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INFLUENCE OF MELATONIN ON ANTIOXIDANT DEFENSE SYSTEM AND YIELD OF WHEAT (*TRITICUM AESTIVUM* L.) GENOTYPES UNDER SALINE CONDITION

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Abstract

Melatonin (N-acetyl-5-methoxytryptamine) has emerged as a new growth regulator in plants due to its positive role in alleviation of abiotic stresses. The regulation effect of melatonin in mediation of salinity stress on antioxidative activities, growth and yield of wheat plants was investigated using genotypes Sarsabz and S-24, grown under 10 dSm^{-1} NaCl salinity stress. Different concentrations of melatonin (50, 100, 300 and 500 µM) were applied foliarly after 45 days of sowing (DAS). Results revealed that melatonin under salinity stress significantly improved the growth and yield of both wheat genotypes as compared to non-treated ones. The yield was enhanced to 5 and 11% by 50 µM melatonin in both wheat cultivars respectively, which increased further 44 and 48% at 500 µM melatonin level in Sarsabz and S-24 respectively, as compared to salinity alone treatment. Furthermore, foliar spray of melatonin was effective in improving the activities of catalase, peroxidase and superoxide dismutase under stress conditions in wheat genotypes. The application of 500 µM melatonin was more effective in reducing the oxidative damage under salinity stress in terms of activities of antioxidant enzymes with an alternate decrease in malondialdehyde content. The increased activities of antioxidant enzymes are positively related with an enhanced biomass production and yield showing the ameliorative effect of melatonin under salt stress condition by up regulating the antioxidative defense mechanism. Results suggested that the foliar application of melatonin can be a useful strategy to help plant combat adverse conditions for enhancing yield of wheat plants.

Key words: Plant hormone, Abiotic stress, Growth, Yield, Foliar spray.

Introduction

Salinity is a serious abiotic stress factor limiting crop growth and agricultural productivity (Zafar *et al*., 2015). According to UNEP, approximately 50% of world area is affected by salinity (Yokoi *et al*., 2002). Increasing level of salt stress reduced the plant fresh and dry weights, germination rate plant length, root dry weight, rate of photosynthesis, lipids and energy production (Li *et al*., 2012). Though the melatonin was discovered in earlier 1993-94 but a potential role in plants of various metabolic processes as a hormone was confirmed much later (Hardeland *et al*., 2011). In plants, it is formed in very complicated way from N-acetyl serotonin (Arnao & Hernandez-Ruiz, 2014).

Wheat, is grown in nearly all parts of the world. Pakistan stands 4th in Asia and 11th in the world as far as wheat production are concerned (Saeed *et al*., 2012). In Pakistan, the worst threat for the production of wheat is salinity (Zafar *et al*., 2015). Melatonin is found almost in every plant part but its maximum concentration (ranges from picogram to microgram) in seeds, leaves and fruits (Garcia-Parrilla *et al*., 2009). Melatonin not only regulates the genes related to ethylene transduction, its synthesis and transformation, promoting the ripening of fruits and increases the yield (Sun *et al*., 2015). In plants, melatonin produces auxin like affects under many stresses by decreasing the concentration of ROS. About one melatonin molecule detoxifies 20 free radicals or reactive oxygen species (Tan *et al*., 2007). Melatonin is also involved in the production of abscisic (ABA) and gibberellic acid playing having key role in seed germination (Zhang *et al*., 2014).

Melatonin's most noticeable effects dependents on the concentration applied and are specific for each plant (Reiter *et al*., 2015).

The current study was executed to examine the regulation effect of exogenously applied different concentrations of melatonin on growth attributes, antioxidants and yield in wheat (*Triticum aestivum* L.) genotypes under NaCl salinity stress.

Materials and Methods

Ten seeds of each genotype (Sarsabz and S-24) were sown in plastic pots containing 8 kg of soil (soil properties - Table 1), placed in wire house at Experimental station of Government College University Faisalabad, Pakistan. Seeds were obtained from Ayub Agricultural Research Institute (AARI), Faisalabad. The required level of salinity (10 dSm^{-1}) was obtained after two weeks of sowing by supplying NaCl solution to the soil.

Different levels of melatonin 50, 100, 300 and 500 µM were applied foliarly at vegetative stage (45 days old plants). Factorial (completely randomized) design was applied with three replicates for each treatment.

