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EXPLORING THE ROLE OF MELATONIN FOLIAR APPLICATION ON WHEAT UNDER DROUGHT

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Abstract

The production of wheat, a major essential crop in Pakistan and around the world, is declining for a variety of biotic and abiotic reasons even though the area under farming increases each year. Drought is primarily brought on by water scarcity and climatic shifts, both of which have a negative impact on the yield and production of wheat around the world. Applying biological fertilizers may help to relieve drought. In the present research, three melatonin foliar treatments—MN0 = 0 uM, MN1 = 50 uM and MN2 = 100 uM—were used to reduce the negative impacts of drought on wheat at three key development phases, namely tillering (DTS), blooming (DFS), and grain filling stage (DGFS), in a drought conditions. Wheat growth and production traits were substantially harmed by drought stress at all important development stages, with DGFS stage being the most vulnerable and leading to a sizable yield loss. But foliar melatonin (MN) application significantly lessened the negative effects of drought as compared to the control treatment by increasing plant height (15.74%), fertile tiller count (18.14%), spike length (17.61%), grain count per spike (14.89%), thousand grain weight (11.4%), and biological yield (13.1%). Additionally, MN boosted the metabolic metrics of wheat under dry conditions and increased water utilization efficiency. Using principal component analysis, which links various aspects of our data, we were able to describe how MN treatment impacted wheat development and output under dry circumstances. MN at 100 uM is the useful strategy for reducing the negative impacts of dry stress on wheat crop production.

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INTRODUCTION

Wheat is one of the most essential dietary products in the world, and severe stress has an impact on its production (Kosova et al., 2016). Reactive oxygen species (ROS) accumulate excessively as a typical result of environmental drought stress (Smirnoff, 1998). Since metabolism and photosynthesis both produce ROS, and over accumulation amount of it can harm proteins, DNA, RNA, and enzymatic function (Mittler, 2002).To avoid excessive abundance of ROS, Enzymatic antioxidants such as superoxide dismutase, catalase, and peroxidase, ascorbate peroxidase, monodehydroascorbate reductase, glutathione reductase, and dehydroascorbate reductase, as well as non-enzymatic antioxidants such as ascorbate and glutathione as well as vitamins, and carotenoids and polyphenols (Apel and Hirt, 2004). Particularly, the glutathione-ascorbate (GSH-AsA) cycle, which includes GSH, AsA, and associated antioxidant enzymes (APX, DHAR, MDHAR, and GR) that take part in GSH and AsA repair, is crucial for maintaining ROS equilibrium and allowing the body to withstand stress, including dehydration stress (Apel and Hirt, 2004).

A substance with a low molecular weight called melatonin (N-acetyl-5-methoxytryptamine) is present in all living things, from simple photosynthesis microbes to humans. Melatonin is a significant hormone that is generated by the pineal gland in mammals (Xi et. al., 2015) and has pleiotropic effects. It was first discovered in plants in 1995. It is found in mitochondria, cytoplasm, and chloroplast among other cell divisions because of its amphiphilic character (Su et. al., 2019). Because melatonin plays a variety of roles in plants, including seed germination, biomass production, enhanced respiration, delayed blooming, circadian rhythm, fruit maturation, and reactions to biotic and abiotic stress, study on the hormone is expanding. Melatonin supplementation during seed preparation increases seed quality as well as juvenile growth, plant production, and plant health. In contrast to other antioxidants, melatonin's main role is as a potent antioxidant and free radical scavenger that can neutralize up to ten free radicals (Tan et. al., 2015).

In plants, melatonin production takes place in four sequential stages (Choi and lee 2017). Tryptophan is first converted to tryptamine by the enzyme tryptophan decarboxylase (TDC), which is then followed by the enzyme tryptamine 5-hydroxylase (T5H) in the production of serotonin. Serotonin N-acetyltransferase (SNAT) catalysis the change of serotonin into acetylserotonin in the third stage. N-acetylserotonin is converted into melatonin by the Omethyltransferase (OMT) process, which is catalysed by Acetylserotonin O-Methyltransferase (ASMT) (Ding et. al., 2017). Caffeic Acid O-methyltransferase (COMT) participates in the final stage. Due to its capacity to generate serotonin and O-methylated N-acetylserotonin, ASMT can create melatonin. Additionally, plants that experience particular stress circumstances exhibit higher expression of genes implicated in melatonin production.

Numerous studies have shown that melatonin increases the ability of plants to withstand drought and has a variety of functions in plants, such as postponing the ageing of leaves, controlling water balance, fostering the development of lateral roots and seed germination, preserving the structural integrity of leaves and chloroplasts, and regulating nitro-oxidative homeostasis and proline metabolism. (Wang et al. 2013; Li et al. 2015; Wei et al. 2015;

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Antoniou et al. 2017; Meng et al. 2014). In particular, the melatonin level in drought-stressed plants such as cucumber, apple, Arabidopsis, grape, tomato, soybean, Bermuda grass, rice, and Medicago sativa was linked to the enhanced antioxidant capacity and elevated ROS equilibrium (Antoniou et al., 2017).

Objectives of current study were to investigate the role of melatonin foliar application under drought stress in wheat.

MATERIALS AND METHODS

In order to examine the impact of PGPR on wheat development under dry stress, a wire house trial were conducted in 2020–21 at the Islamia University of Bahawalpur's Area of Research, to examine that how melatonin alleviate the metabolic effectiveness of the wheat (*Triticum aestivum L*.) crop under dry conditions. There were Seven treatments— Tr0=Control, Tr1=DTS, Tr2=DTS + MN Application, Tr3=DFS, T4=DFS + MN Application, Tr5= DGFS, and Tr6=DGFS + MN Application— used in the trial. 10 kg of soil were collected in 1 pot. In it, 10 seedlings were planted. Seeds stored until the conclusion of the study. The trial had three replications and was conducted using a completely randomized design (CRD). Melatonin's at concentrations of 0 uM, 50 uM, and 100 uM were sprayed onto the affected areas in each plant to administer the treatments. When a drought strikes, MN is manually sprayed on the leaves to moisten all the plants upper sections.

Factor A: Melatonin

MN0= Control (0 uM)

MN1= 50 uM

MN2= 100 uM

Factor B: Drought

Dr0= Control

Dr1= Drought at tillering (DTS)

Dr2= Drought at Flowering (DFS)

Dr3= Drought at grain filling (DGFS)

Plant height at maturity (cm)

Plants were arbitrarily chosen from each pot or treatment to evaluate plant height. This measurement is made using a meter stick. The height of the plant was determined using its distance from the soil base and then aggregated.

Number of Fertile Tillers m^{-2}

Fertile tillers were counted from plants of each pot. In a quadrate, fertile tillers were observed and aggregated.

