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Exogenous Spermine Mitigate Adversities of Salinity Induced Oxidative Stress through Antioxidant Metabolites in Wheat

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Change in climatic scenarios due to global warming is characterized by extreme climate variability, land and water degradation which resulted in water scarcity. Accumulation of salts at the surface and sub-surface layers of soils affect crop production of major cereals which is a constraint in sustainable food production. Salinity is a major challenge to tackle wheat cultivation and harness productivity in arid and semi-arid regions of India. In the present investigation, mitigation of salinity induced oxidative stress through exogenous application of spermine (Spm) in four wheat genotypes was studied in relation to antioxidant metabolites. The levels of $O_2^{\cdot-}$ increased with increasing levels of salinity in wheat flag leaves. DBW 88 showed the levels of $O_2^{\cdot-}$ of 11.75 nmol g^{-1} FW and 15.74 nmol g^{-1} FW (at 8 dSm^{-1} and 12 dSm^{-1} respectively) at 21 Days After Sowing (DAS) and application of Spm decreased the $O_2^{\cdot-}$ content under control and saline stressed conditions at 8 dSm^{-1} and 12 dSm^{-1}. Hydrogen peroxide content was increased with increasing levels of salinity in all the wheat varieties at 21 DAS. However, the increase was more in the case of DBW 88 when compared with HD 3086. Treatment of Spm decreased the H_2O_2 content when compared with control and saline

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stressed wheat varieties. The malondialdehyde (MDA) content was increased with increasing levels of salinity at 21 DAS. The highest increase in MDA content was seen in DBW 88 whereas the lowest increase was found in Kharchia 65. Application of Spm decreased the MDA content under control at both levels of salinity treated wheat varieties. The carotenoid content decreased with increasing levels of salinity in all four wheat varieties. However, the decrease was more in DBW 88 when compared with other varieties *viz.* HD 3086, Kharchia 65 and KRL 210 at 21 DAS. Exogenous Spm increased the carotenoids content in all four wheat varieties irrespective of the salinity. The leaves of Kharchia 65 and KRL 210 had higher levels of ascorbic acid as compared to that of DBW 88 and HD 3086. Increased content of carotenoid was observed in Spm-treated wheat. Exogenous application of Spm increased the ascorbic acid content in control at both levels of salt stress. The glutathione content increased with an increase in salinity treatment in all the varieties however, a higher increase was observed in Kharchia 65. Exogenous Spm increased the glutathione content in all the varieties irrespective of salinity stress. The results presented in the study indicated that the exogenous application of Spm improved their tolerance levels under salinity.

Keywords: Wheat (Triticum aestivum L. em Thell); spermine; oxidative stress; antioxidants and salinity.

1. INTRODUCTION

Soil salinity is considered one of the most severe environmental stresses and salinization is a global threat to food security [1]. It is the main factor impeding sustainable crop production, especially in irrigated arid and semi-arid regions of the world where the rainfall is too low to prevent accumulations of ions in the soil and irrigation is the cause of secondary salinization [2,3]. Major increases in salt-affected areas may be due to faulty irrigation practices in irrigation commands, seawater ingress in coastal areas, and climate change.

India’s total salt-affected area is 6.74 m ha, out of which 3.79 m ha is suffering from sodicity and the rest 2.95 m ha is affected with salinity problems (also includes 1.25 m ha coastal saline soils). Soil salinity has considered an important environmental threat that adversely influences crop growth and development by altering a few biochemical processes [4]. Soil salinity affects plants in two most important ways; the capacity of roots to extract water from the soil and the concentration of the salt within the plant’s body. Elevated concentrations of salts suppress many physiological and biochemical processes such as nutrient uptake and assimilation [5].

Polyamines (PAs) such as spermidine (Spd), spermine (Spm) and putrescine (Put) are small aliphatic amines that are ubiquitous in all plant cells [6]. PAs influence plant development in many ways and the response of plants to abiotic stress involves complex physiological and biochemical responses, including changes in the concentration and ratio of different metabolites [7] however, little is known about their mechanism of alleviating drought stress [8]. Since PAs are protonated at normal cellular pH, their biological function is initially associated with the capability of binding different anionic macromolecules (DNA, RNA, chromatin, and proteins), thus confining them as substances with a structural role [9]. The most abundant types of polyamines in plants are putrescine, spermidine and spermine [10]. In plants, they are present not only as free bases but also conjugated to small molecules, mainly hydroxycinnamic acids and also to various macromolecules like proteins [11]. Polyamines increase survival of various plants under salt stress [12], drought [13], acidic [14] and heavy metal stress [15]. PAs play an important role in the antioxidant system and protect membranes from oxidative damage during stress [16]. In the light of foregoing research, a thorough review of the literature reveals that different wheat genotypes showed varying levels of tolerance to salinity, however, work on wheat genotypes such as DDW 88, HD 3086, Kharchia 65, and KRL 210 was limited with regard to salinity tolerance. Therefore, the present study aims to evaluate the oxidative stress under salinity and to find out the role of exogenous spermine treatment in four wheat genotypes and the finding provides much insight on oxidants and antioxidants.

2. MATERIALS AND METHODS

Seeds of the four varieties *viz.* DBW 88, HD 3086, Kharchia 65, and KRL 210 differing in tolerance to salinity was 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.

2.1 Raising of the Crop

Seeds of wheat varieties were surface sterilized by soaking them for 5 minutes 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 located at the premises of College of Agriculture, CCS Haryana Agricultural University-Hisar 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.

2.2 Artificial Saline Treatment

Wheat plants were grown at two levels of salinity (8 and 12 dSm^{-1}) and control. Artificial saline waters of 8 and 12 dSm^{-1} levels were prepared for irrigation of pots by maintaining Na: Ca + Mg ratio as 1:1; Ca: Mg as 1:3 and that of Cl: SO_4 as 7:3. The respective cations and anions ratio and salt levels were maintained by dissolving the required quantities of chloride and sulfate salts of calcium, magnesium, and sodium in distilled water. Plants were irrigated with distilled water to maintain the salinity levels.

2.3 Spermine Treatment

Spermine at 0.5 and 1.0 mM concentrations were sprayed over the plants at 21 days after sowing (DAS).

2.4 Analysis of Sample

Plants were tagged at the time of application of spermine and leaf samples were analyzed for biochemical oxidative stress parameters at 0, 5, 10, and 15 days of spermine treatment. Analysis of oxidants and antioxidants was carried out under laboratory conditions at 21 DAS. The observations for all the parameters were recorded in triplicate.

2.5 Biochemical Studies for Oxidative Stress Parameters

2.5.1 Superoxide ion ($O^{\cdot -}$)

Extraction: Superoxide radical ($O^{\cdot -}$) was measured as described by Elstner & Heupel (1976) with some modifications. Two gram of frozen leaf sample was homogenized with 5 ml of 65 mM potassium phosphate buffer (pH 7.8) and centrifuged at 5000 rpm for 10 min.

Procedure: The incubation mixture contained 0.9 ml of 65 mM phosphate buffer (pH 7.8), 0.1 ml of 10 mM hydroxylamine hydrochloride, and 1 ml supernatant. After incubation at 25 ^{0}C for 20 min, 1 ml of 17 mM sulphanilamide and 1 ml of 7 mM α – naphthylamine were added to the incubation mixture. After reaction at 25 ^{0}C for 20 min, ethyl ether in the same volume was added and centrifuged at 1500 rpm for 5 min. the absorbance in the aqueous solution was read at 530 nm. The standard curve of $NaNO_2$ (0 - 140 μmol NO_2^-) was used to calculate the production rate of $O_2^{\cdot -}$ from the chemical reaction of NO_2^- and hydroxylamine.

