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Exogenous melatonin improves seedling vigour and drought tolerance in wheat

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Melatonin (N-acetyI-5-methoxytryptamine) is a ubiquitous growth regulator suggested as a promising molecule involved in abiotic stress tolerance. In the past few decades, research on melatonin was hastened to reveal its role in agricultural science (Arnao and Hernandez-Ruiz, 2015). Many studies have confirmed its significant role in seed germination, root and shoot growth regulation, growth stimulation and delaying induced leaf senescence under stress in different crops (Wang *et al.*, 2013a; Shi *et al.,* 2015a).Shi *et al.,* (2015b) reported increased abiotic stress tolerance by increasing antioxidant activity and secondary metabolites in bermudagrass. However, many regulatory and functional roles of melatonin are still not understood completely in cereal crops like wheat (Ahmad *et al.,* 2019). Hence, an experiment was conducted to study the effect of melatonin on Indian wheat genotypes with respect to shoot and root growth parameters under moisture deficit condition at seedling stage.

The experiment was conducted at ICAR-Indian Institute of Wheat and Barley Research, Karnal during crop season 2017-18 under controlled condition. Six wheat genotypes namely, C306, NI5439, HD3086, RW5, DBW136, WH147 were sown in two sets with three replications in the germination tray under drought stress condition. Melatonin treatment was given to one set of genotypes at GS 14, GS 22 growth stages according to Zadok's scale (Zadok's 1974) which generally coincides with seedling development stage and tillering initiation stage. The second set of genotypes was kept untreated based on the literature survey 100µM concentration of melatonin was considered as optimal for the experiment (Ye *et al.,* 2016; Gao *et al.*, 2018; Ahmad *et al.*, 2019; Huang *et al.,* 2019; Bai *et al.,* 2020). Data was recorded for Chlorophyll content (SPAD), Normalized Difference Vegetation Index (NDVI) and Chlorophyll fluorescence (CFL) along with seedling growth related traits like shoot fresh weight, shoot dry weight, root fresh weight, root length on per plant basis. Analysis of variance and ranking of genotypes was done using Tukey's Studentized range test at 0.05 (5%) level of significance. Pearson's correlation coefficient (r) was used to indicate the interaction among the traits studied. All the statistical analysis were performed using JASP 0.12.1 statistical package (JASP Team, 2020).

Analysis of variance (Table-1) showed significant differences among the treatments for most of the traits like, SPAD, CFL, Shoot fresh weight, shoot dry weight and root fresh weight except for root to shoot ratio. The genotypes performed better under melatonin treatment for almost all parameters studied (except R:S ratio) compared to drought stress. This is in close compliance with those obtained by Ahmad *et al.,* 2019.When the genotypes were compared for different physiological traits then more variation was found in melatonin treated set of wheat genotypes as compared to drought. Lower mean SPAD value (47.56) was recorded under drought condition as compared to melatonin treated (51.49) condition(Table-2). Chlorophyll fluorescence is the widely used most important mean for accessing the maximal photochemical efficiency of PSII (Zhou *et al.,* 2010).Data recorded for CFL showed substantial impact of melatonin spray on wheat genotypes and the values were higher (0.809) as compared to drought (0.803). The similar results were also reported by Ye *et al,* 2016.

In case of plant growth related traits, overall increase in plant weight (root and shoot) was observed under melatonin treated condition as compared to drought. The mean shoot fresh weight under drought stress was 0.974g however under treated set of genotypes the values were 1.44g. The same trend was followed for root fresh weight for the genotypes under untreated (0.405g) and treated (0.478g) conditions (Table-2). Melatonin also effected the growth in root length and the mean root length under treated (18.02cm) condition was higher as compared to the drought (15.27cm) condition (Table-2). Ye et al (2016) also reported the better rooting as well as root water uptake efficiency under drought due to melatonin application in maize seedlings.

Two-way ANOVA revealed that, in the treated set of wheat genotypes no significant difference has been recorded for SPAD values among the genotypes except DBW136 which was significantly low value (45.86). However, among the genotypes under drought condition C306 was having highest chlorophyll content (50.36) while DBW136 showed lowest SPAD value (42.83).