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Spike Length (cm)

Ten wheat plants were arbitrarily selected from each area or treatment, and the spike length—measured from the beginning of the spike to its top—was measured and averaged.

Number of Spikelets Spike^{-1}

Ten plants were arbitrarily chosen from each pot or treatment, and the spikelets on each stem were recorded. Typical amount of spikelets observed.

Number of Grains Spike^{-1}

Ten plants were chosen at random from the pot or treatment, and their spines were physically broken and agitated. Take the average after calculating the number of granules from each spike.

1000 seeds weight (g)

Count and divide each thousand granules as you are threshing the grain. On an automated scale, weight them. The weight was given in gram.

Grain Yield (g/pot)

At the time of harvesting grain yield of each treatment recorded after manual threshing and then converted into g/pot.

Biological yield (g/pot)

The crop harvested at maturity. Biological yield of each treatment recorded with the help of hand-held weighing balance and then calculated according to g/pot.

Leaf chlorophyll contents (%)

Using a chlorophyll meter, the chlorophyll concentration of leaves was determined.

Photosynthesis rate (Pn= μ mol m-2 s-1)

The amount of photosynthesis activity was measured using an infrared gas analyser.

Antioxidant enzymes (U/mg protein)

According to a method described by Nakano and Asada (1981), the activities of CAT (catalase), APX (ascorbate peroxidase), SOD (superoxide dismutase) and POD (peroxidase), were determined.

Grain nitrogen content (mg g-1 Dw)

Take 0.1 g powdered grounded wheat grain in digestion flask. Fill each container with 5mL of pure H2SO4. Keep them at ambient temperature for the entire night. Add 1 mL of 35% H2O2 to the processing container. Put tubes in the stomach block, heat them to 3500 degrees Celsius, and then maintain the heat for 30 minutes. Place the containers back in the stomach block after cooling the mixture and adding 1 milliliter of H2O2. The process was repeated until the metabolized substance was white and chilled. In volumetric flasks, the extract was produced up to an amount of 50 ml. Clean the residue before using Kjeldahl's technique to determine its nitrogen concentration.

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Grain phosphorus content (mg g-1 Dw)

The portion of 5 mL was taken from the volumetric beaker. Introduce 10 mL of Barton chemicals and fill the container to the specified level with purified water. Using purified water and KH2PO4, BR volume was used to make the standards. This example was left for a short while to develop the hues. Spectrophotometer is used for the measurement of phosphorus.

Grain potassium, calcium and sodium content (mg g-1 Dw)

Count 0.1 gram of desiccated, powdered wheat grain tubes. Each container should contain 5 mL CON. H2SO4. Keeps it incubating all the night. With the processing tube, add 1ml of the 35% H2O2. Heat the block with the conduit inside at 3500 C to release any vapors additional heat for 30 minutes. Remove the digestive tube from the block after cooling it placed the tubes back in the stomach block after adding 1 milliliter of H2O2. Continue the aforementioned procedures up until the metabolized substance was white. Create 50 ml of the amount in containers. Separate the extract and use it to measure the amounts of K, Ca, and Na using flame photometers to create standard curves with ranges of 10-100 milligrams L-1 for each nutrient (Na, K, and Ca).

Enzyme determination

The method of Bates et al (1973) was used to determine proline. 0.3 g of FL substance was purified with sulphosalicylic acid and shaken continuously for 7.5 min at 100 °C. Proline was determined using the acid ninhydrin technique (Zhang et al., 2009). Kolari and Sarjala's (1995) method and Racusen and Foote's (1965) technique were used to estimate peroxidases and identify acid phosphatases, respectively. According to the therapies, every enzyme prediction was investigated.

Statistical analysis

Using the computer program Statistix 8.1, the data were scientifically evaluated and examined at a 5% level of probability.

RESULTS & DISCUSSION

Plant height (cm)

Data on plant height reveals that M0 (61.24 cm) had the highest plant height (PH) during the drought, followed by Dr3 (51.71 cm) and Dr2 (50.11 cm), while Dr1 had the lowest (47.6 cm). Compared to the control treatment (MN0), the administration of MN1 and MN2 substantially increased plant height by 18.7 and 24.2%, respectively. There was no statistically significant interaction between irrigation schedules and Melatonin.

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Table 1: Influence of PH by Melatonin application under drought in wheat

Treatments	MN0 (0 uM)	MN1 (50 uM)	MN2 (100 uM)	Mean
DR0(C)	52.24 c	58.2 b	61.24 a	57.20 A
Dr1(DTS)	36.54 f	43.02 e	47.63 d	42.40 C
Dr2(DFS)	32.41 g	47.31 d	50.11 cd	43.27 C
Dr3(DGFS)	38.41 f	47.91 d	51.71 c	46.00 B
Mean	39.90 C	49.09 B	52.67 A	

DTS= Drought at Tillering stage

DFS= Drought at Flowering stage

DGFS= Drought at Grain Filling stage

LSD 0.05 for Comparison of PH for Melatonin * Drought = 3.044

LSD 0.05 for Melatonin = 1.5222

Spike length (cm)

Spike length data reveals that M0 (11.2 cm) had the highest spike length (SL) during the drought, followed by Dr3 (8.91 cm) and Dr2 (8.7 cm), and Dr1 (7.93 cm) had the lowest. In comparison to the control situation (MN0), the administration of MN1 and MN2 greatly increased the spike duration by 13.5 and 17.2%, respectively. There was no statistically significant interaction between irrigation schedules and Melatonin.

Table 2: Influence of spike length (SL) by Melatonin under drought in wheat

Treatments	MN0 (0 uM)	MN1 (50 uM)	MN2 (100 uM)	Mean
DR0(C)	9.603 abc	10.757 ab	11.233 a	10.53 A
Dr1(DTS)	6.933 c	7.817 bc	7.937 bc	7.62 B
Dr2(DFS)	6.900 c	8.100 bc	8.800 abc	7.93 B
Dr3(DGFS)	7.200 c	8.800 abc	8.910 abc	8.33 B
Mean	7.68 B	8.86 AB	9.26 A	

LSD 0.05 for Comparison of SL for Melatonin * Drought = 3.044

LSD 0.05 for Melatonin = 1.522

Number of spikelets per spike (NSPS)

The number of spikelets per spike data indicates that M0 had the highest NSPS among the droughts (15.4), followed by Dr1 (12.9) and Dr2 (11.3), and Dr3 had the lowest (9.5). Comparing the administration of MN1 and MN2 to the control (MN0), the NSPS was considerably improved by 7.3 and 16.6%, respectively. There was no statistically significant interaction between irrigation schedules and Melatonin.