2.5.2 Hydrogen peroxide (H2O2)

Extraction: Two gram of leaf tissue was macerated in 5 ml of ice-cold 0.01 M phosphate buffer (pH 7.0), centrifuged at 8000 x g for 10 min. and the supernatant was used for estimation of H_2O_2 content (Sinha, 1972).

Procedure: To 0.4 ml of extract, 0.6 ml of 0.1 M phosphate buffer (pH 7.0) and 3 ml mixture of 5 % (*w/v*) potassium dichromate and glacial acetic acid (1:3, *v/v*) were added. The tube was kept in a boiling water bath for 10 min. and then cooled. The absorbance at 570 nm against the reagent blank was recorded. The quantity of H_2O_2 was estimated by using the standard curve of H_2O_2 (0-160 μmole).

2.5.3 Malondialdehyde (MDA)

Extraction: Two grams of leaf tissue was homogenized from the control and treated plants in 5 ml of prechilled 0.1 per cent Trichloroacetic acid (*w/v*) and centrifuged at 10,000 rpm for 15 min. The clear supernatant was used for the estimation of malondialdehyde by the method of Heath & Packer, 1968.

Procedure: To 0.5 ml of supernatant 2.3 ml of 20% (*w/v*) trichloroacetic acid containing 0.5 % thiobarbituric acid was added. The mixture was heated in a water bath at 95° C for 30 min and quickly cooled in an ice bath. The absorbance was recorded at 532 nm and the value of nonspecific absorption at 600 nm were subtracted. The concentration of malondialdehyde in the sample was calculated using the extinction coefficient of 155 mM cm^{-1}.

Table 1. Effect of spermine at 21 days after sowing on generation of superoxide ion in wheat flag leaf under salt stress

		Superoxide ion (nmol 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**	**Spm1.0 mM**	
DBW 88	**0 DAT**	8.68	8.68	8.68	11.75	11.75	11.75	15.74	15.74	15.74	**12.06**
	5 DAT	8.86	7.30	6.84	12.12	10.84	10.77	15.90	13.67	12.62	**10.99**
	10 DAT	9.29	7.55	7.74	12.86	11.00	11.19	16.48	13.69	12.86	**11.41**
	15 DAT	9.53	7.91	8.35	12.88	11.03	11.41	17.12	12.43	13.07	**11.53**
HD 3086	**0 DAT**	7.63	7.63	7.63	10.07	10.07	10.07	12.92	12.92	12.92	**10.21**
	5 DAT	7.82	6.17	5.68	10.41	9.39	9.34	13.10	11.42	10.64	**9.33**
	10 DAT	8.12	6.30	6.50	10.94	9.47	9.62	13.47	11.40	10.78	**9.62**
	15 DAT	8.43	6.71	7.18	11.10	9.62	9.93	14.13	10.61	11.09	**9.87**
Kharchia 65	**0 DAT**	6.32	6.32	6.32	7.73	7.73	7.73	9.81	9.81	9.81	**7.95**
	5 DAT	7.42	5.23	4.59	9.14	8.41	8.36	11.43	9.64	9.43	**8.18**
	10 DAT	7.64	5.23	5.49	9.49	8.47	8.55	11.66	9.63	9.51	**8.41**
	15 DAT	7.65	5.46	6.06	9.34	8.36	8.53	11.79	8.94	9.46	**8.40**
KRL 210	**0 DAT**	7.08	7.08	7.08	8.89	8.89	8.89	11.53	11.53	11.53	**9.16**
	5 DAT	7.24	5.44	4.90	9.15	8.34	8.29	11.67	9.71	9.47	**8.24**
	10 DAT	7.91	5.81	6.04	10.09	8.90	8.99	12.63	10.26	10.11	**8.97**
	15 DAT	8.07	6.13	6.66	10.11	8.94	9.14	13.02	9.62	10.25	**9.11**
Mean		**7.98**	**6.56**	**6.61**	**10.38**	**9.45**	**9.54**	**13.28**	**11.31**	**11.21**	

CD at 5 % level								Where,	
a →	0.08	ab →	0.14	bd →	0.14	acd→	NS	a →	Varieties
b →	0.07	ac→	0.14	cd →	0.14	bcd →	0.23	b →	Artificial saline treatment
c →	0.07	ad →	0.16	abd →	0.27	abcd →	NS	c →	Spermine treatment
d →	0.08	bc →	0.12	abc →	0.23			d →	Days after treatment (DAT)

Table 2. Effect of spermine at 21 days after sowing on hydrogen peroxide content in wheat flag leaf under salt stress

		Hydrogen peroxide content (µmole g^{-1} FW)									
		Saline and spermine treatment									
Variety	**21 DAS**	**Control**			**8 dSm^{-1}**			**12 dSm^{-1}**			**Mean**
		Spm^0	**Spm0.5mM**	**Spm1.0mM**	**Spm^0**	**Spm0.5mM**	**Spm1.0mM**	**Spm^0**	**Spm0.5mM**	**Spm1.0 mM**	
DBW 88	**0 DAT**	51.90	51.90	51.90	60.18	60.18	60.18	98.64	98.64	98.64	**70.24**
	5 DAT	66.09	60.21	55.83	77.18	70.58	65.07	126.29	90.93	108.15	**80.04**
	10 DAT	84.35	73.98	75.71	99.46	83.01	87.98	174.83	124.62	149.41	**105.93**
	15 DAT	94.13	81.10	89.77	112.33	88.66	103.28	205.45	145.39	175.36	**121.72**
HD 3086	**0 DAT**	49.32	49.32	49.32	57.08	57.08	57.08	90.74	90.74	90.74	**65.71**
	5 DAT	60.85	56.54	52.83	70.91	65.32	60.65	112.47	82.54	97.11	**73.25**
	10 DAT	73.78	66.29	67.68	86.71	73.53	77.52	147.13	106.88	126.76	**91.81**
	15 DAT	78.37	69.34	75.93	93.08	75.09	86.20	163.89	118.22	141.00	**100.12**
Kharchia 65	**0 DAT**	45.01	45.01	45.01	50.19	50.19	50.19	75.34	75.34	75.34	**56.85**
	5 DAT	55.16	52.05	49.73	61.85	57.86	54.54	92.78	71.02	83.25	**64.25**
	10 DAT	64.89	59.86	60.69	73.26	64.14	66.90	116.80	88.50	104.79	**77.76**
	15 DAT	71.14	64.92	69.06	81.06	68.16	76.13	133.98	100.71	120.15	**87.26**
KRL 210	**0 DAT**	44.45	44.45	44.45	49.20	49.20	49.20	72.25	72.25	72.25	**55.30**
	5 DAT	54.74	51.35	48.83	60.91	57.24	54.17	89.43	69.05	78.97	**62.74**
	10 DAT	64.89	59.35	60.28	72.67	64.20	66.76	113.17	86.37	99.60	**76.36**
	15 DAT	71.98	65.05	69.65	81.32	69.16	76.67	131.18	99.24	115.17	**86.60**
Mean		**64.44**	**59.42**	**60.42**	**74.21**	**65.85**	**68.28**	**121.52**	**95.03**	**108.54**	

