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EFFECT OF MELATONIN ON THE MORPHOLOGICAL AND PHYSIOLOGICAL CHARACTERISTICS OF VIGNA RADIATA L. PLANT EXPOSURE TO DROUGHT STRESS CONDITIONS

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ABSTRACT

A field experiment was conducted in the growth season 2019-2020 at the botanical garden of the Biology Department at the College of Education for Pure Sciences/Ibn Al-Haitham, University of Baghdad-Iraq. The experiments' purpose is to determine the effects of different melatonin concentrations on some morphological and physiological characteristics of mung (*Vigna radiata* L.) plant under the influence of three periods of drought (5, 10, 15 days). The results were showed that the increasing of water stress periods had significant effects in the reducing rates of the studied characteristics, especially at the period 15 days. The external treatment of different concentrations of melatonin reduced the drought effects and that's led to significant increasing in the rates of the studied characteristics. It was noted that the concentration of 200 ppm caused significant increasing of the root length rate by 47.00%, and the water content relative was also increased by 25.00%. The saturation water was decreased by 31.78%, and the Abcisic acid (ABA) content rate was also decreased by 87.88%. The 150-ppm concentration, caused auxin hormone content rate increasing by 1866.26% compared to control plants. This work also aimed to determine the melatonin effects on the damages reduction which caused by drought stress like the increasing of certain morphological characteristics rates such as root length, water relations (relative water content, saturation water deficit) and some physiological traits such as ABA content and auxin hormone.

1. INTRODUCTION

Vigna radiata L. is one of the plants of the Leguminosae (Al-Kateb, 1988), an annual herb summer crop. Iraq is one of the countries interested in the cultivation of mung with an estimated area of 13.84 thousand hectares, producing 11.49 tons for the period from 1970 to 2010 (Bashar, 2013). Mung is important because its seeds are rich in protein ranging from 19-29% used in the bread industry, in addition to its use in preparations and pasta because it contains anti-irritating substances, in addition to using its stems as animal feed and is a feed that improves soil properties (Ali *et al*., 1990). The worsening drought problem in large parts of the world, which has increased in recent years with low rainfall, has reduced the water content of the soil and caused plants to suffer from water deficits accompanied by severe evaporation as a result of high temperatures (Touati, 2002). This prompted researchers to pay attention and seek ways to reduce the effect of water stress as much as possible and to clarify some of the mechanisms that allow the plant to adapt or increase its tolerance to this phenomenon (Abdul Al-Malik, 2015). One of the side effects of water stress is oxidative melatonin and increased free radical formation (Mittler, 2002). Melatonin is a direct antioxidant that can sweep free radicals with high efficiency (Tan *et al*., 2000), which was discovered in plants in 1995 and since this period it has been diagnosed and measured in roots and stems, fruit, leaves and seeds of various plant species (Young *et al*., 2002). It has been noted that the external treatment of melatonin modifies antioxidant enzymes by increasing the regulation of both transcription and activity levels of these antibiotics by regenerating antioxidants such as glutathione (GSH) and vitamins such as vitamin E, C (Zhang *et al*., 2013). It was also found to regulate levels of gene cloning that encode antioxidants, so melatonin performs direct actions, such as scavenging free radicals and indirect activation of antioxidant enzymes (Fischer *et al*., 2014).

2. MATERIALS AND METHODS

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The experiment is designed by Randomized Complete block design (RCBD) as a working experience (3x5) and with three replicates with a total number of 45 experimental units. The treatment of one experimental unit is 1.8 m^2 and in dimensions of 1.50x 1.20 m. The drought treatments were attended for three periods according to the method of Yassin (1992), which is the treatment of irrigation control (5 days, 10-day irrigation treatment and 15-day irrigation treatment).

2.1. Preparing the standard solution for melatonin

The standard solution for melatonin was prepared by dissolving 1 gm of melatonin in a liter of distilled water and then attended the following compositions (0, 50, 100, 150 and 200) ppm and according to the following dilution law:

Size taken from standard solution = desired concentration× desired size/standard solution concentration

The plants were sprayed with treatment early in the morning until full wet.