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Table 3: Effect of NSPS by Melatonin application under drought in wheat

Treatments	MN0 (0 uM)	MN1 (50 uM)	MN2 (100 uM)	Mean
DR0(C)	14.233 abc	15.440 ab	16.727 a	15.467 A
Dr1(DTS)	11.533 cde	12.917 bcd	14.327 abc	12.926 B
Dr2(DFS)	10.500 de	11.100 de	12.400 cde	11.333 BC
Dr3(DGFS)	9.500 e	9.900 e	11.400 cde	10.267 C
Mean	11.442 B	12.339 AB	13.713 A	

LSD 0.05 for Melatonin = 1.4981

LSD 0.05 for Comparison of NSPS for Melatonin* Drought = 3.044

Number of grains per spike (NGPS)

According to statistics on the number of grains per spike, M0 had the highest NGPS during the drought (37.2), followed by Dr1 (31.3) and Dr2 (29.3), and Dr3 had the lowest (26.9). Comparing the administration of MN1 and MN2 to the control (MN0), the NGPS was substantially increased by 5.6 and 11.9%, respectively. There was no statistically significant interaction between irrigation schedules and Melatonin.

Table 4: Effect of NGPS by Melatonin application under drought in wheat

Treatments	MN0 (0 uM)	MN1 (50 uM)	MN2 (100 uM)	Mean
DR0(C)	35.23 b	37.10 ab	39.33 a	37.224 A
Dr1(DTS)	28.93 de	30.91 d	34.12 bc	31.326 B
Dr2(DFS)	27.30 ef	29.40 de	31.20 cd	29.300 C
Dr3(DGFS)	25.70 f	26.80 ef	28.40 def	26.967 D
Mean	29.292 C	31.156 B	33.265 A	

LSD 0.05 for Comparison of NGPS for Melatonin*Drought 3.044

LSD 0.05 for MN = 1.5222

Number of Fertile Tillers (NFT)

According to statistics on the number of viable tillers, Dr0 had the highest NFT during the drought (8.2), followed by Dr1 (7.1) and Dr2 (6.2), and Dr3 had the lowest NFT (5.7). In comparison to the control (MN0), the administration of MN1 and MN2 greatly increased the NFT by 15.8 and 25.6%, respectively.

Table 5: Effect of NFT by Melatonin application under drought in wheat

Treatments	MN0 (0 uM)	MN1 (50 uM)	MN2 (100 uM)	Mean
DR0(C)	6.833 bcd	6.016 cde	6.200 cde	6.3500 A
Dr1(DTS)	7.633 ab	7.126 abc	3.900 g	6.2200 A
Dr2(DFS)	8.233 a	4.500 fg	4.930 efg	5.8878 AB
Dr3(DGFS)	5.133 efg	5.540 def	5.700 def	5.4578 B
Mean	6.9583 A	5.7958 B	5.1825 B	

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LSD 0.05 for comparison of NFT for Melatonin * Drought = 3.045

LSD 0.05 for Melatonin = 0.6539

1000-grain weight (g)

According to statistics on 1000 grain weight, Dr0 had the highest 1000GW during the drought (34.9), followed by Dr1 (30.2) and Dr2 (27.7), and Dr3 had the lowest 1000GW (26.6). As compared to the control (MN0), the administration of MN1 and MN2 greatly increased the 1000GW by 9.3 and 14.3%, respectively.

Table 6: Effect of 1000 GW (g) by Melatonin application under drought in wheat

Treatments	MN0 (0 uM)	MN1 (50 uM)	MN2 (100 uM)	Mean
DR0(C)	30.33 bc	29.41 cd	27.70 cd	29.150 A
Dr1(DTS)	32.50 ab	30.21 bc	21.40 f	28.041 AB
Dr2(DFS)	34.93 a	23.50 f	24.20 ef	27.544 AB
Dr3(DGFS)	27.13 de	26.80 de	26.60 de	26.844 B
Mean	31.227 A	27.483 B	24.975 C	

LSD 0.05 for comparison of 1000W for Melatonin * Drought = 3.044

LSD 0.05 for Melatonin = 1.5222

Grain yield (g/pot)

According to grain production (GY) statistics, Dr0 had the highest GY during the drought (10.5), followed by Dr1 (9.2) and Dr2 (8.9), and Dr3 had the lowest GY (8.5). When MN1 and MN2 were applied, the GY was greatly improved by 5.8 and 8.9%, respectively, compared to control (MN0).

Table 7: Effect of GY (g/pot) by Melatonin application under drought in wheat

Treatments	MN0 (0 uM)	MN1 (50 uM)	MN2 (100 uM)	Mean
DR0(C)	6.74 bc	6.93 bc	6.93 bc	6.8698 A
Dr1(DTS)	7.83 ab	7.53 ab	4.30 d	6.5556 AB
Dr2(DFS)	8.33 a	4.41 d	5.90 c	6.2156 AB
Dr3(DGFS)	5.83 c	6.20 c	6.20 c	6.0778 B
Mean	7.1865 A	6.2700 B	5.8325 B	

LSD 0.05 for comparison of GY for Melatonin * Drought = 3.044

LSD 0.05 for Melatonin = 0.6460

Biological yield (g/pot)

According to biological yield (BY) statistics, Dr0 (16.3) had the highest BY during the drought, followed by Dr1 (14.4) and Dr2 (12.4), and Dr3 (11.8) had the lowest BY. In comparison to the control (MN0), the use of MN1 and MN2 greatly increased **BY** by 7.2% and 12.3%, respectively.

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Table 8: Effect of BY (g/pot) by Melatonin application under drought in wheat

Treatments	MN0 (0 uM)	MN1 (50 uM)	MN2 (100 uM)	Mean
DR0(C)	15.53 a	13.53 abc	12.40 cd	13.822 A
Dr1(DTS)	15.43 ab	14.43 abc	7.800 f	12.556 AB
Dr2(DFS)	16.28 a	9.84 def	8.900 ef	11.677 B
Dr3(DGFS)	12.43 bcd	11.83 cde	10.20 def	11.488 B
Mean	14.921 A	12.41 B	9.825 C	