CD at 5 % level									
								Where,	
a →	0.66	ab →	1.14	bd →	1.14	acd→	2.28	a →	Varieties
b →	0.57		1.14	cd →	1.14	bcd→	1.98	b →	Artificial saline treatment
		ac→							
c →	0.57	ad →	1.32	abd→	2.28	abcd→	NS	c →	Spermine treatment
d →	0.66	bc →	0.99	abc→	1.98			d →	Days after treatment (DAT)

Table 3. Effect of spermine at 21 days after sowing on malondialdehyde content in wheat flag leaf under salt stress

		Malondialdehyde content (µmole 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**	**Spm1.0 mM**	
DBW 88	**0 DAT**	4.25	4.25	4.25	5.25	5.25	5.25	6.31	6.31	6.31	**5.27**
	5 DAT	4.92	4.44	4.19	6.27	5.67	5.31	7.32	6.90	6.95	**5.78**
	10 DAT	5.67	5.01	5.03	7.33	6.29	6.05	8.63	8.03	8.24	**6.70**
	15 DAT	6.53	5.82	6.22	8.51	7.20	7.53	10.13	9.23	9.82	**7.89**
HD 3086	**0 DAT**	3.90	3.90	3.90	4.68	4.68	4.68	5.86	5.86	5.86	**4.81**
	5 DAT	4.29	3.81	3.59	5.22	5.12	4.99	6.53	6.02	6.24	**5.09**
	10 DAT	4.97	4.31	4.21	6.10	5.24	5.92	7.68	7.03	7.37	**5.87**
	15 DAT	5.67	4.97	5.31	6.97	6.16	6.70	8.89	8.07	8.53	**6.81**
Kharchia 65	**0 DAT**	3.59	3.59	3.59	4.29	4.29	4.29	5.10	5.10	5.10	**4.33**
	5 DAT	3.98	3.44	3.20	4.82	4.55	4.79	5.70	5.27	5.32	**4.56**
	10 DAT	4.37	3.67	4.09	5.35	4.64	4.95	6.36	5.92	6.01	**5.04**
	15 DAT	4.98	4.24	4.61	6.16	5.15	5.44	7.34	6.84	7.09	**5.76**
KRL 210	**0 DAT**	3.49	3.49	3.49	4.34	4.34	4.34	5.06	5.06	5.06	**4.30**
	5 DAT	4.39	3.84	3.55	5.59	5.19	5.25	6.38	5.89	5.85	**5.10**
	10 DAT	4.79	4.06	4.55	6.11	5.37	5.38	7.01	6.58	6.62	**5.61**
	15 DAT	5.09	4.37	4.78	6.49	5.43	5.65	7.55	7.04	7.14	**5.95**
Mean		**4.68**	**4.20**	**4.28**	**5.84**	**5.29**	**5.41**	**6.99**	**6.57**	**6.72**	

CD at 5 % level								Where,	
a →	0.04	ab →	0.07	bd →	0.07	acd→	NS	a →	Varieties
b →	0.03	ac→	0.07	cd →	0.07	bcd→	0.12	b →	Artificial saline treatment
c →	0.03	ad →	0.08	abd →	0.14	abcd →	NS	c →	Spermine treatment
d →	0.04	bc →	0.06	abc →	0.12			d →	Days after treatment (DAT)

Table 4. Effect of spermine at 21 days after sowing on carotenoid content in wheat flag leaf under salt stress

Variety	21 DAS	Carotenoid content (mg g^{-1} FW)									Mean
		Saline and spermine treatment									
		Control			8 dSm^{-1}			12 dSm^{-1}			
		Spm^0	Spm0.5mM	Spm1.0mM	Spm^0	Spm0.5mM	Spm1.0mM	Spm^0	Spm0.5mM	Spm1.0 mM	
DBW 88	**0 DAT**	2.64	2.64	2.64	2.23	2.23	2.23	1.71	1.71	1.71	**2.19**
	5 DAT	2.66	2.88	2.90	2.27	2.45	2.41	1.69	2.00	1.95	**2.36**
	10 DAT	2.80	3.04	2.98	2.39	2.62	2.55	1.80	2.17	1.99	**2.48**
	15 DAT	2.84	3.07	2.99	2.42	2.67	2.62	1.85	2.22	2.00	**2.52**
HD 3086	**0 DAT**	2.96	2.96	2.96	2.55	2.55	2.55	2.03	2.03	2.03	**2.52**
	5 DAT	2.92	3.17	3.20	2.54	2.72	2.67	1.98	2.28	2.23	**2.63**
	10 DAT	3.08	3.37	3.29	2.68	2.91	2.84	2.11	2.47	2.30	**2.78**
	15 DAT	3.12	3.39	3.29	2.71	2.96	2.90	2.16	2.52	2.30	**2.82**
Kharchia 65	**0 DAT**	3.24	3.24	3.24	2.88	2.88	2.88	2.41	2.41	2.41	**2.85**
	5 DAT	3.16	3.50	3.53	2.83	3.01	2.95	2.33	2.67	2.63	**2.95**
	10 DAT	3.31	3.70	3.60	2.97	3.18	3.10	2.46	2.85	2.71	**3.10**
	15 DAT	3.23	3.57	3.45	2.89	3.12	3.05	2.43	2.79	2.63	**3.02**
KRL 210	**0 DAT**	3.19	3.19	3.19	2.80	2.80	2.80	2.30	2.30	2.30	**2.76**
	5 DAT	3.10	3.39	3.42	2.74	2.93	2.86	2.20	2.57	2.52	**2.86**
	10 DAT	3.26	3.60	3.51	2.89	3.12	3.03	2.34	2.76	2.61	**3.01**
	15 DAT	3.21	3.52	3.40	2.84	3.08	3.01	2.34	2.73	2.56	**2.96**
Mea n		**3.05**	**3.26**	**3.22**	**2.67**	**2.83**	**2.78**	**2.13**	**2.41**	**2.31**	

CD at 5 % level							Where,	
a →	0.03	ab →	0.05	bd →	NS	acd→ NS	a →	Varieties
b →	0.02	ac→	NS	cd →	0.05	bcd → NS	b →	Artificial saline treatment
c →	0.02	ad →	0.05	abd →	NS	abcd → NS	c →	Spermine treatment
d →	0.03	bc →	004	abc →	NS		d →	Days after treatment (DAT)

Table 5. Effect of spermine at 21 days after sowing on ascorbic acid content in wheat flag leaf under salt stress