2.2. Studied characteristics

1. Root length (cm): The root length of three plants were measured from each experimental unit after taken off and washed it with running water and calculate the mean.

2. Relative water content (RWC): The relative water content was calculated by taking full-sized leaves at the age of 50 days from three areas of the plant and then weighed according to its fresh weight. Then the leaves were placed in test dishes containing distilled water and left for 24 hours. The leaves were removed and dried with filter paper and calculated the full weight of it, then dried by an electric oven for three hours and then calculating its dry weight and applied the following equation:

$$\mathbf{RWC=\frac{FW-DW}{TW-DW} \times 100}$$

Whereas:

FW= Fresh weight, DW=Dry weight and TW= Turgid weight (Tuner, 1981).

Saturation water deficit: Calculated from the following equation:

WSD= 100-RWC

Whereas:

RWC= Relative water content (Yassin, 1992).

3. Abscisic acid (ABA) content (mg/ml): It was calculated by Wei Lei *et al*. (2016). One gm of fresh plant sample was weighed and grinded by placing it with liquid nitrogen and then crushed well. Then 15 ml of methanol (80%) was added to the powder and kept for 12 hours at 4°C. The sample is then filtered for two hours and the extract is taken and dissolved by adding HCl (36%) and three ethyl acetates (10%) was added. Drying of extract was done by reducing the concentration of pressure in the vacuum. The extract was taken then and the volume was supplemented to 10 ml by adding methanol. Finally, filtering of the samples was done through filtration papers 0.45 μm before injecting them with HPLS advice (SYKAM 2015, German).

4. The conditions of the HPLC were as follows; Motile phase=acetonitrile: distilled water: phosphoric acid (2:13:85), Column = ODS-C18 (25 cm × 4.6 mm), Speed of flow of the conveyor phase = (1 ml/min) and the Detector= UV-260 nm.

5. Plant hormones: auxins (μg/g soft weight) were detected for drought stress by using HPLC technique. This analysis was conducted in the laboratories of the Department of Environment and Water of the Ministry of Science and Technology, Baghdad-Iraq.

HPLC conditions were as follows; Motile phase = methanol 2%: acetic acid (70:30), Column = C18-ODS (25 cm × 4.6 mm), Detector = UV-273 nm and the flow velocity of the conveyor phase= 1.2 ml/min (Sweetser and Zfager, 1973; Hussain, 2018).

3. RESULTS AND DISCUSSION

3.1. The effect of melatonin on root length (cm)

Table (1) results indicated that drought stress had significant effects on the root length rate. It was noted that when dry periods increased from 5 to 10 days, the root length rate increased from 14.79 cm to 15.10 cm and an increase rate of 2.09% compared to control plants. The reason for the increased root length rate may be due to the fact that one of the mechanisms used by the plant during periods of non-severe drought is the process of avoiding drought, which is the plant's ability to retain water in quantities that enable it to continue its metabolic processes by maintaining the process of water absorption and reducing the process of loss in the process of transpiration, and in this process the plant maintains a high water stress that enables it to absorb water (Blum *et al*., 1988). Therefore, plants will perform several morphological processes to resist stress, including increased root length (Westg *et al*., 1985), in addition to the depletion of soil water near the water absorption area, the roots expand in search of distant water sources, and divisions in the meristematic cells in the cap root area and the gathering of cytokinins increasing the length of the roots (Gergory, 2006). Also, when dry periods diverged to 15 days, the root length rate decreased to 12.27 cm and decreased rate by 17.04%. This decrease in root length may be attributed to the fact that increased drought periods have led to increased accumulation of ABA, which inhibits the growth of the apical meristematic and thus reduces vegetative growth. This is due to the lack of access of the

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metabolic product and nutrients to the root system from the leaves, which hinder their growth and expansion (Benjamen and Nielson, 2006; Sathyamoorthi *et al*., 2008).