Table 1. Physico-chemical properties of the soil.

Soil texture	Sandy loam	Cl^- (meq/L)	2.32
EC_e (dS m^{-1})	0.50	Ca^+ Mg (meq/L)	4.3
pH	7.11	Na^+ (ppm)	2.81
Organic matter (%)	0.29	Soluble K^+ (ppm)	23
Saturation (%)	37	Total N (%)	0.071
CO_3^{2-} (meq/L)	Nil	Available P(ppm)	8.41
HCO_3^- (meq/L)	2.94	Zn (ppm)	2

Three plants from each pot were harvested 15 days after melatonin application for the determination of plant growth, antioxidants and MDA contents. Yield attributes were recorded at maturity stage of wheat crop. Plants were oven dried for 72 hours for dry weight.

Chlorophyll contents were extracted from leaves according to the method suggested by Arnon (1949) and were calculated using formula by Davies (1976).

Leaves were homogenized in 50 mM phosphate buffer 7.0 pH, having 1 mM dithiothreitol (DTT). Superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) were analyzed by using spectrophotometer (Dixit *et al*., 2001). The activity of CAT was determined in 3 ml 7.0 pH phosphate buffer (50 mM) having 5.9 mM H_2O_2 and enzyme extract (0.1 ml) as reported by Chance & Maehly (1955). Activity of CAT enzyme measured at 240 nm absorbance past each 20 sec. Every unit of activity is a change of 0.01 unit min^{-1}.

Peroxidase is the peroxidation as an electron donor of hydrogen peroxide and guaicol (Chance & Maehly, 1955). Add 50 mM, phosphate buffer (pH 5), 20 mM of guaicol, H_2O_2 (40 mM) and 0.1 mL enzyme extract. Enhanced absorbance at 470 nm by tetra guaicol formed measured after each 20 sec. A single enzyme unit was responsible for enhancing OD value of 0.01 min^{-1}. SOD activity (Giannopolitis & Ries, 1977) was measured as the inhibition reduction of nitroblue tetrazolium (NBT) by xanthine oxidase. Absorbance was measured by UV-visible (IRMECO U2020) spectrophotometer at 560 nm. A single unit activity exhibited 50% photochemical inhibition of NBT.

The malondialdehyde (MDA) contents were estimated by the technique of Camak & Horst (1991). One gram fresh leaves were ground in 20 ml (0.1%) tri-chloroacetic acid (TCA) and centrifuge for 10 min. Take 1 ml of the supernatant, 4 ml of mixture having 20% TCA with 0.5% thiobarbituric acid added. Allowed it to heat at 95°C for half an hour; cooled and centrifuged again for 10 minutes, absorbance of supernatant measured at 532 and 600 nm was calculated:

$$MDA\ (nmol) = \Delta\ (A\ 532\ nm\text{-}A\ 600\ nm)/1.56\times10^5$$

Fertile spikelets per spike, 1000-grain weight, biomass $plant^{-1}$ and yield $plant^{-1}$ were estimated at crop maturity. The collected data was analyzed by the software program Statistix version 8.1. Microsoft Excel-2007 was used to evaluate mean and standard errors, the difference of means was calculated at 5% probability level by least significant difference (LSD) test. XLSTAT software version 2014.5.03 was used to find out the significant correlations, dendrogram and principle component analysis among studied attributes.

Results

Response of two wheat genotypes (Sarsabz and S-24) to foliarly applied melatonin under saline and non-saline conditions were assessed in this study. Exhibit 17% and 9% reduction in shoot length was observed whereas 37% and 22% decrease in root length in Sarsabz and S-24 was noted respectively under saline conditions (Table 2). Application of melatonin exerted a positive influence on growth traits. Maximum shoot and root length in both wheat genotypes were observed by foliar application of 500 µM melatonin. Foliar applied melatonin significantly increased shoot in conjunction with root fresh and dry weight under saline but a non-significant increase under non-saline conditions. Under saline conditions, S-24 yielded high shoot, root fresh and dry weights. Sarsabz and S-24 exhibited a significant ($p\leq0.01$) reduction (58%, 56%) in root dry weight under NaCl stress respectively.