Variety	21 DAS	Ascorbic acid content (µg g^{-1} FW)									Mean
		Saline and spermine treatment									
		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**	219.59	219.59	219.59	231.36	231.36	231.36	181.91	181.91	181.91	**210.95**
	5 DAT	253.61	322.99	295.93	269.77	262.44	265.25	211.08	246.03	271.90	**266.56**
	10 DAT	239.10	304.97	275.44	251.96	236.01	246.96	195.43	258.51	228.46	**248.54**
	15 DAT	226.61	292.49	266.55	193.47	218.52	232.48	193.47	250.13	221.55	**232.81**
HD 3086	**0 DAT**	232.23	232.23	232.23	243.43	243.43	243.43	196.36	196.36	196.36	**224.01**
	5 DAT	267.19	347.16	317.40	282.51	275.56	278.23	226.86	260.00	284.53	**282.16**
	10 DAT	250.65	322.70	293.33	262.78	247.74	258.07	209.44	268.96	240.61	**261.59**
	15 DAT	241.91	310.11	287.51	210.07	234.13	247.55	210.07	264.50	237.04	**249.21**
Kharchia 65	**0 DAT**	268.12	268.12	268.12	229.33	229.33	229.33	209.82	209.82	209.82	**235.76**
	5 DAT	305.27	404.20	340.36	263.15	357.74	306.83	248.22	310.50	276.97	**312.58**
	10 DAT	288.10	375.96	318.44	245.38	337.51	282.07	234.53	291.82	262.36	**292.91**
	15 DAT	272.03	366.65	309.70	232.63	327.47	275.58	224.98	288.28	253.73	**283.45**
KRL 210	**0 DAT**	255.48	255.48	255.48	219.71	219.71	219.71	202.19	202.19	202.19	**225.79**
	5 DAT	296.68	380.77	328.51	252.37	343.09	288.51	237.45	281.65	258.67	**296.41**
	10 DAT	268.59	352.34	305.16	232.99	317.38	268.49	217.98	262.91	240.50	**274.04**
	15 DAT	268.90	354.07	293.36	230.93	312.67	261.40	222.67	266.33	241.77	**272.46**
Mean		**259.63**	**319.36**	**287.95**	**240.74**	**274.63**	**258.45**	**213.90**	**252.49**	**238.02**	

CD at 5 % level								Where,	
a →	1.19	ab →	2.05	bd →	2.05	acd→	4.11	a →	Varieties
b →	1.03	ac→	2.05	cd →	2.05	bcd→	3.56	b →	Artificial saline treatment
c →	1.03	ad →	2.37	abd →	4.11	abcd →	7.11	c →	Spermine treatment
d →	1.19	bc →	1.78	abc→	3.56			d →	Days after treatment (DAT)

Table 6. Effect of spermine at 21 days after sowing on total glutathione content in wheat flag leaf under salt stress

Variety	21 DAS	Glutathione content (μmol g^{-1} FW)									Mean
		Saline and spermine treatment									
		Control			8 dSm^{-1}			12 dSm^{-1}			
		Spm^0	Spm0.5mM	Spm1.0mM	Spm^0	Spm0.5mM	Spm1.0mM	Spm^0	Spm0.5mM	Spm1.0 mM	
DBW 88	**0 DAT**	8.73	8.73	8.73	11.44	11.44	11.44	13.55	13.55	13.55	**11.24**
	5 DAT	8.81	9.07	9.23	11.45	12.64	13.03	13.71	14.30	14.74	**11.89**
	10 DAT	9.25	9.47	9.58	11.55	12.65	12.95	13.71	14.92	15.11	**12.13**
	15 DAT	9.37	9.71	9.77	11.97	13.04	13.23	13.75	15.02	14.85	**12.30**
HD 3086	**0 DAT**	9.33	9.33	9.33	12.72	12.72	12.72	15.42	15.42	15.42	**12.49**
	5 DAT	9.49	9.80	9.99	12.97	14.02	14.48	15.71	17.05	17.31	**13.43**
	10 DAT	9.70	9.95	10.07	13.53	14.00	13.94	15.81	17.10	17.48	**13.51**
	15 DAT	10.14	10.54	10.60	13.97	14.49	14.23	15.91	17.61	17.67	**13.91**
Kharchia 65	**0 DAT**	11.07	11.07	11.07	16.21	16.21	16.21	20.48	20.48	20.48	**15.92**
	5 DAT	11.31	11.79	12.08	16.53	18.12	19.37	21.47	22.10	23.22	**17.33**
	10 DAT	11.63	12.03	12.21	17.66	18.42	19.24	21.42	22.48	23.43	**17.61**
	15 DAT	12.00	12.61	12.71	18.04	18.39	19.12	22.40	22.90	23.18	**17.93**
KRL 210	**0 DAT**	10.38	10.38	10.38	15.16	15.16	15.16	18.62	18.62	18.62	**14.72**
	5 DAT	10.58	10.99	11.24	15.45	16.78	17.50	18.73	19.50	20.73	**15.72**
	10 DAT	10.87	11.20	11.36	16.52	17.03	17.09	18.66	19.59	20.78	**15.90**
	15 DAT	11.23	11.75	11.84	17.35	17.78	17.45	18.57	20.02	20.81	**16.31**
Mean		**10.24**	**10.53**	**10.64**	**14.53**	**15.18**	**15.45**	**17.37**	**18.17**	**18.59**	

CD at 5 % level							Where,	
a →	0.10	ab →	0.17	bd → 0.17	acd→ NS		a →	Varieties
b →	0.09	ac→	0.17	cd → 0.17	bcd→ 0.30		b →	Artificial saline treatment
c →	0.09	ad →	0.20	abd→ 0.35	abcd →NS		c →	Spermine treatment
d →	0.10	bc →	0.15	abc → 0.30			d →	Days after treatment (DAT)

Carotenoids: The carotenoids content in leaves was estimated as per the method of Hiscox & Israelstam [17]. The finely chopped leaf sample 100 mg (excluding veins) were placed in a tube containing 10 ml DMSO, allowed for overnight. The carotenoids which were extracted in DMSO were read at 480, 645, and 663 nm and calculated (Wellburn & Lichtenthaler, 1984) from the following formulae:

$$\text{Chlorophyll a} = (12.19 \text{ x } A_{663} - 3.45 \text{ x } A_{645}) \text{ x } \frac{\text{Volume}}{1000 \text{ x Weight}}$$

$$\text{Chlorophyll b} = (21.19 \text{ x } A_{645} - 5.32 \text{ x } A_{663}) \text{ x } \frac{\text{Volume}}{1000 \text{ x Weight}}$$

$$\text{Carotenoids} = \frac{1000 \text{ x}(A_{480} - 2.14) \text{ x (chl. a} - 70.16) \text{ x (chl. b)}}{220}$$

2.5.4 Ascorbate

Extraction: Ascorbic acid content was estimated by the method of Mukherjee & Choudhuri [18], which is based on the reduction of 2, 4 – dinitrophenylhydrazine. Two gram of leaf sample was homogenized in 5 ml of 6% TCA, centrifuged at 10,000 rpm for 15 min. and the supernatant was used for estimation.

Procedure: To the properly diluted aliquot of 0.1 ml, 1.9 ml of distilled water, 1 ml of 2% 2, 4 dinitrophenylhydrazines (in acidic medium), and one drop of 10% thiourea (in 70% ethanol) was added, the contents were mixed thoroughly and kept in boiling water bath for 15 min. then cooled to room temperature, 2 ml of 80% (*v/v*) H_2SO_4 was added to the mixture at 0^0 C (in an ice bath). The absorbance was read at 530 nm. The quantity of ascorbic acid was determined from the standard curve of ascorbic acid (10-100 µg).

2.5.5 Total glutathione

Extraction: Two grams of leaf tissue was homogenized in 5 ml of 5% (*w/v*) sulphosalicylic acid at 4^0 C, centrifuged at 10,000 rpm for 25 min. and the supernatant was used for estimation of total glutathione by the method of Smith [19].