LSD 0.05 for comparison of BY for Melatonin * Drought 3.044

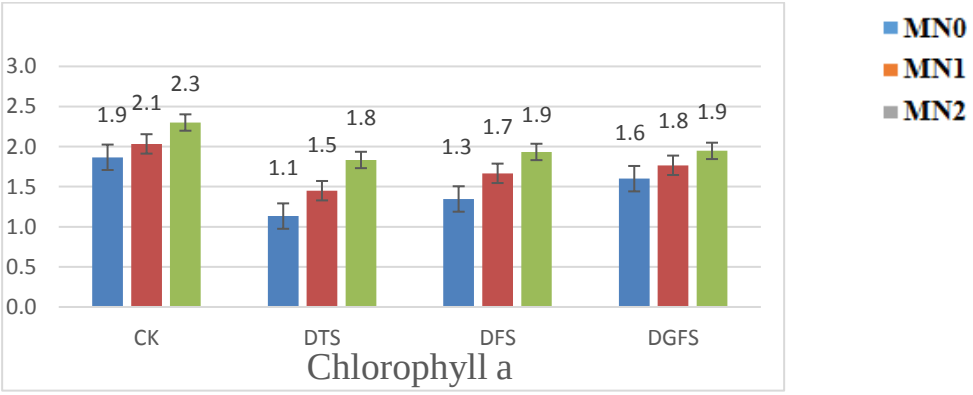
LSD 0.05 for Melatonin = 1.5013

Chlorophyll Contents

Chlorophyll a

The highest chlorophyll a concentration during the drought was found in Dr0 (2.3), followed by Dr3 (1.97) and Dr2 (1.84), and the lowest concentration (1.67) was found in Dr1. In comparison to the control (MN0), the administration of MN1 and MN2 greatly increased the chlorophyll a content by 14.12% and 25.8%, respectively. There was no statistically significant interaction between watering regimes and Melatonin.

Fig 1: Influence of melatonin on chlorophyll a at different growth stages of wheat.



LSD 0.05 for Melatonin = 0.1783

Chlorophyll b Contents

According to statistics on chlorophyll levels, the highest impact of dryness on chlorophyll b was observed in Dr2 (13%) followed by Dr1 (9.6%), and the lowest (4.5%) was observed in Dr3 when compared to control. In comparison to the control (MN0), the treatments of MN1 and MN2 greatly increased the chlorophyll b by 10.9% and 23.1%, respectively. There was no statistically significant interaction between irrigation schedules and Melatonin.

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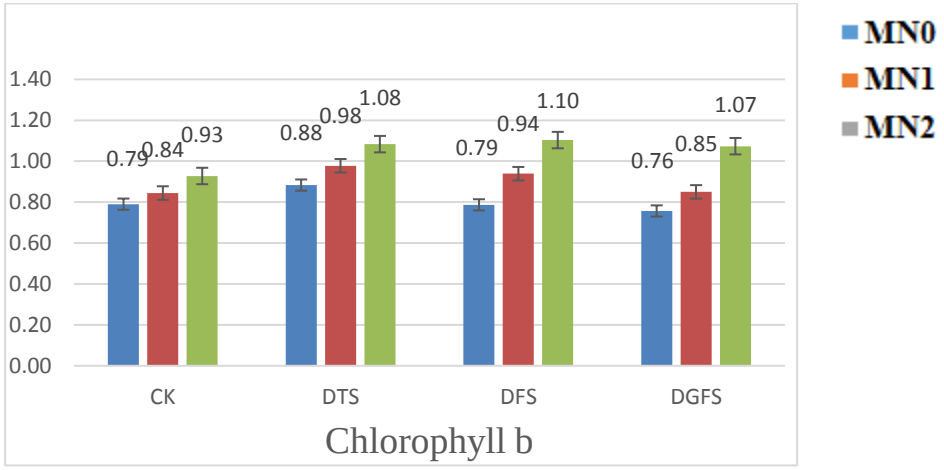
Fig 2: Influence of melatonin on chlorophyll b at different growth stages of wheat

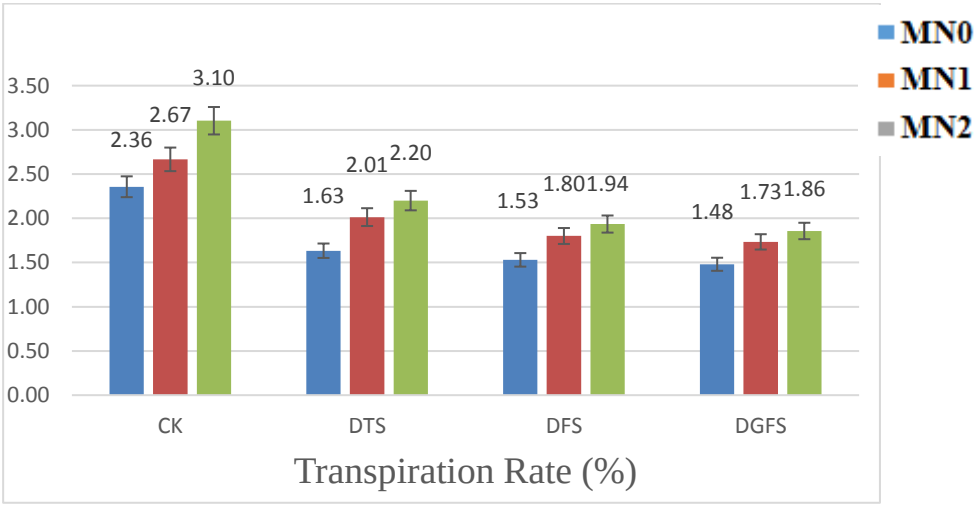


LSD 0.05 for Melatonin = 0.957

Transpiration rate

Transpiration rate data indicates that the highest Tr during the drought was noted in Dr1 (60.3%), followed by Dr2 (54.4%), and the lowest Tr (39.0%) was noted in Dr3 when compared to the control. Comparing the treatments of MN1 and MN2 to the control (MN0), the Tr was substantially increased by 14.8% and 23.04%, respectively (MN0). Melatonin and watering schedules did not interact in a significant way.

Fig 3: Influence of melatonin on transpiration rate at different growth stages of wheat



LSD 0.05 for Melatonin = 0.1269

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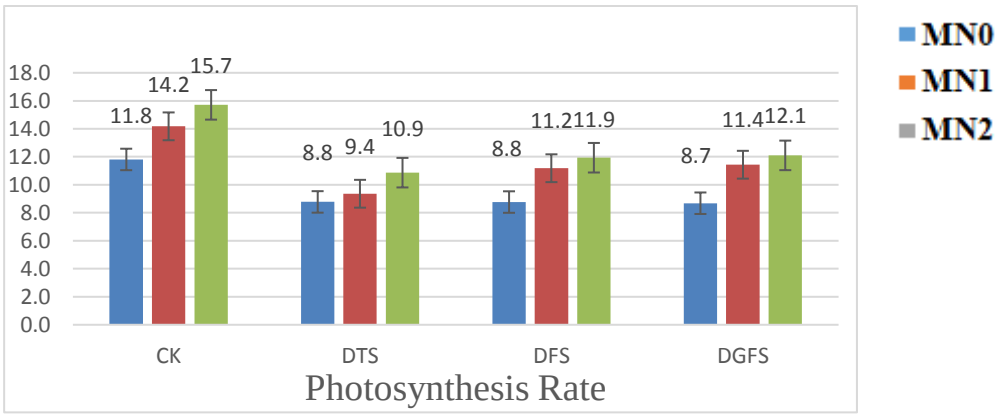
Photosynthesis rate

Data on photosynthesis rate reveals that Dr3 (43.8%) had the highest Pr during the drought, followed by Dr2 (30.7%), and Dr1 (29.5%) had the lowest Pr in comparison to control. In comparison to the control (MN0), the application of MN1 and MN2 greatly increased Pr by 17.6% and 24.9%, respectively. There was no statistically significant interaction between irrigation schedules and Melatonin.

