04 acd→ 0.09				a →	Varieties		

b →	0.02	ac→	0.04	cd →	0.04	bcd→	NS	b →	Artificial saline treatment
c →	0.02	ad →	0.05	abd →	NS	abcd→	NS	c →	Spermine treatment
d →	0.02	bc →	0.04		abc→	NS		d →	Days after treatment (DAT)

Table 2: Changes in glutathione reductase activity in wheat flag leaf under different levels of salinity and spermine treatment at 90 days after sowing

Glutathione reductase activity (units g-1 FW)											
Variety	**90 DAS**	**Saline and spermine treatment**									**Mean**
		Control			**8 dSm-1**			**12 dSm-1**			
		Spm0	**Spm0.5mM**	**Spm1.0mM**	**Spm0**	**Spm0.5mM**	**Spm1.0mM**	**Spm0**	**Spm0.5mM**	**Spm1.0 mM**	
DBW 88	0 DAT	2.62	2.96	3.04	3.50	3.67	3.80	4.64	4.83	5.14	3.80
	5 DAT	2.51	2.83	2.94	3.48	3.72	3.77	4.36	4.95	4.86	3.71
	10 DAT	2.26	2.52	2.56	2.96	3.22	3.27	3.83	4.39	4.20	3.25
	15 DAT	2.16	2.45	2.37	2.81	3.10	2.99	3.56	3.97	3.81	3.02
HD 3086	0 DAT	2.71	3.17	3.29	3.79	3.99	4.15	5.27	5.52	5.91	4.20
	5 DAT	2.67	3.10	3.27	3.87	4.18	4.24	5.07	5.85	5.72	4.22
	10 DAT	2.35	2.71	2.76	3.21	3.52	3.59	4.35	5.07	4.82	3.60
	15 DAT	2.25	2.65	2.53	3.05	3.40	3.27	4.03	4.57	4.35	3.34
Kharchia 65	0 DAT	2.93	3.80	4.02	4.56	4.92	5.22	6.81	7.37	8.20	5.31
	5 DAT	2.86	3.68	4.00	4.67	5.19	5.35	6.56	7.98	7.86	5.35
	10 DAT	2.54	3.20	3.31	3.83	4.35	4.50	5.56	6.82	6.59	4.52
	15 DAT	2.43	3.17	2.95	3.63	4.20	4.04	5.11	6.08	5.88	4.17
KRL 210	0 DAT	2.86	3.60	3.78	4.37	4.71	4.98	6.46	6.98	7.75	5.05
	5 DAT	2.80	3.49	3.76	4.48	4.98	5.11	6.26	7.56	7.40	5.09
	10 DAT	2.49	3.06	3.15	3.70	4.20	4.31	5.31	6.49	6.28	4.33
	15 DAT	2.40	3.03	2.84	3.52	4.08	3.89	4.91	5.78	5.63	4.01
Mean		2.55	3.09	3.16	3.71	4.09	4.16	5.13	5.89	5.90	
CD at 5% level								Where,			
a →	0.03	ab →	0.05	bd →	0.05	acd→	NS	a →	Varieties		
b →	0.03	ac →	0.05	cd →	0.05	bcd→	0.09	b →	Artificial saline treatment		
c →	0.03	ad →	0.06	abd →	0.10	abcd→	NS	c →	Spermine treatment		
d →	0.03	bc →		0.04	abc→	0.09		d →	Days after treatment (DAT)		

Table 3: Changes in glutathione peroxidase activity in wheat flag leaf under different levels of salinity and spermine treatment at 21 days after sowing