Procedure: The reaction mixture consisted of 0.1 ml of 0.5 M potassium phosphate buffer (pH 7.5), 0.5 ml of 0.1 M sodium phosphate buffer (pH 7.5) containing 5 mM of EDTA, 0.1 ml of 2 mM NADPH (0.1M sodium phosphate buffer, pH 7.5), 0.1 ml of glutathione reductase (2.5 units ml^{-1}), 0.15 ml of 0.6 mM 5, 5'-dithiobis-(2-nitrobenzoic acid) (DTNB) and 0.05 ml supernatant. The reaction was initiated by the addition of GR and the reduction rate of DTNB was monitored at 412 nm for 3 min. The total glutathione was calculated from the standard curve in which the GSH (200 - 400 ng) equivalents were plotted against the rate of increase of absorbance at 412 nm.

3. RESULTS

3.1 Effect of Exogenous Spermine on Antioxidant Metabolites under Salinity

3.1.1 Superoxide ion

The effect of spermine and salinity on the generation of superoxide ion in wheat flag leaves at 21 DAS is illustrated in Table 1. The levels of $O_2^{\cdot-}$ increased with increasing levels of salinity in wheat flag leaf in all the varieties and the highest increase was found in DBW 88 *i.e.,* from 8.68 nmol g^{-1} FW (control) to 11.75 nmol g^{-1} FW and 15.74 nmol g^{-1} FW (at 8 dSm^{-1} and 12 dSm^{-1} respectively) at 21 DAS. The application of Spm decreased the mean $O_2^{\cdot-}$ content in all the varieties under control *i.e.,* from 7.98 (Spm^0) to 6.56 ($Spm^{0.5mM}$) and 6.61 nmol g^{-1} FW ($Spm^{1.0mM}$), as well as saline stressed conditions (*i.e.,* from 10.38 (Spm^0) to 9.45 ($Spm^{0.5mM}$) and 9.54 nmol g^{-1} FW ($Spm^{1.0mM}$) at 8 dSm^{-1} and from 13.28 (Spm^0) to 11.31 ($Spm^{0.5mM}$) and 11.21 nmol g^{-1} FW ($Spm^{1.0mM}$) at 12 dSm^{-1}).

3.1.2 Hydrogen peroxide content

Hydrogen peroxide content progressively increased with increasing levels of salinity in all the wheat varieties at 21 DAS (Table 2). However, the increase was more in case of DBW 88 *i.e.*, from 51.90 µmole g^{-1} FW in control to 60.18 µmole g^{-1} FW at 8 dSm^{-1} and 98.64 µmole g^{-1} FW at 12 dSm^{-1} when compared with HD 3086 (from 49.32 µmole g^{-1} FW in control to 57.08 µmole g^{-1} FW at 8 dSm^{-1} and 90.74 µmole g^{-1} FW at 12 dSm^{-1}), KRL 210 (From 44.45 µmole g^{-1} FW in control to 49.20 µmole g^{-1} FW at 8 dSm^{-1} and 72.25 µmole g^{-1} FW at 12 dSm^{-1}) and Kharchia 65 (from 45.01 µmole g^{-1} FW in control to 50.19 µmole g^{-1} FW at 8 dSm^{-1} and 75.34 µmole g^{-1} FW at 12 dSm^{-1}).

Application of Spm decreased the H_2O_2 content when compared with non-spermine treated control and saline stressed wheat varieties. The mean H_2O_2 content gradually increased with a duration from 21 DAS to 15 DAT in all the varieties. Table 2 depicts that the H_2O_2 content increased with increasing levels of salinity and

the highest value recorded was 282.72 µmole g^{-1} FW for DBW 88 at 12 dSm^{-1} and 15 DAT. However, the basal levels of H_2O_2 were more in case of DBW 88 and HD 3086. The H_2O_2 content gradually increased throughout the observation with days after sowing under control conditions at 21 DAS (Table 2). Application of Spm with concentration 0.5 mM was found to be more effective when compared with 1.0 mM Spm and non-spermine treated wheat varieties irrespective of saline treatments.

3.1.3 Malondialdehyde content

The malondialdehyde content was increased with increasing levels of salinity at 21 DAS (Table 3). The highest increase in MDA content was seen in DBW 88 *i.e.*, from 4.25 µmol g^{-1} FW in control to 5.25 and 6.31 µmol g^{-1} FW at 8 dSm^{-1} and 12 dSm^{-1} respectively whereas the lowest increase was found in Kharchia 65 *i.e.*, from 3.59 µmol g^{-1} FW in control to 4.29 and 5.10 µmol g^{-1} FW at 8 dSm^{-1} and 12 dSm^{-1} respectively. Application of Spm decreased the mean MDA content under control (from 4.68 to 4.20 and 4.28 µmol g^{-1} FW) and at both levels of salinity treated wheat varieties (from 5.84 to 5.29 and 5.41 µmol g^{-1} FW at 8 dSm^{-1} and from 6.99 to 6.57 and 6.72 µmol g^{-1} FW at 12 dSm^{-1}). The higher mean MDA content was found at 15 DAT in all four varieties.

3.1.4 Carotenoid content

The response to salt stress and spermine treatment on carotenoid content in the flag leaf of wheat was presented in Table 4. The carotenoid content decreased linearly with increasing levels of salinity in all four varieties. However, the decrease was more in DBW 88 *i.e.,* from 2.64 in control to 2.23, 1.71 mg g^{-1} FW at 8 dSm^{-1} and 12 dSm^{-1} respectively when compared with other varieties *viz.* HD 3086, Kharchia 65 and KRL 210 at 21 DAS.

Exogenous Spm increased the carotenoids content in all the varieties irrespective of the salinity treatments. Higher levels of mean carotenoids content were noted at 15 DAT in DBW 88 (2.52 mg g^{-1} FW) & HD 3086 (2.82 mg g^{-1} FW) and at 10 DAT in Kharchia 65 (3.10 mg g^{-} FW) and KRL 210 (3.01 mg g FW).

3.1.5 Ascorbic acid

Results in Table 5 show the effect of spermine on ascorbic acid content in the flag leaf of wheat under salinity. The leaves of Kharchia 65 and KRL 210 had higher basal levels of ascorbic acid (268.12 and 255.48 µg g^{-1} FW respectively) as compared to that DBW 88 and HD 3086 (219.59 and 232.23 µg g^{-1} FW respectively). The ascorbic acid content gradually decreased with increasing levels of salinity in Kharchia 65 and KRL 210 whereas, in DBW 88 and HD 3086 there was a slight increase at lower levels of salinity (8 dSm^{-1}) and then declined at higher levels of salinity (12 dSm^{-1}) at 21 DAS. Exogenous Spm increased the ascorbic acid content in control at 12 dSm^{-1} in all the varieties. However, at lower levels of salt stress (8 dSm^{-1}) exogenous Spm marginally decreased the ascorbic acid content in DBW 88 and HD 3086 but not in Kharchia 65 and KRL 210.

3.1.6 Total glutathione

Salinity and spermine induced changes in total glutathione content is depicted in Table 6 The glutathione content increased progressively with an increase in salinity treatment in all the varieties, however, a higher increase was observed in Kharchia 65 (from 11.07 µmol g^{-1} FW in control to 16.21 and 20.48 mg g^{-1} FW at 8 dSm^{-1} and 12 dSm^{-1}respectively).

Exogenous Spm increased the glutathione content in all the varieties irrespective of salinity stress. The mean glutathione content was more at 15 DAT in all the varieties (17.93, 16.31, 13.91 and 12.30 mg g^{-1} FW in Kharchia 65, KRL 210, HD 3086 and DBW 88 respectively).