Fig 4: Effect of melatonin application on photosynthesis rate at different growth stages in wheat

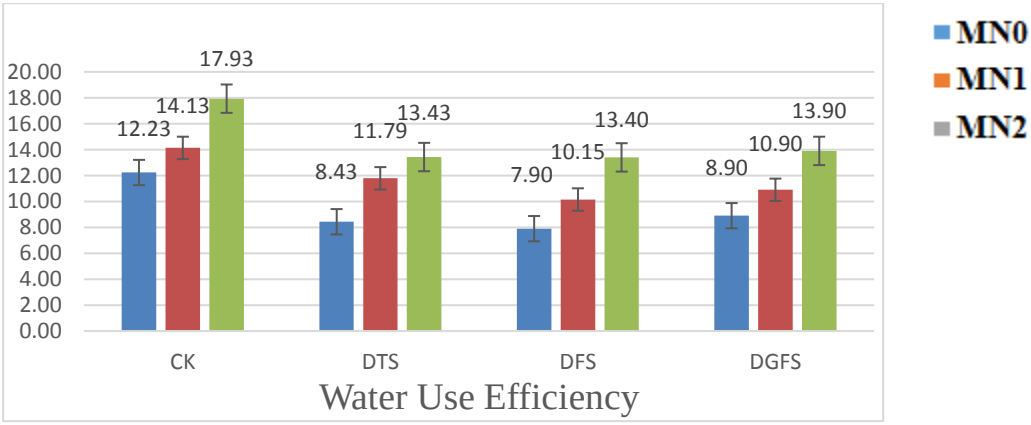


LSD 0.05 for Melatonin = 1.2462

Water Use Efficiency

Data on water use efficiency reveals that Dr3 (40.9%) had the highest WUE during the drought, followed by Dr1 (34.7%), and Dr2 (31.5%) had the lowest WUE when compared to control. In comparison to the control (MN0), the treatments of MN1 and MN2 greatly increased WUE by 20.3% and 36.2%, respectively. There was no statistically significant interaction between watering regimes and Melatonin.

Fig 5: Effect of melatonin application on water use efficiency at different growth stages of wheat



LSD 0.05 for Melatonin = 1.1823

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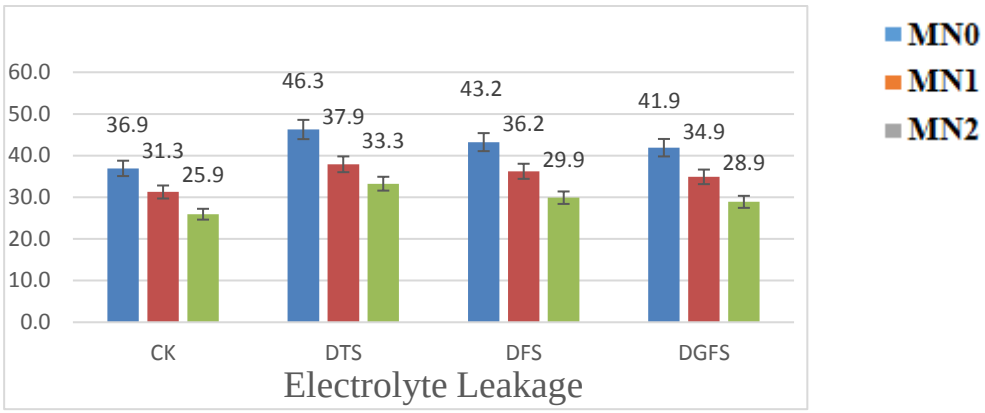
Electrolyte Leakage

Electrolyte leakage data reveals that, when compared to control, Dr1 had the highest EL during the drought (19.9%), followed by Dr2 (14%) and Dr3, which had the lowest EL (11%) levels. In comparison to the control (MN0), the treatments of MN1 and MN2 greatly increased the EL by 20% and 42.7%, respectively. There was no statistically significant interaction between watering schedules and Melatonin.

Fig 6: Effect of melatonin application on electrolyte leakage at different growth stages of wheat

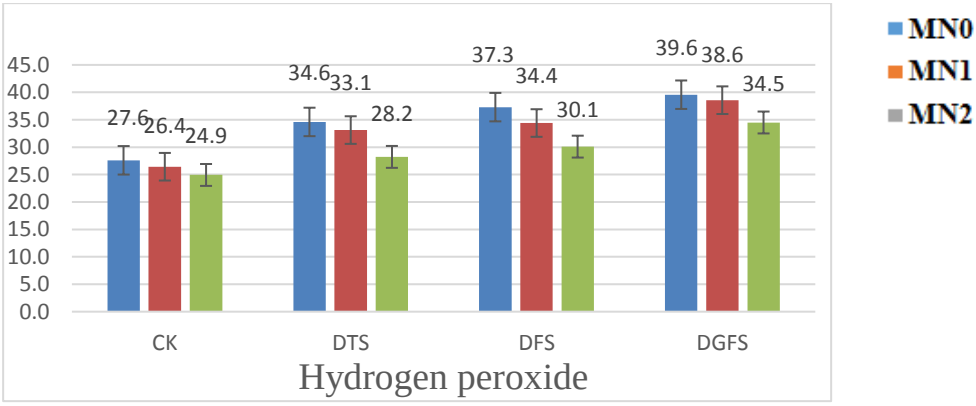


LSD 0.05 for Melatonin = 1.5258

Hydrogen Peroxide

According to hydrogen peroxide statistics, the highest H2O2 during the drought was found in Dr3 (29.9%), followed by Dr2 (22.5%), and the lowest (17.7%) was found in Dr1 when compared to Dr0. In comparison to the control (MN0), the administration of MN1 and MN2 greatly increased the H2O2 by 11.4% and 18.1%, respectively. There was no statistically significant interaction between watering schedules and Melatonin.