Glutathione peroxidase activity (units g-1 FW)											
Variety	**21 DAS**	**Saline and spermine treatment**									**Mean**
		Control			**8 dSm-1**			**12 dSm-1**			
		Spm0	**Spm0.5mM**	**Spm1.0mM**	**Spm0**	**Spm0.5mM**	**Spm1.0mM**	**Spm0**	**Spm0.5mM**	**Spm1.0 mM**	
DBW 88	0 DAT	0.83	0.83	0.83	1.07	1.07	1.07	1.30	1.30	1.30	1.07
	5 DAT	0.85	1.00	0.93	1.17	1.14	1.23	1.27	1.49	1.60	1.19
	10 DAT	0.86	1.00	0.93	1.23	1.19	1.15	1.30	1.50	1.50	1.18
	15 DAT	0.87	0.99	0.94	1.20	1.18	1.08	1.28	1.44	1.39	1.15
HD 3086	0 DAT	0.87	0.87	0.87	1.10	1.10	1.10	1.41	1.41	1.41	1.13
	5 DAT	0.89	1.04	0.97	1.20	1.17	1.25	1.38	1.61	1.73	1.25
	10 DAT	0.90	1.03	0.97	1.25	1.21	1.17	1.41	1.62	1.62	1.24
	15 DAT	0.91	1.03	0.98	1.23	1.21	1.12	1.39	1.56	1.51	1.21
Kharchia 65	0 DAT	1.00	1.00	1.00	1.37	1.37	1.37	2.06	2.06	2.06	1.48
	5 DAT	1.01	1.17	1.11	1.31	1.71	1.78	2.08	2.56	2.74	1.72
	10 DAT	1.03	1.17	1.11	1.39	1.87	1.58	2.24	2.83	2.72	1.77
	15 DAT	1.04	1.17	1.12	1.39	2.08	1.68	2.21	2.86	2.71	1.81
KRL 210	0 DAT	0.94	0.94	0.94	1.24	1.24	1.24	1.75	1.75	1.75	1.31
	5 DAT	0.96	1.12	1.05	1.19	1.49	1.54	1.77	2.14	2.27	1.50
	10 DAT	0.97	1.11	1.06	1.25	1.62	1.39	1.90	2.34	2.26	1.55
	15 DAT	0.98	1.11	1.06	1.25	1.78	1.47	1.88	2.37	2.25	1.57
Mean		0.93	1.04	0.99	1.24	1.40	1.33	1.66	1.93	1.93	
CD at 5% level								Where,			
a →	0.01	ab →	0.02	bd →	0.02	acd→	0.03	a →	Varieties		
b →	0.01	ac→	0.02	cd →	0.02	bcd→	0.03	b →	Artificial saline treatment		
c →	0.01	ad →	0.02	abd→	0.03	abcd→	0.06	c →	Spermine treatment		
d →	0.01	bc →		0.01	abc→	0.03		d →	Days after treatment (DAT)		

Table 4: Changes in glutathione peroxidase activity in wheat flag leaf under different levels of salinity and spermine treatment at 90 days after sowing

Glutathione peroxidase activity (units g-1 FW)											
Variety	90 DAS	Saline and spermine treatment									Mean
		Control			8 dSm-1			12 dSm-1			
		Spm0	Spm0.5mM	Spm1.0mM	Spm0	Spm0.5mM	Spm1.0mM	Spm0	Spm0.5mM	Spm1.0 mM	
DBW 88	0 DAT	0.84	1.00	1.18	1.51	1.17	1.26	1.54	1.75	1.82	1.34
	5 DAT	0.78	1.00	0.88	1.37	1.17	1.09	1.37	1.66	1.58	1.21
	10 DAT	0.84	1.02	0.90	1.42	1.33	1.08	1.43	1.82	1.55	1.26
	15 DAT	0.92	1.15	0.99	1.44	1.55	1.09	1.52	1.95	1.57	1.36
HD 3086	0 DAT	0.88	1.03	1.19	1.49	1.19	1.26	1.68	1.89	1.97	1.40
	5 DAT	0.82	1.02	0.91	1.37	1.18	1.11	1.50	1.80	1.72	1.27
	10 DAT	0.87	1.05	0.94	1.42	1.33	1.10	1.56	1.96	1.69	1.33
	15 DAT	0.96	1.18	1.04	1.45	1.55	1.13	1.67	2.11	1.72	1.42
Kharchia 65	0 DAT	1.01	1.15	1.31	1.35	1.64	1.96	2.42	2.67	3.38	1.88
	5 DAT	0.95	1.14	1.04	1.19	1.67	1.88	2.40	2.99	2.89	1.79
	10 DAT	1.00	1.17	1.06	1.27	1.74	1.83	2.49	3.08	2.62	1.81
	15 DAT	1.10	1.31	1.18	1.39	1.98	1.82	2.67	3.30	2.76	1.95
KRL 210	0 DAT	0.95	1.10	1.26	1.21	1.43	1.67	2.01	2.20	2.74	1.62
	5 DAT	0.89	1.10	0.99	1.07	1.44	1.60	1.99	2.43	2.36	1.54
	10 DAT	0.95	1.13	1.01	1.15	1.50	1.57	2.07	2.52	2.17	1.56
	15 DAT	1.04	1.26	1.12	1.26	1.70	1.58	2.22	2.70	2.37	1.70
Mean		0.93	1.11	1.06	1.33	1.47	1.44	1.91	2.30	2.18	
CD at 5% level								Where,			
a →	0.01	ab →	0.02	bd →	0.02	acd→	0.04	a →	Varieties		
b →	0.01	ac→	0.02	cd →	0.02	bcd→	0.03	b →	Artificial saline treatment		
c →	0.01	ad →	0.02	abd→	NS	abcd→	0.07	c →	Spermine treatment		
d →	0.01	bc →		0.02	abc→	0.03		d →	Days after treatment (DAT)		