4. DISCUSSION

4.1 Biochemical Response of Non-Enzymatic Antioxidant System during Salt Stress and Spermine Treatment

Environmental stresses such as drought, salinity, chilling, metal toxicity led to the enhanced generation of ROS in plants due to disruption of cellular homeostasis [20]. ROS and its reaction products have been credibly evidenced to play a significant role in cell signaling, redox-sensing mechanism, and plant survival under abiotic stresses [21,22]. In the present investigation, salt stress continuously increased the accumulation of $O_2^{\cdot-}$ and H_2O_2 in wheat leaves (Tables 1 and 2). The more accumulation of $O_2^{\cdot-}$ and H_2O_2 in the case of salt-sensitive varieties such as DBW 88 and HD 3086 might be due to the lower efficiency of the antioxidant system to scavenge ROS when compared with tolerant varieties such

as Kharchia 65 and KRL 210. The increase in $O_2^{\cdot-}$ and H_2O_2 content during salt stress has earlier been reported in wheat [23,24]. Ashraf et al. [25] studied the changes in the accumulation of H_2O_2 in wheat under salt stress and reported a high accumulation of H_2O_2 in *cv*. MH-97 (salt-sensitive cultivar) when compared with *cv*. S-24 (salt tolerant cultivar). However, the $O_2^{\cdot-}$ and H_2O_2 content decreased with the application of Spm in control and salt-stressed wheat varieties. Further, a lower level of Spm (0.5 mM) treatment was found effective in reducing these ROS in wheat leaves under salinity. Numerous previous studies discovered that there was a cross-talk between reactive oxygen species and PAs for plant adaptive response and PA could reduce ROS levels under osmotic stress [26,27]. Exogenously applied spermidine had a significantly lower level of $O_2^{\cdot-}$ and H_2O_2 than non-spermine treated creeping bentgrass under drought stress [28]. Verma & Mishra [29] studied the alleviation of PAs in salt-stressed *Brassica juncea* and reported an increase of 2-3 folds in $O_2^{\cdot-}$ and 58 % increase in H_2O_2 content under high salinity stress. However, exogenous putrescine reduces both $O_2^{\cdot-}$ and H_2O_2 considerably. It was found in the present study that Spm treatment potentially suppresses the levels of $O_2^{\cdot-}$ and H_2O_2 content in leaves of wheat under salt stress. The biological toxicity of $O_2^{\cdot-}$ is due to its capacity to inactivate iron-sulfur cluster containing enzymes, thereby liberating free iron in the cell, which can undergo Fenton reaction and generate highly reactive hydroxyl radicals ($OH^{\cdot}$). Jiang et al., [30] reported that salt stress caused a significant increase in the contents of $O_2^{\cdot-}$ and H_2O_2 as compared with the controls in the chloroplast of rice seedlings and also suggested that application of spermidine alone did not affect the ROS production.

Lipid peroxidation is considered the most damaging process in living organisms. Since MDA is one of the end-products of lipid peroxidation in biomembranes, it is usually used to represent the level of lipid peroxidation and membrane injury. The results presented in Table 3 indicates that the MDA content increased progressively with the levels of salinity in all the varieties at 21 DAS. However, the increment of MDA was more in the case of DBW 88 and HD 3086 when compared with Kharchia 65 and KRL 210 under salinity. This indicates that the lipid peroxidation was more in sensitive varieties than tolerant varieties. The MDA content is an important criterion in evaluating the stress tolerance of plants under stress conditions. The increase in MDA content under salt stress had been well documented in *Triticum aestivum* [31], rice [32], wheat seedlings [33], *Cucumis sativus* L. [34], *Catharanthus roseus* [35]. However, the stability of biological membranes has been taken as an effective screening tool to assess salinity stress effects [35]. Spermine protection on membranes was evidenced by the reduction of MDA content in all the varieties when the exogenous Spm was foliar sprayed at a stage of 21 DAS. However, lower levels of spermine (0.5 mM) application were found effective in reducing the lipid peroxidation and thereby maintaining the membrane integrity in both control and salts tressed wheat plants. Similarly, the exogenous Spm decreased the MDA content in *Cucumis sativus* L. under salt stress [34[. In addition, lipid peroxidation gradually increased throughout the stages of development of the wheat crop observed in non-spermine controls in all the varieties. Ashraf et al. [31] reported that the MDA content increased with the growth stages *viz*., vegetative stage, booting stage, and reproductive stage in two genetically diverse wheat cultivars under salt stress. These results showed that salt stress increases lipid peroxidation and Spm treatment reverted stress-induced membrane damage to a large extent as a result plant are protected from salt stress damage. Jing et al., [36] reported that MDA content showed first an increasing and then decreasing trend in grains during filling in wheat under high-temperature stress and Spm treatment decreased the MDA content.

Carotenoids are indispensable components of the photosynthetic structures and enhance the efficiency of light-harvesting complex and electron transfer. In the present investigations, the carotenoid contents decreased with increasing levels of salinity in wheat leaves (Table 4). However, less reduction in carotenoid content was observed under salt stress in tolerant varieties as compared with susceptible varieties. Carotenoids are responsible for scavenging singlet oxygen, and hence their comparative levels in a genotype will determine its relative tolerance [37]. The decrease in chlorophyll and carotenoid contents in susceptible genotypes has also been reported in wheat under stress [38,39,40]. Exogenous Spm increased the carotenoid contents in all the varieties irrespective of the salinity treatments. Carotenoids is one of the antioxidants required for salt tolerance in crop plants and increased levels of carotenoid suggest efficient scavenging of ROS and free radicals which is mediated by

exogenous spermine. However, Spm of lower concentration (0.5 mM) was more effective in increasing the carotenoids content in wheat varieties. In addition, carotenoids play a major role in protecting PS I and PS II core complexes against photodamage and in the assembly and stabilization of the entire photosynthetic machinery [41]. Similarly, putrescine alleviated the salt-induced damages and increased the level of glutathione and carotenoids in *Brassica juncea* [29]. Mohammad et al., [42] had reported increased carotenoid content in durum wheat plants when inoculated with plant growth-promoting bacteria (PGPB) under mild salt stress conditions and protect photosynthetic reaction centres.

Ascorbate is a major primary antioxidant that can react directly with $OH^{\bullet}$ & $O_2^{\bullet-}$ and also a powerful secondary antioxidant that maintains α-tocopherol in reduced form [43]. The ascorbic acid content decreased with increasing levels of salinity in tolerant varieties but a slight increase at 8 dSm^{-1} was observed in susceptible varieties at 21 DAS (Table 5). However, the basal level of ascorbic acid content in tolerant varieties was more than in susceptible varieties. The reduced pool of ascorbate represents an antioxidant reserve, so that it may assume great importance in adaptive responses of plants to stress conditions. Sairam et al. [44] also reported that ascorbate content decreased under salinity in wheat genotypes. In spite of a decrease in ascorbic acid content under salinity stress, tolerant varieties maintained higher ascorbic acid content as compared to susceptible varieties. Application of Spm increased the ascorbate content in both control and salt-stressed plants except at the lower level of salinity stress where a slight decrease in ascorbate content was observed in susceptible varieties at 21 DAS. Jain et al. [45] also reported that under higher levels of salt stress and 1.0 mM Spm treatment elevated levels of ascorbic acid of up to 28.3 percent was recorded in rice cultivar. An increase in ascorbic acid content under salt stress was also reported in two cultivars of maize [46].