Significant reductions in chlorophyll a (Chl_a) and chlorophyll b (Chl_b) content of both wheat genotypes was recorded under salinity stress (Fig. 1). Sarsabz and S-24 exhibited 54% and 9% reduction in Chl_b respectively. However, application of melatonin prevents damage caused by stress on chlorophyll. Chl_b content was increased with increasing level of melatonin from 50 to 500 µM. Under salinity stress, maximum Chl_a and Chl_b content were observed at 500 µM melatonin followed by 300 µM, 100 µM, 50 µM under saline conditions and minimum was observed at salinity alone.

Antioxidant activities were significantly increased in salinity stressed wheat genotypes (Fig. 1). Melatonin alleviated oxidative stress as manifested by further enhancing the activities of SOD, CAT, POD and a decrease in MDA contents in melatonin treated plants as compared to at salinity alone. The applied dose of 500 µM melatonin under saline conditions was helpful in minimizing the catalase activity from 134 to 116 unit min^{-1} in Sarsabz and from 122 to 106 unit min^{-1} in S-24. Overall, maximum activities of SOD, CAT and POD in both wheat genotypes were recorded in salinity alone and minimum was recorded at 500 µM melatonin. Increase in MDA content was observed under saline conditions in comparison with control in Sarsabz and S-24. However, genotype S-24 showed better response regarding MDA contents and SOD activity under saline conditions.

The most encouraging results regarding thousand grain weight in Sarsabz and S-24 were recorded in plants treated with 500 µM melatonin (39.06g, 42.29g) followed by 300 µM (38.95g, 39.79g) under salinity stress. Numbers of fertile spikelets $spike^{-1}$ were significantly decreased in Sarsabz (16%) and S-24 (17%) under salinity stress as compared to control. An amplifying trend towards this attribute was observed with 500 µM melatonin followed by 300 µM melatonin under saline conditions. Data regarding biomass $plant^{-1}$ and yield $plant^{-1}$ revealed a decrease of 40 and 16% in Sarsabz, and 32 and 19% in S-24 respectively under salinity stress (Table 3).

The dendrogram analysis (Fig. 2) classified all studied attributes into three major classes. Parameters grouped in classes 1 and 2 exhibited a positive correlation among themselves in a single class as well as among the class (Table 4). However, the class 3 (antioxidants) presented a negative correlation with all other classes. Principle component analysis (PCA) (Fig. 3) grouped all the studied attributes in close relation with each other except to that of antioxidants. Of the extracted components, F1 and F2 (90.81%) showed major contribution as compared with others. The component F1 contributed 80.22% as compared to F2 10.59%. Yield $plant^{-1}$ was correlated positively with chlorophyll contents and biomass. Results showed that imposition of salinity stress enhanced antioxidant activity (MDA, POD, CAT and SOD) and decreased growth attributes.

Table 2. Effect of melatonin on growth attributes of wheat genotypes under saline conditions.