The presence of significant genotypic differences among the studied genotypes was observed for SPAD and root fresh weight under drought while SPAD, CFL and Shoot dry weight under treated conditions (Table-2). The observations revealed that higher CFL values were recorded for C306(0.813), HD3086 (0.813) and DBW136 (0.812) followed by NI5439(0.808) under melatonin treated set. While in drought condition DBW136 (0.800) has showed minimum CFL value and no significant difference was recorded for other genotypes. RW5 (0.803)

Table 1. ANOVA for different physiological and plant growth traits in six wheat genotypes

Source of variation	df	SPAD	NDVI	CFL	Shoot Fresh Wt	Shoot Dry Wt	Root Length	Root Fresh Wt	R:S Ratio
Treat	1	< .001	0.001	< .001	< .001	< .001	0.006	< .001	0.096
gen	5	< .001	0.035	0.001	0.048	0.002	0.032	0.010	0.053
Treat gen	5	0.851	0.760	< .001	0.390	0.297	0.797	0.999	0.461

Table 2. Comparative effect of melatonin on different physiological and plant growth traits in wheat genotypes under drought and treated conditions

Treatment	Genotypes	SPAD	NDVI	CFL	Shoot Fresh Wt (g)	Shoot Dry Wt (g)	Root Length cm	Root fresh Wt (g)	R:S Ratio
Drought	C306	50.367^{aAB}	0.227^{aA}	0.804^{aBC}	0.880^{aB}	0.490^{aB}	16.26^{aAB}	0.497^{aABC}	0.563^{aA}
	DBW136	42.833^{bC}	0.197^{aA}	0.800^{aC}	1.157^{aAB}	0.843^{aB}	15.63^{aAB}	0.441^{abABC}	0.387^{aAB}
	HD3086	47.667^{abABC}	0.247^{aA}	0.804^{aBC}	1.043^{aB}	0.643^{aB}	16.20^{aAB}	0.379^{abABC}	0.367^{aAB}
	NI5439	48.767^{abABC}	0.233^{aA}	0.804^{aBC}	0.890^{aB}	0.517^{aB}	16.76^{aAB}	0.407^{abABC}	0.463^{aAB}
	RW5	48.833^{abABC}	0.197^{aA}	0.802^{aBC}	1.010^{aB}	0.667^{aB}	14.13^{aAB}	0.365^{abBC}	0.360^{aAB}
	WH147	46.900^{abABC}	0.217^{aA}	0.803^{aBC}	0.863^{aB}	0.587^{aB}	12.66^{aB}	0.340^{bC}	0.390^{aAB}
Mean		47.56	0.22	0.803	0.974	0.625	15.27	0.405	0.42
Drought+ Melatonin treatment	C306	52.700^{aA}	0.280^{aA}	0.813^{aA}	1.317^{aAB}	0.837^{bB}	20.40^{aAB}	0.553^{aA}	0.433^{aAB}
	DBW136	45.867^{bBC}	0.223^{aA}	0.812^{aA}	2.007^{aA}	1.630^{aA}	16.83^{aAB}	0.545^{aAB}	0.273^{aB}
	HD3086	53.100^{aA}	0.277^{aA}	0.813^{aA}	1.123^{aB}	0.800^{bB}	17.40^{aAB}	0.473^{aABC}	0.433^{aAB}
	NI5439	52.867^{aA}	0.270^{aA}	0.808^{abAB}	1.437^{aAB}	0.923^{abB}	21.73aA	0.490^{aABC}	0.377^{aAB}
	RW5	53.000^{aA}	0.257^{aA}	0.803^{bBC}	1.453^{aAB}	1.167^{abAB}	16.50^{aAB}	0.424^{aABC}	0.347^{aAB}
	WH147	51.400^{aAB}	0.230^{aA}	0.802^{bBC}	1.307^{aAB}	0.877^{abB}	15.30^{aAB}	0.385^{aABC}	0.350^{aAB}
Mean		51.49	0.26	0.809	1.44	1.03	18.02	0.478	0.36

Mean values that do not share a letter (small: Within treatment, Capital: across treatments) are significantly different at p=<0.05

and WH147 (0.802) did not show any differential effect of melatonin treatment for CFL values.

Among the genotypes highest shoot fresh weight was obtained by DBW136 (2.007g) followed by RW5 (1.453g) under melatonin treatment and the same trend was observed for shoot fresh weight under drought. Under melatonin treated condition, DBW136 also recorded the maximum shoot dry weight (1.630g) followed by RW5 (1.167g) and NI5439 (0.923g), but there was no significant difference observed among genotypes under drought environment.Minimum root length and root weight under drought was recorded for WH147 (12.66cm and 0.340g) while others were at par with each other. But under melatonin treatment maximum root length was obtained by NI5439(21.73cm). Although C306 (20.40cm) was succeeded by NI5439 (21.73cm) in terms of root length but it maintained highest root fresh weight when treated with melatonin.

The results highlighted that melatonin application improved seedling vigour, and other morpho-physiological traits along with overall plant performance as compared to the non-treated set of wheat seedlings under drought stress. Though, the experiment is preliminary at seedling stage but the results are promising. However,the research findings entail that melatonin can improve drought stress tolerance and could be considered as a potential growth regulator for yield improvement in arid and semi arid areas.

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