Glutathione, a non-enzymatic antioxidant, is involved in both direct and indirect control of ROS levels in the cellular environment and plays a protective role in stress tolerance. As a component of the ascorbate-glutathione pathway, it takes part in the removal of excess H_2O_2 in a reaction in which glutathione is oxidized [47,48]. The glutathione content increased progressively with an increase in salinity levels in all the varieties (Table 6). However, a higher increase was noted in tolerant varieties under salinity than susceptible varieties. Similar reports were reported by Jain et al. [45] that imposition of salt stress increased total glutathione content in tolerant rice cultivar and Spm application further triggered the GSH level in stressed plants. In the present investigation, exogenous Spm treatment increased the total glutathione in both control and stressed plants. Similar results were also reported, Groppa et al., [49] showed that Spm restored the glutathione content which was reduced by cadmium and copper stress in wheat seedlings. Verma & Mishra [29] reported a considerable decline in glutathione content under low salinity while reduced little on an increase in salinity level and putrescine elevated the GSH level in *Brassica juncea* seedlings. An increase in cellular GSH level, by improving GSH biosynthetic capacity or through manipulation of GR activity that converts GSSG back into GSH, has been shown to enhance resistance to oxidative stress as well as to abiotic stresses in plants [47]. Alterations in quantitative changes of glutathione during the day in wheat genotypes exposed to drought were reported and further glutathione amount increased at the expense of regeneration of oxidized glutathione Durna et al., [50].

5. CONCLUSION

- The oxidative stress as judged by the production of $O_2^{\bullet-}$ and H_2O_2 and was significantly higher in salt-sensitive varieties which might be due to the lower efficiency of the antioxidant system to scavenge ROS when compared with tolerant varieties. Exogenously applied Spm potentially suppressed the accumulation of salt-induced levels of $O_2^{\bullet-}$ and H_2O_2.
- The peroxidation of membrane lipids as measured by the increase in MDA content was more in DBW 88 and HD 3086 relative to tolerant varieties under salinity. Spermine protection on membranes is evident from the decrease of MDA content in flag leaves of all four varieties under salt stress.
- The carotenoid content decreased with increasing levels of salinity in all the four varieties, however, the decrease was more in DBW 88 when compared with other varieties.

- The ascorbic acid content decreased under salinity stress; the tolerant varieties maintained higher ascorbate content than susceptible varieties. The reduced pool of ascorbate under salt stress was reversed with Spm application in tolerant varieties.
- The glutathione content increased under salt stress with much more increase in tolerant varieties under salinity than susceptible varieties. In addition, exogenous Spm further increased the glutathione content in all the varieties irrespective of salt stress.

6. FUTURE SCOPE

Further studies are needed to evaluate the role of exogenous application of spermine in other wheat genotypes with respect to induction of oxidants and antioxidant metabolites under salinity.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Priyanka S, Mahesh MM, Nagendra KS, Dinesh K, Kanika K. Physiological and molecular response under salinity stress in bread wheat (*Triticum aestivum* L.). Journal of Plant Biochemistry and Biotechnology. 2020;29:125-133.
2. Hu X, Xu Z, Xu W, Li J, Zhao N, Zhou Y. Application of γ- aminobutyric acid demonstrates a protective role of polyamine and GABA metabolism in muskmelon seedlings under Ca $(NO_3)_2$ stress. Plant Physiology and Biochemistry. 2015;92:1-10.
3. Calanca PP. Effects of Abiotic Stress in Crop Production. Quantification of Climate Variability, Adaptation and Mitigation for Agricultural Sustainability (eds M. Ahmed, C.O. Stockle), Springer. 2017;165–180.
4. Yassin M, EL-Sabagh A, Mekawy AMM, Islam MS, Hossain A, Barutcular C, et al. Comparative performance of two bread wheat (*Triticum aestivum* L.) genotypes under salinity stress. Applied Ecology and Environmental Research. 2019;17(2): 5029-5041.
5. Mahboob W, Khan MA, Shirazi M. Induction of salt tolerance in wheat (*Triticum aestivum* L.) seedlings through exogenous application of proline. Pakistan Journal of Botany. 2016;48:861–867.
6. Bouchereau A, Aziz A, Larher F, Martin-Tanguy J. Polyamines and environmental challenges: Recent development. Plant Science. 1999;140:103–125.
7. El-Bassiouny H, Bekheta M. Role of putrescine on growth, regulation of stomatal aperture, ionic contents and yield by two wheat cultivars under salinity stress. Egyptian Journal of Physiological Science. 2004;2:239-258.
8. Nemat H, Heb Heba H, Alshafei. Exogenous application of spermine p utrescine mitigate adversities of drought stress in wheat by protecting membranes chloroplast ultra-structure. Physiology and Molecular Biology of Plants. 2021;26:233-245.
9. Ahmad P, Kumar A, Gupta A, Hu X, Azooz MM, Sharma S. Polyamines: Role in plants under abiotic stress. Crop production for agricultural improvement (eds M. Ashraf, M. Ozturk, M. Sajid, A. Ahmad & A. Aksoy), Springer. 2012;491–512.
10. Assad KA. Reduction of the harmful effects of water stress by exogenous spermine and amino fertilizer of wheat plants. Eurasian Journal of Biosciences. 2020;14:6429-6436.
11. Tiburcio AF, Altabella T, Bitrián M, Alcázar R. The roles of polyamines during the

lifespan of plants: From development to stress. Planta. 2014;240:1–18.