Table (1) results also showed that the external treatment of different melatonin concentrations gave significant results in an increase in root length, increasing by 14.90%, 34.81%, 41.90%, 47.0%, and melatonin treatments 50, 100, 150 and 200 ppm, respectively compared to the control treatment. The increasing of root length caused by melatonin and that may be due to the fact that melatonin improves internal growth factors resulting in an increase in root length (Zeng *et al*., 2018). The intensity of external treatment with regular physiological concentrations to form the mean roots, the primary root and lateral roots and cross roots and generally affects the length and number of roots in a similar way to auxin (Murch *et al*., 2001). The interaction was significant and gave the highest value to root length when treated with irrigation every 5 days and the concentration of melatonin 200 ppm was 17.00 cm. The lowest value of root length was when treated with irrigation every 15 days and the concentration of melatonin zero ppm, which was 7.67 cm.

Table 1. The melatonin effect on root length of the mung plant exposed to drought stress

Melatonin concentration	Irrigation interval (day)			Mean Melatonin (Mean±S.E)
	5 (Control)	10	15	
	Root length (cm) (Mean±S.E)			
0	12.76±0.67	12.67±0.33	7.67±1.45	11.00±1.67
50	12.93±0.07	14.00±0.58	11.00±0.58	12.64±0.88
100	14.67±0.33	16.83±0.44	13.00±0.58	14.83±1.11
150	16.33±0.67	15.83±0.17	14.67±1.45	15.61±0.49
200	17.33±1.33	16.17±1.17	15.00±2.00	16.17±0.67
Mean irrigation	14.79±0.92	15.10±0.77	12.27±1.35	-
L.S.D (0.05)	Melatonin= 1.57	Irrigation=1.22	Interaction= N. S	

3.2. The effect of melatonin on the relative water content %

The results of Table (2) indicated that drought stress when drought periods increased from 5 to 10 days resulted significant increasing in the relative water rate to 76.33% and 4% increasing over control plants. The reason may be due to the increase caused by drought in this period in root length and as shown in Table (1), which has enhanced plant efficiency in absorbing water and nutrients and transporting them to the aerobic parts (Ahmad *et al*., 2019). When droughts diverge to 15 days, there was a significant decrease in the relative water content rate to 45.83% and a decrease of 37.13% compared to control plants. The reason may be that the lack of water in the soil causes drought, which leads to a lack of water reaching the leaves, and increased evaporation from the stomata caused by high temperatures leads to a decrease in relative water content (Pugnair and Valladares, 2007; Salloum *et al*., 2011). Table (2) results also showed that the external treatment of various melatonin concentrations 50, 100, 150 and 200 ppm gave significant increasing in the relative water content rate with an increase rate 10%, 22%, 24% and 25% compared to the treatment of control and all the above concentrations, respectively. The increasing caused by melatonin in relative water content may be due to the fact that melatonin regulates the work of stomata that reduce water loss from drought stress plants (Li *et al*., 2015; Cui *et al*., 2017; Li *et al*., 2017). Melatonin was found to increase stomatic conductivity accompanied by a simultaneous increase in the relative water content of the leaves (Ahmad *et al*., 2019), as well as the role of melatonin in reducing Reactive Oxygen Species (ROS) damage as melatonin kept cells from rupture and water retention, increasing relative water content (Meng and Fang, 2014). The results of the same table indicated that the interaction was significant between drought stress and melatonin and gave the highest value to the relative water content when treated with irrigation every 5 days and the concentration of melatonin 150 ppm, which amounted to 81.35%. The lowest value of relative water content was when treated with irrigation every 15 days and the concentration of melatonin zero ppm, which was 33.18%.