Treatments			Shoot Attributes			Root Attributes		
Genotypes (G)	**Salinity (S)**	**Mel (µM)**	**FW (g)**	**DW(g)**	**SL(cm)**	**F W (g)**	**DW (g)**	**RL (cm)**
Sarsabz	Control	0	6.41a	1.80bc	42.33ab	0.95a	0.81ab	7.10abc
		50	6.56a	0.95abc	44.00ab	0.96a	0.86a	7.20abc
		100	6.62a	2.18ab	44.33ab	0.97a	0.90a	7.25abc
		300	6.64a	0.36a	45.66a	0.98a	0.91a	7.61ab
		500	6.61a	2.40a	45.67a	0.96a	0.92a	8.30a
	dSm^{-1}	0	4.97b	1.27d	36.33d	0.54c	0.34c	4.45d
		50	5.40ab	1.45cd	38.66cd	0.61b	0.36bc	5.61c
		100	5.44ab	1.62cd	41.66bc	0.63b	0.42bc	6.31bc
		300	5.60ab	1.70bcd	42.67ab	0.63b	0.47b	6.35bc
		500	5.64ab	1.73bcd	43.33ab	0.64ab	0.48b	7.50ab
	Control	0	6.64a	1.99b	43.00cd	0.97a	0.83a	6.83bc
S-24		50	6.65a	2.01b	43.33bcd	0.97a	0.89a	7.66ab
		100	6.67a	2.13ab	44.00bc	0.98a	0.9a	7.67ab
		300	6.68a	2.39a	46.67ab	0.97a	0.90a	8.20ab
		500	6.70b	2.43a	47.66a	0.97ab	0.93a	8.43a
	100mM	0	5.60a	1.39d	39.00e	0.59d	0.36c	5.33d
		50	5.67a	1.56cd	40.33de	0.64c	0.42bc	5.93cd
		100	5.77a	1.57cd	42.33cde	0.66c	0.45bc	7.01abc
		300	5.78a	1.84bc	43.33bcd	0.68bc	0.52b	7.33abc
		500	5.79ab	1.86bc	45.67abc	0.70b	0.53b	7.86ab
	F_g		1.05ns	0.55ns	4.82ns	4.03ns	2.14ns	4.951*
	F_s		22.96***	48.93**	48.13***	187.4**	614.0*	27.55***
	F_m		1.45ns	13.55**	63.33***	6.98***	6.62**	10.32***
	$F_g \times F_s$		0.44ns	1.32ns	12.15ns	7.32**	1.04ns	0.39ns
	$F_s \times F_m$		1.23ns	1.03ns	1.93ns	2.94*	0.68ns	0.18ns
	$F_g \times F_m$		0.33ns	0.53ns	1.82ns	0.25ns	0.09ns	0.98ns
	$F_g \times F_s \times F_m$		0.28ns	0.09ns	0.48ns	0.08ns	0.09ns	0.75ns

Means with same alphabet are significantly similar. *, ** and *** Means significant difference at 0.05, 0.1 and 0.01 probability levels. Mel; Melatonin, FW; Fresh weight, DW; Dry weight, SL; Shoot length, RL; Root length

Fs, Fm and Fg are the F values from analysis of variance of the data of wheat genotypes affected by different levels of melatonin grown under salt stress