12. Legocka J, Kluk A. Effect of salt and osmotic stress on changes in polyamine content and arginine decarboxylase activity in *Lupinus luteus* seedlings. Journal of Plant Physiology. 2005;162:662–668.
13. Yamaguchi K, Takahashi Y, Berberich T, Imai A, Takahashi T, Michael AJ, Kusano T. 7 A protective role for the polyamine spermine against drought stress in Arabidopsis. Biochemical and Biophysical Research Communications. 2003;52:486–490.
14. Capell T, Bassie L, Christou P. Modulation of the polyamine biosynthetic pathway in transgenic rice confers tolerance to drought stress. Proceedings of the National Academy of Sciences of the United States of America. 2004;101:9909–9914.
15. Zhao H, Yang H. Exogenous polyamines alleviate the lipid peroxidation induced by cadmium chloride stress in Malus hupehensis Rehd. Scientia Horticulturae. 2008;116:442–447.
16. Hasnaa SA, Yousef ARM, El-Moursi A. Improving fruit and oil quality of picual olive through the exogenous application of putrescine and stigmasterol. New York Science Journal. 2011;4:40-45.
17. Hiscox JT, Israelstam G. A method for the extraction of chlorophyll from leaf tissue without maceration. Canadian Journal of Botany. 1979;57:1332–1334.
18. Mukherjee S, Choudhuri M. Implications of water stress-induced changes in the levels of endogenous ascorbic acid and hydrogen peroxide in Vigna seedlings. Physiologia Plantarum. 1983;58:166–170.
19. Smith IK. Stimulation of glutathione synthesis in photorespiring plants by catalase inhibitors. Plant Physiology. 1985;79:1044–1047.
20. Sharma P, Jha AB, Dubey RS, Pessarakli M. Reactive oxygen species, oxidative damage, and antioxidative defence mechanism in plants under stressful conditions. Journal of Botany; 2012. DOI: 10.1155/2012/217037
21. Miller G, Suzuki N, Ciftci-Yilmaz S, Mittler R. Reactive oxygen species homeostasis and signalling during drought and salinity stresses. Plant, Cell & Environment. 2010;33:453–467.
22. Gill SS, Anjum NA, Gill R, Tuteja N. Abiotic Stress Signaling in Plants–An Overview. Abiotic Stress Response in Plants (eds N. Tuteja & S. S. Gill). Wiley. 2016;3-12.
23. Kumar M, Hasan M, Arora A, Gaikwad K, Kumar S, Rai RD, Singh A. Sodium chloride-induced spatial and temporal manifestation in membrane stability index and protein profiles of contrasting wheat (*Triticum aestivum* L.) genotypes under salt stress. Indian Journal of Plant Physiology. 2015;20:271–275.
24. Nouri H, Navabpour S, Yamchi A, Zeyaee F. Differential response of parent and advanced mutant lines of wheat (*Triticum aestivum* L. cv. Tabasi) genotypes in antioxidant activity to salinity stress at seedling stage. International Journal of Biosciences (IJB). 2015;6:133–147.
25. Ashraf MA, Ashraf M, Shahbaz M. Growth stage-based modulation in antioxidant defence system and proline accumulation in two hexaploid wheat (*Triticum aestivum* L.) cultivars differing in salinity tolerance. Flora-Morphology, Distribution, Functional Ecology of Plants. 2012;207:388–397.
26. Xu YC, Wang J, Dong X, Xu YC, Li MM, Shan L, Lib MM. Effect of exogenous polyamines on glycolate oxidase activity and active oxygen species accumulation in wheat seedlings under osmotic stress. Israel Journal of Plant Sciences. 2001;49:173–178.
27. Pottosin I, Velarde-Buendía AM, Bose J, Zepeda-Jazo I, Shabala S, Dobrovinskaya O. Cross-talk between reactive oxygen species and polyamines in regulation of ion transport across the plasma membrane: Implications for plant adaptive responses. Journal of Experimental Botany. 2014;65:1271–1283.
28. Li Z, Zhou H, Peng Y, Zhang X, Ma X, Huang L, Yan Y. Exogenously applied spermidine improves drought tolerance in creeping bentgrass associated with changes in antioxidant defense, endogenous polyamines and phytohormones. Plant Growth Regulation. 2015;76:71–82.
29. Verma S, Mishra SN. Putrescine alleviation of growth in salt stressed *Brassica juncea* by inducing antioxidative defence system. Journal of Plant Physiology. 2005;162: 669–677.
30. Jiang DX, Chu X, Li M, Hou JJ, Tong X, Gao ZP, Chan GX. Exogenous spermidine enhances salt stressed rice photosynthetic performance by stabilizing structure and function of chloroplast and thylakoid

membranes. Phtosynthetica. 2020;58(1): 61-71.
31. Ashraf MA, Ashraf M, Ali Q. Response of two genetically diverse wheat cultivars to salt stress at different growth stages: Leaf lipid peroxidation and phenolic contents. Pakistan Journal of Botany. 2010;42:559–565.
32. Sobahan MA, Akter N, Murata Y, Munemasa S. Exogenous proline and glycine betaine mitigate the detrimental effect of salt stress on rice plants. Silpakorn University Science and Technology Journal. 2016;10:38–43.
33. Mandhania S, Madan S, Sawhney V. Antioxidant defence mechanism under salt stress in wheat seedlings. Biologia Plantarum. 2006;50:227–231.
34. Shu S, Yuan LY, Guo SR, Sun J, Yuan YH. Effects of exogenous spermine on chlorophyll fluorescence, antioxidant system and ultrastructure of chloroplasts in Cucumis sativus L. under salt stress. Plant Physiology and Biochemistry. 2013;63:209–216.
35. Jaleel CA, Gopi R, Sankar B, Manivannan P, Kishorekumar A, Sridharan R, Panneerselvam R. Studies on germination, seedling vigour, lipid peroxidation and proline metabolism in *Catharanthus roseus* seedlings under salt stress. South African Journal of Botany. 2007;73:190–195.
36. Jing J, Gua S, Li Y, Weihua L. The alleviating effects of exogenous polyamines on heat stress susceptibility of different heat resistant wheat (*Triticum aestivum* L.) varieties. Scientific Reports. 2020;10:7476.
Available:https://doi.org/10.1038/s41598-020-64468-5
37. Sairam R, Saxena D. Oxidative stress and antioxidants in wheat genotypes: Possible mechanism of water stress tolerance. Journal of Agronomy and Crop Science. 2000;184:55–61.
38. Sairam R, Srivastava G. Changes in antioxidant activity in subcellular fractions of tolerant and susceptible wheat genotypes in response to long term salt stress. Plant Science. 2002;162:897–904.
39. Amirjani M. Estimation of wheat responses to high heat stress. American-Eurasian Journal of Sustainable Agriculture. 2012;6: 222–233.
40. Morad M, Sara M, Mohammad D, Javad R, Majid R. Effect of salicylic acid on alleviation of salt stress on growth and some physiological traits of wheat. International Journal of Biosciences. 2013;3:20–27.
41. Domonkos I, Kis M, Gombos Z, Ughy B. Carotenoids, versatile components of oxygenic photosynthesis. Progress in Lipid Research. 2013;52:539–561.
42. Mohammad Y, Beniamino L, Carmine C. Photosynthetic responses of durum wheat to chemical/microbial fertilization management under salt and drought stress. Acta Physiologiae Plantarum. 2021;43:123.
Available:https://doi.org/10.1007/s11738-021-03289-z
43. Agrawal S, Rathore D. Changes in oxidative stress defence system in wheat (*Triticum aestivum* L.) and mung bean (*Vigna radiata* L.) cultivars grown with and without mineral nutrients and irradiated by supplemental ultraviolet-B. Environmental and Experimental Botany. 2007;59:21–33.
44. Sairam R, Srivastava G, Agarwal S, Meena R. Differences in antioxidant activity in response to salinity stress in tolerant and susceptible wheat genotypes. Biologia Plantarum. 2005;49:85–91.
45. Jain V, Vart S, Verma E, Malhotra SP. Spermine reduces salinity-induced oxidative damage by enhancing antioxidative system and decreasing lipid peroxidation in rice seedlings. Journal of Plant Biochemistry and Biotechnology. 2015;24:316–323.
46. Parveen S, Hussain A. Methionine induced changes in growth, glycinebetaine, ascorbic acid, total soluble proteins and anthocyanin contents of two *Zea mays* L. varieties under salt stress. The Journal of Animal and Plant Sciences. 2021;31(1):131-142.
47. Kumar B, Singla-Pareek SL, Sopory SK. Glutathione homeostasis: Crucial for abiotic stress tolerance in plants. Abiotic Stress Adaptation in Plants (eds A. Pareek, S. K. Sopory, H. J. Bohnert & Govindjee), 2009;263–282. Springer.
48. Szalai G, Kellős T, Galiba G, Kocsy G. Glutathione as an antioxidant and regulatory molecule in plants under abiotic stress conditions. Journal of Plant Growth Regulation. 2009;28:66–80.
49. Groppa MD, Tomaro ML, Benavides MP. Polyamines and heavy metal stress: The antioxidant behavior of spermine in

cadmium and copper-treated wheat leaves. Biometals. 2007;20:185–195.
50. Durna RA, Lala MA, Ismayil SZ, Irada HM. Diurnal changes of the ascorbate-glutathione cycle components in wheat genotypes exposed to drought. Functional Plant Biology. 2020;47(11):998-1006.

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