Fig 7: Effect of melatonin application on hydrogen peroxide at different growth stages of wheat



LSD 0.05 for Melatonin = 1.7617

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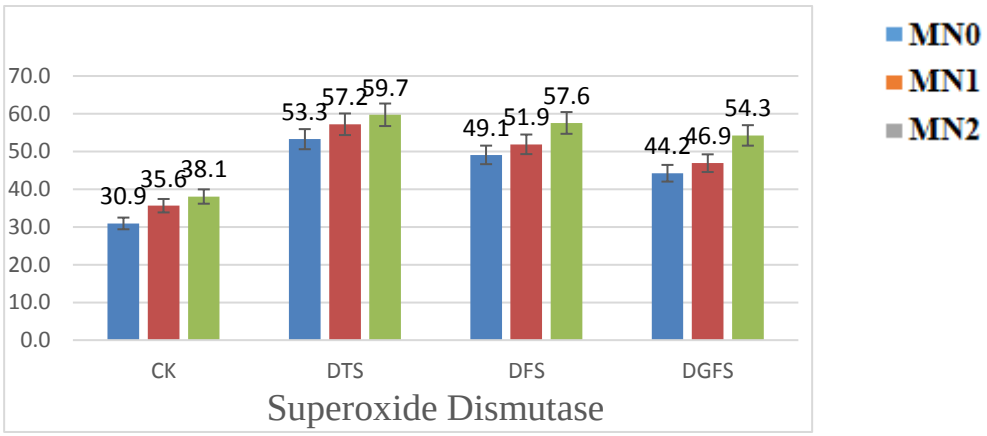
Superoxide Dismutase

According to SOD statistics, Dr1 had the highest SOD during the drought (38.6%), followed by Dr2 (34.1%), and Dr3 had the lowest SOD (28.43%) when compared to Dr0. In comparison to the control (MN0), the application of MN1 and MN2 greatly increased SOD by 7.4% and 15.4%, respectively (MN0). There was no statistically significant interaction between watering schedules and Melatonin.

Fig 8: Influence of melatonin application on superoxide dismutase at different growth stages of wheat

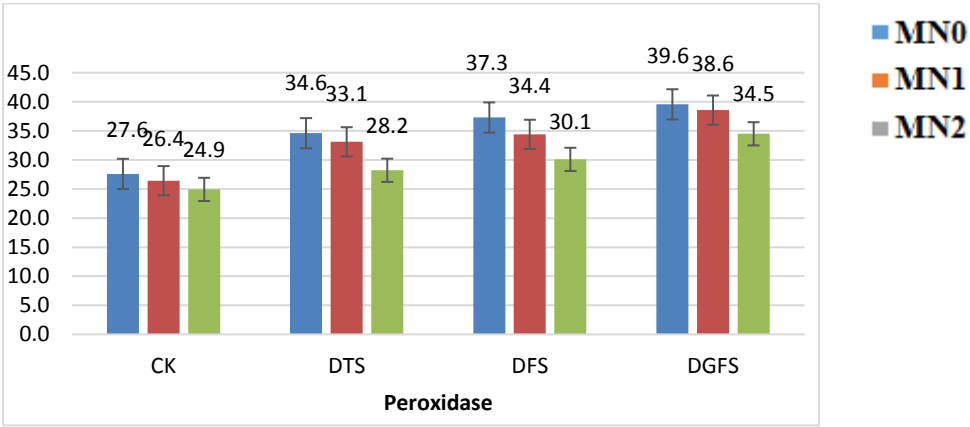


LSD 0.05 for Melatonin = 1.5268

Peroxidase

According to peroxidase data, Dr1 had the lowest POD value (17.7%), Dr2 had value (22.5%), and Dr3 had the highest percentage of peroxidase effect (29.90%) when compared to DR0. In comparison to the control (MN0), the application of MN1 and MN2 greatly increased the POD value by 12.8% and 18.10%, respectively. There was no statistically significant interaction between watering regimes and Melatonin.

Fig 9: Influence of melatonin on peroxidase at different growth stages of wheat.



LSD 0.05 for Melatonin = 1.525

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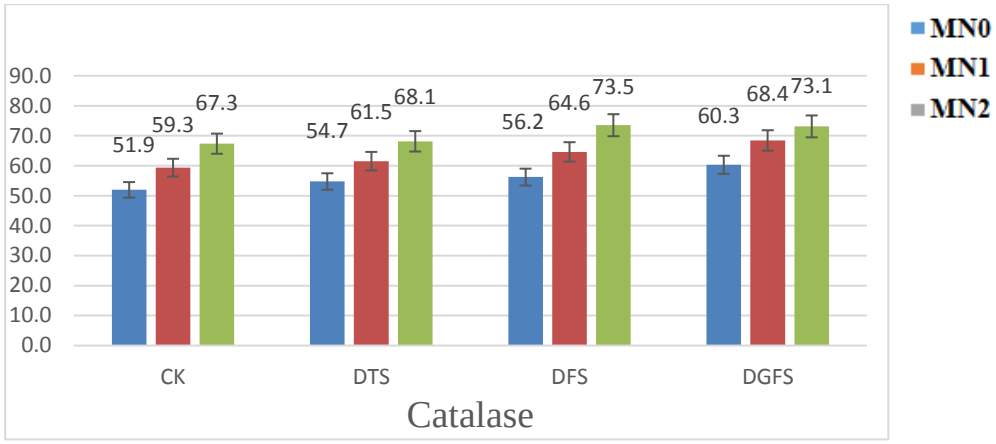
Catalase

According to catalase statistics, Dr3 had the highest CAT during the drought (4.3%), followed by Dr2 (8.09%), and Dr1 had the lowest CAT (11.5%) when compared to the Dr0. When compared to the control treatment (MN0), the treatments of MN1 and MN2 greatly improved the CAT by 12.10% and 20.9%, respectively. There was no statistically significant interaction between watering schedules and melatonin.

Fig 10: Effect of melatonin application on catalase at different growth stages of wheat

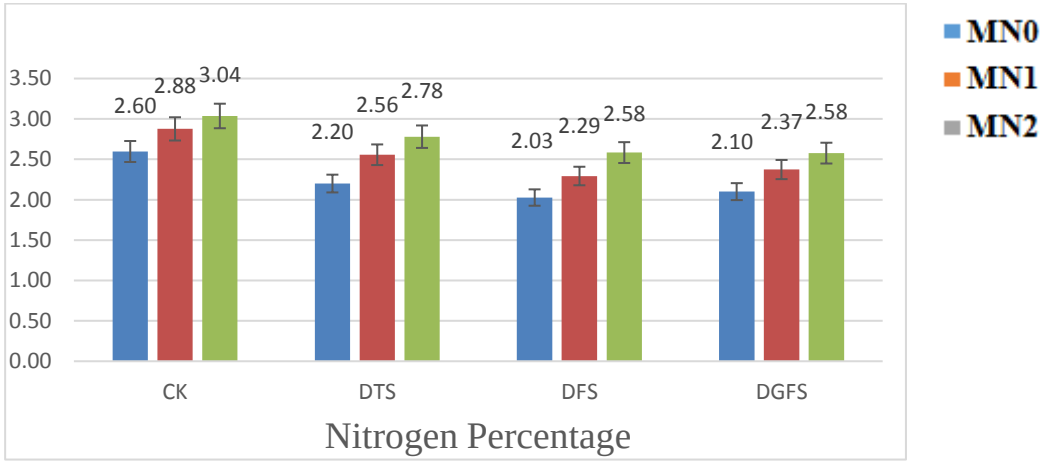


LSD 0.05 for Melatonin = 1.5258

Nitrogen Percentage in Seed

According to nitrogen statistics, the Dr1 and Dr2 droughts had the highest N% values, respectively (20.8% and 23.3%), and Dr3 had the lowest (13%). In comparison to the control (MN0), the administration of MN1 and MN2 greatly increased the N% by 11.7% and 18.8%, respectively. There was no statistically significant interaction between watering schedules and Melatonin.