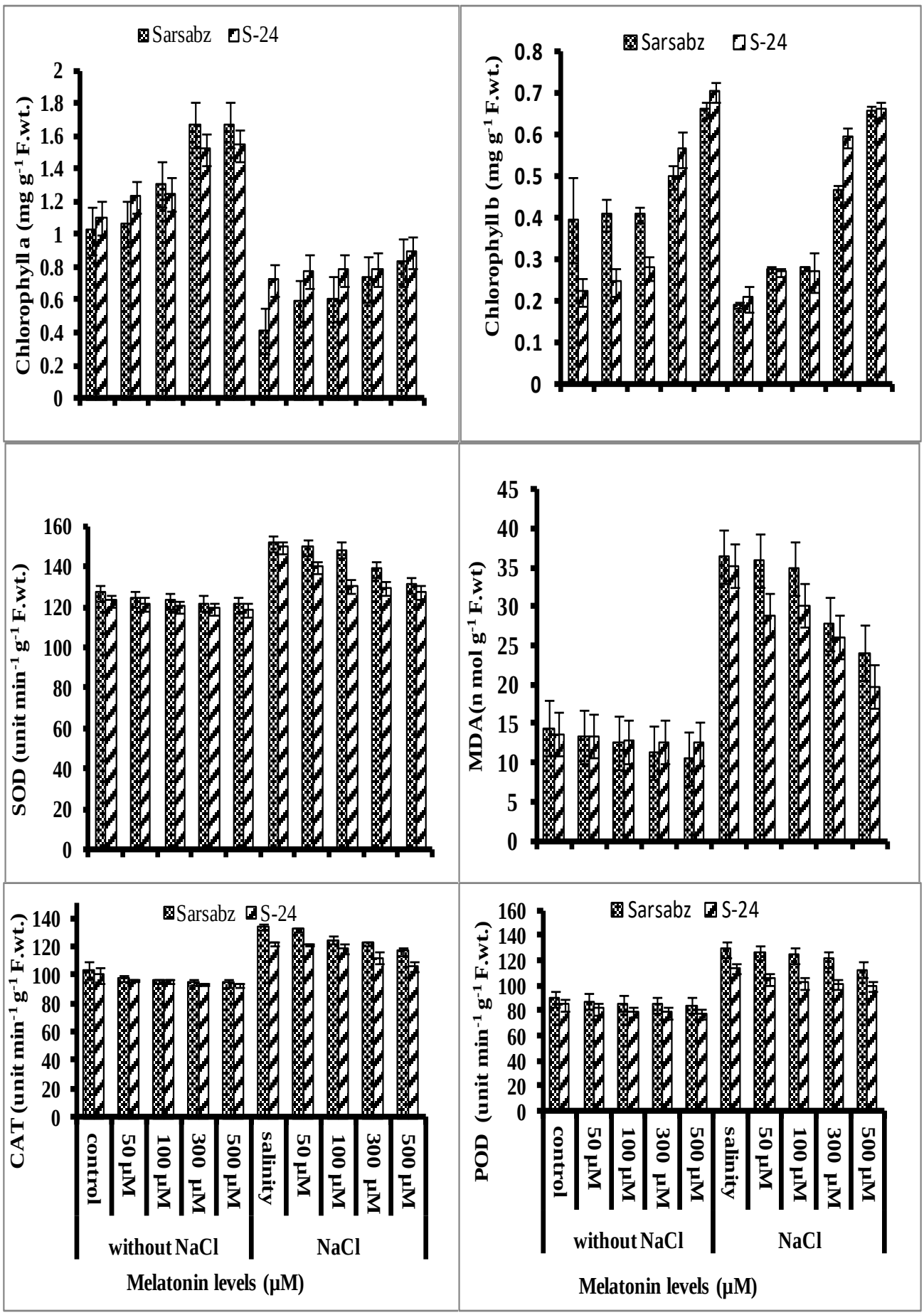

Fig. 1. Effect of melatonin on chlorophyll contents and antioxidants of two wheat genotypes under saline and non-saline conditions.

Table 3. Effect of melatonin on yield attributes of wheat genotypes under saline conditions.

Genotypes (G)	Salinity (S)	Mel (µM)	1000 grain wt.(g)	Fertile spklt/spk	Biomass/ plant(g)	Yield/ plant(g)
Sarsabz	Control	0	34.84b	11.67abc	6.67bcd	2.70a
		50	38.62ab	11.89abc	7.33bc	2.80a
		100	39.97a	12.21a	8.07ab	2.96a
		300	39.57a	12.33a	8.26ab	2.83a
		500	40.34a	12.78a	9.00a	2.98a
	100mM	0	26.53d	9.78d	4.00e	1.83d
		50	33.64c	10.33cd	5.67d	1.93cd
		100	37.43abc	10.56bcd	6.00cd	2.06bc
		300	38.95ab	11.56abc	7.67ab	2.39abc
		500	39.06ab	12.00ab	7.83ab	2.63ab
	Control	0	38.00bc	13.00bcd	7.00def	3.39bcd
S-24		50	41.02ab	14.33ab	7.50cde	3.81abc
		100	42.64ab	14.50ab	8.67bc	4.12a
		300	42.88ab	14.77a	8.52bc	4.20a
		500	43.95a	15.52a	10.00a	4.21a
	100mM	0	32.08d	10.77e	5.83f	2.73d
		50	34.18cd	11.67de	6.50ef	3.04d
		100	39.35abc	12.72cd	7.68bcd	3.31cd
		300	39.79ab	13.04bcd	8.33bc	3.75abc
		500	42.29ab	14.00abc	8.83ab	4.05ab
	F_g		12.76***	64.52***	15.73***	145.62***
	F_s		25.55***	47.95***	34.73***	46.72***
	F_m		16.40***	10.31***	25.04***	10.57***
	$F_g * F_s$		0.16ns	1.86ns	3.06ns	1.12ns
	$F_s * F_m$		2.19ns	0.69ns	1.38ns	2.01ns
	$F_g * F_m$		0.46ns	0.76ns	0.51ns	0.43ns
	$F_g*F_s*F_m$		0.30ns	0.15ns	0.39ns	0.43ns

Means with same alphabet are significantly similar. *, ** and *** Means significant difference at 0.05, 0.1 and 0.01 probability levels
Fs, Fm and Fg are the F values from analysis of variance of the data of wheat genotypes affected by different levels of melatonin grown under salt stress

Table 4. Correlation coefficient between physiological traits for salinity tolerance.