Discussion

In the present investigation, exogenous Spermine (Spm) treatment increased the total glutathione in both control and stressed plants. Similar results were also reported, Groppa *et al*., (2007) [6] showed that Spm restored the glutathione content which was reduced by cadmium and copper stress in wheat seedlings. Verma & Mishra (2005) [19] reported a considerable decline in glutathione content under low salinity while reduced little on increase in salinity level and putrescine elevated the GSH (reduced glutathione) level in *Brassica juncea* seedlings. Increase in cellular GSH level, by improving GSH biosynthetic capacity or through manipulation of GR activity that converts GSSG (Oxidized glutathione) back into GSH, has been shown to enhance resistance to oxidative stress as well as to abiotic stresses in plants (Kumar *et al.,* 2009). The activity of GR linearly increased with increasing salinity levels in all four varieties (Table 1 & 2). However, the higher basal levels of GR were maintained by tolerant varieties. Salinity induced increase in glutathione reductase and Ascorbate peroxidase (Apx) activities in salinity tolerant cultivars have been reported by various workers (Comba *et al*., 1998; Hernandez *et al.,* 2000; Sairam *et al*., 2005) [2, 7, 17]. The present study also corroborates with previous reports of Dionisio-Sese & Tobita (1998) [3] who showed high GR activity in salt tolerant rice and wheat (Sairam & Srivastava, 2002) [16]. Further, application of Spm increased the activity of GR in all the varieties. Similarly, Verma & Mishra (2005) [19] reported an increase in GR activity by exogenous Put under salinity in the salt tolerance varieties in *Brassica* seedlings. Besides the GSH-ascorbate cycle, GSH also participates in H_2O_2 degradation in a reaction catalysed by GPx (Szalai *et al.,* 2009) [18]. Spm increases enzymatic antioxidant defence system and GR is regulated by exogenous application of Spm in plants under salinity stress (Hasan *et al.,* 2021). GR plays an important role in maintaining ratio of GSH/GSSG which is detrimental to plant tolerance to abiotic stress (Ahmed *et al*., 2019) [1].

The results presented in Table 3 & 4 indicate that the activity of the GPx increased with increasing salinity levels in tolerant varieties. In addition, application of Spm further enhanced the salt-induced activity of GPx in tolerant varieties. Exogenous Spm treatment decreased the GPx activity at lower levels of salt stress in susceptible varieties. The present investigation is in corroboration with that of Roychoudhury *et al.* (2011) [14] who showed that GPx activity was induced under salt stress with Spermidine (Spd) and Spm treatments highly enhanced the GPx activity in rice cultivars. Glutathione peroxidase is also a vital enzyme for the detoxification of H_2O_2 and xenobiotics (Mirza *et al.,* 2020) [10]. The relative gene expression and activity of GPX increased significantly in both roots and leaves under lead stress condition (Saeid *et al.,* 2020) [15].

Conclusions

The increased activity of GR with increasing levels of salinity was further promoted by the exogenous Spm in all four wheat varieties. The activity of GPx increased with salinity stress in tolerant varieties however there was no much variation was observed in susceptible varieties at higher levels of salt stress. Application of Spm increased the activity of GPx in tolerant varieties but a slight decrease in the activity was observed in susceptible varieties at lower levels of salinity.

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Conflict of Interest

The authors declare that there is no conflict of interest exist.

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