Fig 11: Effect of melatonin application on N% at different growth stages of wheat



LSD 0.05 for Melatonin = 0.1255

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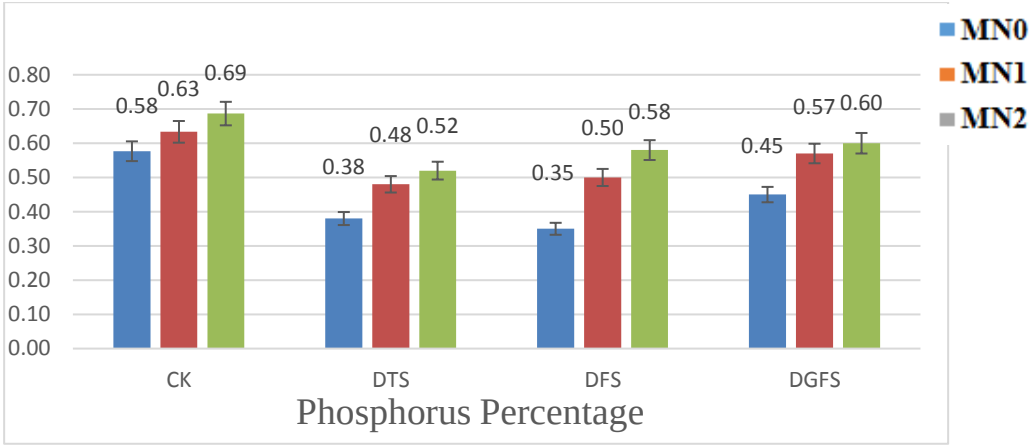
Phosphorus Percentage in Seed

According to phosphorus statistics, the greatest effects of the drought on P% were observed in Dr1 (37.5%), Dr2 (32.7%), and Dr3 (17.17%) when compared to the control conditions Dr0. In comparison to the control (MN0), the administration of MN1 and MN2 greatly increased the P% by 19.6% and 26.4%, respectively. There was no statistically significant interaction between watering schedules and Melatonin.

Fig. 12: Effect of melatonin application on P% at different growth stages of wheat

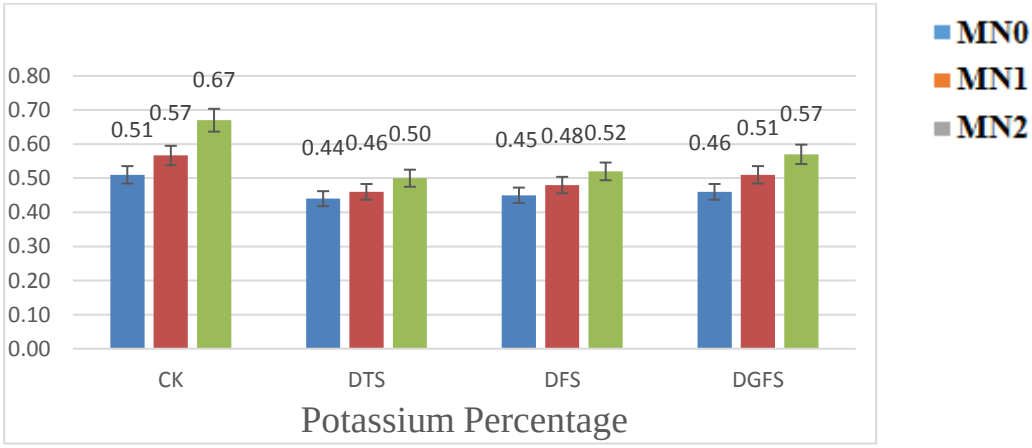


LSD 0.05 for Melatonin = 0.0506

Potassium Percentage in Seed

According to potassium statistics, the greatest effects of the drought on K% were observed in Dr1 (24.8%), Dr2 (20.5%), and Dr3 (13.5%) when compared to the Dr0. In comparison to the control (MN0), the administration of MN1 and MN2 greatly increased the K% by 11.4% and 17.7%, respectively. There was no statistically significant interaction between watering schedules and Melatonin.

Fig 13: Influence of melatonin application on K% at different growth stages of wheat



LSD 0.05 for Melatonin = 0.0452

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DISCUSSION

In many regions of the world, drought stress limits development, which lowers wheat harvests (Raza et al., 2017). Although more than half of the world's population consumes wheat as a staple food and it has a high nutritional value, global warming-related factors like rising temperatures, water scarcity, and high transpiration are escalating on a regular basis, affecting wheat plant physiology, growth, and yield (Swaminathan and Kesavan, 2012; Dusenge et al., 2019). There are many ways to deal with these problems, but applying melatonin in agriculture has gained a lot of attention recently due to its ability to reduce these stresses (Zhao et al. 2016).

Drought can cause harm to plant height. Wheat plant development is significantly impacted by Melatonin applications. Zafar *et. al.,* (2019) demonstrated that water stress had a substantial effect on cell growth. Dehydration and diminished turgidity of plant cells interfere with protoplasmic functions, which reduce cell reproduction and, as a result, lower plant height. Plant growth regulators are essential in this situation because they increase the height of the plant. At any stage of its development cycle, environmental duress reduced the plant's height (Su et al., 2019). Hormone changes may have a significant impact on plant height in environments with limited water supplies (Xia et al., 2020). According to our research, the plant height increased by 18.7% and 24.2%, respectively, when MN1 and MN2 were compared to the control.

Higher spike length on a plant increases the possibility that there will be more spikelets and a higher output (Zafar et al., 2020). According to Ihsan et al. (2015), a water-limited environment reduces the amount of spike duration by slowing down plant biochemical processes as a result of the absence of water in various plant functions. According to Meng et. Al., (2014), the administration of MELATONIN may improve the supply of nutrients, increasing the spike duration. Cui et al. (2017) discovered that MELATONIN increase proline production during dry circumstances, directly enhancing spike elongation and plant biochemical processes. According to our findings, the administration of MN1 and MN2 increased the spike length by 13.5 and 17.2%, respectively.