Variables	SFW	SDW	SL	RFW	RDW	RL	Chl. a	Chl. b	SOD	CAT	POD	MDA	1000 GW	F SPKT	Biomass	Yield/ plant
SFW	1															
SDW	0.893***	1														
SL	0.768***	0.910***	1													
RFW	0.983***	0.874***	0.715***	1												
RDW	0.977***	0.913***	0.758***	0.992***	1											
RL	0.773***	0.877***	0.960***	0.723***	0.760***	1										
Chl. a	0.909***	0.963***	0.827***	0.886***	0.916***	0.808***	1									
Chl. b	0.280ns	0.589**	0.744***	0.240 ns	0.321 ns	0.717***	0.488*	1								
SOD	-0.909***	-0.893***	-0.882***	-0.876***	-0.895***	-0.912***	-0.859***	-0.512*	1							
CAT	-0.971***	-0.929***	-0.853***	-0.952***	-0.965***	-0.854***	-0.918***	-0.433 ns	0.950***	1						
POD	-0.965***	-0.869***	-0.784***	-0.935***	-0.933***	-0.808***	-0.883***	-0.324 ns	0.944***	0.971***	1					
MDA	-0.954***	-0.908***	-0.817***	-0.956***	-0.968***	-0.823***	-0.899***	-0.440 ns	0.946***	0.975***	0.939***	1				
1000 GW	0.677**	0.792***	0.927***	0.599**	0.636**	0.925***	0.679**	0.613**	-0.815***	-0.745***	-0.696***	-0.686**	1			
F SPKT	0.710***	0.757***	0.820***	0.641**	0.671**	0.847***	0.689**	0.488*	-0.839***	-0.774***	-0.807***	-0.711***	0.860***	1		
BM	0.672**	0.827***	0.942***	0.587**	0.640**	0.932***	0.747***	0.759***	-0.837***	-0.759***	-0.720***	-0.715***	0.935***	0.848***	1	
Yield/plant	0.621**	0.627**	0.710***	0.536*	0.551*	0.739***	0.567*	0.390 ns	-0.754***	-0.695**	-0.764***	-0.606**	0.766***	0.956***	0.765***	1

Discussion

Salinity stress significantly reduced root and shoot length of both wheat genotypes. However, external application of melatonin improved growth of both the wheat genotypes (Table 2). Similarly, melatonin induced growth improvement was reported in maize (Jiang *et al*., 2016), *Vicia faba* (Dawood & El-Awadi, 2015) and soybean (Wei *et al*., 2014).

The analysis of data revealed that salinity stress significantly reduced the chlorophyll contents of wheat genotypes. The findings are in harmony with Zafar *et al*., (2018) in wheat and Sabra *et al*., (2012) in *Echinacea purpurea.* Santos (2004), reported that chlorophyll contents were decreased because of the reduction of 5-aminolinolic acid under salt stress. Exogenous application of 500 µM melatonin improved the chlorophyll contents and was the most effective treatment for minimizing the harmful effects of salinity (Li *et al*., 2012).

The accumulation of reactive oxygen species (ROS) is harmful to plant structure and function; thereby, to restore cellular redox balance antioxidants are activated to detoxify the toxic levels of ROS (*Ahmad et al*., 2009). Enzyme activities are enhanced in leaves of both wheat genotypes, suggesting that wheat activates its antioxidant system under saline condition. Foliar application of melatonin maintained the SOD, POD & CAT activities under saline conditions. It is reported that melatonin activates antioxidant defense system in plants to reduce oxidative damage (Zhang *et al*., 2014).

Malondialdehyde (MDA) is produced by degradation of polyunsaturated lipids; it is accumulated in tissues as a lipid peroxidation product (Parida & Das, 2005). Exogenous application of melatonin reduced MDA content, accounting for enhanced antioxidant response and the protective role of membranes thus increasing the plant tolerance to damage. A reduction in wheat grain yield was observed in our study which might be due to reduction in yield contributing traits like fertile spikelets spike^{-1}, 1000 grain weight and biomass plant^{-1} under saline conditions, attributed to reduced photosynthetic activity of plants (Plaut *et al*., 2004). Salinity stress curtail grain filling period, significantly reduce final grain weight (Javed *et al*., 2003). Reduction in plant yield under stress is a common observation, however, tolerant varieties successfully maintain higher yield than sensitive ones (Anjum *et al.,* 2011). Results of present study showed that S-24 maintained higher yield and yield components with exogenous application of melatonin under salt stress. However, genotype S-24 was found superior in managing biomass plant^{-1}, thousand grain weight and yield plant^{-1}. Our results were similar with Tan *et al.,* (2012) who reported melatonin-treated corn plants with greater production. It is believed that melatonin has auxin like growth promoting effects as both are structurally similar. An improvement in crop yield potential by melatonin application is reported by Wei *et al*., (2014) in soybean plants.

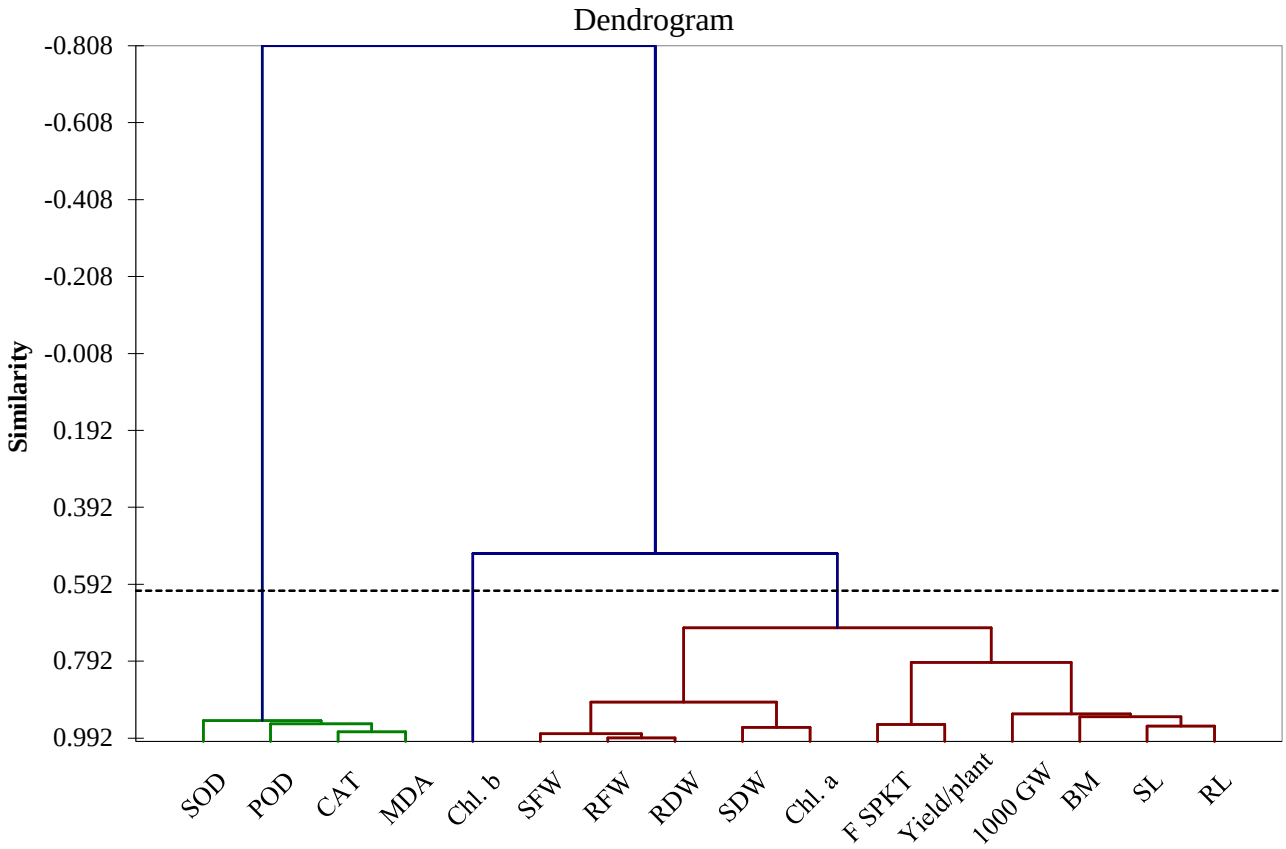

Fig. 2. Dendogram showing relationship among different physiological traits under salinity stress Principle component analysis (PCA)