By decreasing the amount of water available to plants, drought stress reduces the quantity of viable tillers. Reduced plant biochemical activity results in fewer tillers as a result of decreased water supply (Su et al., 2019). Drought causes a decrease in the quantity of tillers of 14.6% at the tillering stage, 20.4% at the blooming stage, and 27.4% at the grain filling stage. Under famine, Melatonin worked best for increasing the quantity of tillers, 15.8 and 25.6% with MN1 and MN2 treatment. Cui et al. (2017) noted that various MELATONINS have varying capacities to enhance plant development in various environments. The quantity of spikelets per spike has a significant impact on the economic output. Dry stress shortens spikes, which results in fewer spikelets being produced per spike (Su et al., 2019). When famine was administered to wheat at the grain filling stage, there was a 19.8% decrease in spikelets per spike. Melatonin yields noteworthy outcomes as well. When wheat seedlings were stimulated with MN2, the highest spikelets per spike were observed. According to Cui et al. (2017), distinct Melatonin have varying effects on various crops, and

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Melatonin is useful in enhancing plant vegetative development by the creation of chemicals that reduce the effects of dehydration.

According to Su et al. (2019), arid stress had a substantial impact on cereals per rise at any level. The amount of seeds per spike reduces under dry stress, which leads to shorter spikes and fewer spikelets. According to (Zhang et al. 2014), Melatonin can enhance plant development. Melatonin boosts plant proline synthesis, which enhances plant development. When compared to the baseline, seed stimulation of MN1 and MN2 increased grains per spike by 5.69% and 11.9%, respectively.

According to Su et al. (2019), water scarcity causes bad plant development and fertilizer absorption, which results in a reduction in crop weight. Melatonin synthesizes proline and produces the substance indole-3-acetic acid, both of which contribute to the shortage (Zafar et al., 2020). Plants that produce growth-regulating compounds have improved metabolic processes and increased sink capacity as a consequence of higher cereal weights per 1000 grains. MN1 and MN2 showed 9.3% and 14.3% more 1000-grain weight in the current study, respectively. Biological output was affected by drought stress to a degree of up to 7.08% at tillering, 19.5% at blooming, and 26.3% at grain filling. Due to the reduction in cereal weight and plant height brought on by the drought, the biological output was reduced (Li et al. 2012). Melatonin supports in promoting plant development (Tan et al. 2012). Melatonin helps in enhancing nutrient absorption, which induces metabolic changes in plants and promotes increased plant development, which increases biological output. According to Yan et al. (2016), various Melatonin have varying capacities to enhance plant development. Application of MN1 and MN2 increased biological output by 8.2% and 14.4%, respectively.

31% less grain was produced during blooming, 40.4% less during grain filling, and 13.1% less during tillering. According to Raza et al. (2012), the anthesis period is when arid stress has the greatest negative effects on crop production. In water-stress situations, nutrient absorption is also disrupted, which reduces cereal production and weight. When compared to the control treatment under dry circumstances, MN1 and MN2 cereal production rose by 21.05% and 26.9%, respectively, in terms of Melatonin seed priming. Under the controlled circumstances, water use effectiveness improved. When comparing tillering, blooming, and grain filling deficit, the control solution was found to have greater WUE by 8.89%, 16.17%, and 23.17%, respectively. According to Zhang et al. (1998), WUE increased with the full usage of all of its associated components. MELATONIN also aids in enhancing plant health and plant WUE in wheat crops. Under dry circumstances, seed stimulation of MN1 and MN2 revealed 20.2 and 36.1% more WUE as compared to control. Chlorophyll levels in leaves are crucial for the creation of the sustenance in plants. According to Gill and Tuteja (2010), when foliage area dropped, the amount of chlorophyll also decreased. Raza *et al*. (2012) also observed that dry stress reduced the plant's LA, and as a result, when LA is reduced, chlorophyll levels are also reduced. According to Raza et al. (2017), dehydration stress raised ROS that harm chlorophyll. Melatonin treatment enhanced plant metabolism, reduced ROS, and increased cell development (Zhang et al. 2013). Under dry circumstances, seed stimulation of MN1 and MN2 increased chlorophyll levels by 14.02% and 25.7%, respectively, over control.

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A plant's biochemical activity may be impacted by oxidative stress in a number of ways, including lipid breakdown and nucleic acid degradation (Ahanger et al., 2017; Dusenge et al., 2019). A correct equilibrium of ROS production and degradation is required for optimal plant development (Ahanger et al., 2017). As a consequence of their inability to eliminate ROSs, plants are vulnerable to oxidative stress (Rizwan et al., 2016). Our findings demonstrated that while SOD and CAT activities decreased in drought-stressed plants, EL, H2O2 content, and POD activity increased. Additionally, the preservation and healing of the cell membrane in the wheat plant with the administration of Melatonin was responsible for the greatly reduced levels of reactive oxygen species. Furthermore, according to Kumar et al. (2005), when plants experience biotic and abiotic stress, it unbalances the electrical transport chain and increases the generation of ROS, which are thought to be harmful to cells. Then, using antioxidant enzymes like SOD, CAT, and POD, the plant defense system defends the cells (Hasheminasab et al., 2012). These results are consistent with earlier studies that found wheat and olive varieties' drought endurance was increased by elevated antioxidant enzyme rates (Ahmed et al., 1999). (Wang et al. 2013). Additionally, increased SOD activity following Melatonin application shows the effectiveness of the antioxidant system regulator that shields plants from reactive harm under pressure. As a result, applying MN increases the activities of SOD, glutathione synthase (Zhang et al. 2013), and catalase (Li et al. 2012), which partially counteracts the negative impact of dehydration on plants.

Different fertilizers and dry stress interventions produce varying outcomes in terms of N-P-K absorption and cereal protein composition. The plant's N-uptake is lowest under regulated conditions without dry duress, and it is greatest at the kernel filling period. At the cereal loading step, there was a 12.8% and 11.6% greater N-uptake detected compared to the control therapy. P-uptake increased by 10.3% and 9.5%, as indicated by maximum readings under MN2 administration. The patterns seen in the N-uptake statistics are also seen in K-uptake and protein levels. Melatonin enhances nitrogen absorption, which promotes plant development (Zafar et al. 2020).

CONCLUSION

One of the primary abiotic stressors that ultimately causes a sharp decline in the end cereal production is drought stress. When compared to other development phases, the grain filling stage experienced the greatest cereal production decrease due to drought stress. Melatonin is advised for preparing wheat seedlings because it works best under dry conditions in wheat crops.

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