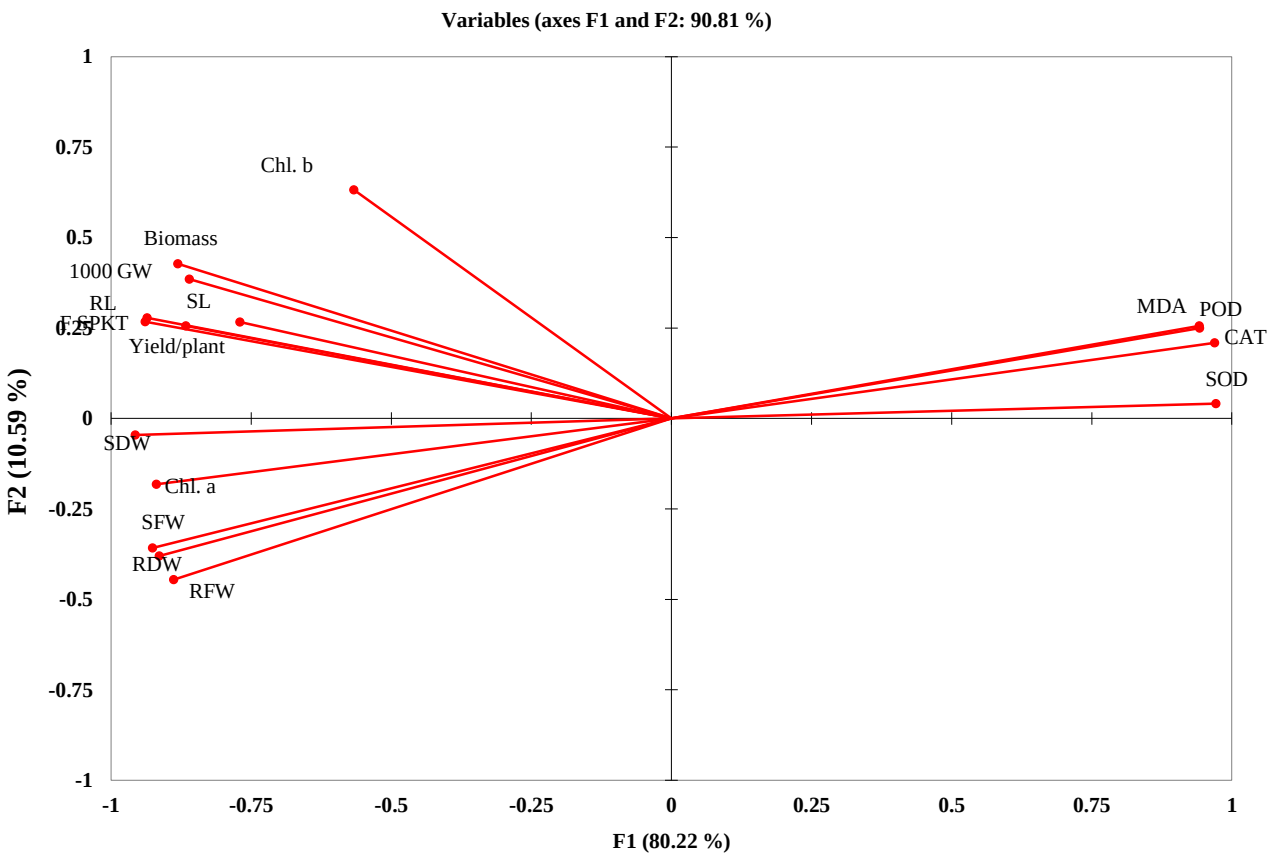

Fig. 3. PCA analysis showing physiological traits as salt tolerance indicators (Chl; chlorophyll, GW; grain weight, SL; shoot length, RL; root length, FSPKT; fertile spikelet per spike, SDW; shoot dry weight, SFW; shoot fresh weight, RDW; root dry weight, RFW; root fresh weight, BM; biomass, MDA; malondialdehyde, POD; peroxidase, CAT; catalase, SOD; superoxide dismutase).

Conclusion

Current study suggest the beneficial role of exogenously applied melatonin on growth, improvement of the antioxidant potential and yield of wheat plants under saline environment. Exogenous application of 500 µM melatonin is an effective approach to decrease the effect of salinity stress in wheat genotypes.

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