Table 2. The melatonin effect on the relative water content of the mung plant exposed to drought stress

Melatonin concentration	Irrigation interval (day)			Mean Melatonin (Mean±S.E)
	5 (Control)	10	15	
	Relative water content (%) (Mean±S.E)			
0	65.76±1.76	68.25±0.52	33.18±2.65	55.73±11.30
50	68.60±0.42	74.62±0.42	41.52±2.40	61.65±10.20
100	76.05±0.92	79.53±0.53	48.96±0.81	68.18±9.66
150	76.79±0.57	81.35±0.71	50.40±0.40	69.51±9.65
200	77.09±1.04	77.91±1.09	55.08±0.94	70.03±7.48
Mean irrigation	72.90±2.35	76.33±2.30	45.83±3.84	-
L.S.D (0.05)	Melatonin= 1.77	Irrigation=1.37	Interaction=3.06	

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3.3. The effect of melatonin on saturation water deficit %

The effect of melatonin on saturation water deficit % results were showed on Table (3). There are significant effects of drought stress on the saturation water deficit rate, and that was observed when droughts increased from 5 to 10 days led to decreasing of saturation water deficit rate from 27.10% to 23.80% and decreasing by 12.18% compared to control plants. This is due to an increase in the relative water content in that period as indicated in Table (2). When droughts increased to 15 days, the saturation water deficit increased to 54.17% and an increase of 99.89% compared to control plants. The reason is due to the low relative water content rate in the mung leaves as shown in Table (2), in addition to the high temperatures led to increased evaporation and transpiration, resulting in the depletion of soil water and the lack of water absorbed from the roots by the plant and increased the deficit of saturation water (Siddique *et al*., 2000; Yang and Milao, 2010). The results in Table (3) also showed significant effects of melatonin composition in reducing the saturation water deficit rate by 3.37%, 28.00%, 31.00%, and 31.78% for melatonin treatments 50, 100, 150 and 200 ppm compared to control treatment. The reason may be due to the active role of melatonin in increasing the relative water content in the leaves as explained in Table (2), as melatonin regulates root growth through physiological processes similar to auxin work, which increased the effectiveness of water absorption (Bleiss and Ehwald, 1993). The increasing in the roots has enhanced plant efficiency in acquiring water and nutrients from soil and transporting them to green parts and reducing saturation water deficits (Ahmad *et al*., 2019). The results in the same table showed that the interaction between drought and melatonin concentrations was significant reducing of the saturation water deficit, and was less valuable when treated with irrigation every 5 days and melatonin concentration of 150 ppm to 18.65%, While the highest saturation water deficit was at irrigation treatment every 15 days and the concentration of melatonin was zero ppm and was valued at 66.82%.

Table 3. The melatonin effect on saturation water deficit of the mung plant exposed to drought stress

Melatonin concentration	Irrigation interval (day)			Mean Melatonin (Mean±S.E)
	5 (Control)	10	15	
	Saturation water deficit (%) (Mean±S.E)			
0	34.24±1.76	31.75±0.52	66.82±2.65	44.27±11.3
50	31.20±0.42	25.38±0.42	58.48±2.40	38.35±10.20
100	23.95±0.92	20.47±0.53	51.04±0.81	31.82±9.66
150	23.19±0.59	18.65±0.71	49.60±0.40	30.48±9.65
200	22.91±1.40	22.75±0.75	44.62±0.94	30.20±7.36
Mean irrigation	27.10±2.35	23.80±2.28	54.17±3.84	-
L.S.D (0.05)	Melatonin= 1.71	Irrigation= 1.32	Interaction=2.96	

3.4. The effect of melatonin on the rate of abscisic acid content (mg/ml)

Table (4) results showed significant effect of drought stress in the content of abscisic acid (ABA), and it was noted when the drought period increased from 5 to 10 days, the abscisic acid content rate increased from 21.69 to 24.07 mg/ml and an increase of 10.97% compared to control plants. When the drought period increased to 15 days, the ABA content rate increased to 28.63 mg/ml and an increase of 31.99% compared to control plants. The reason may be that ABA plays a key role in water stress resistance (Tardien and Davies, 1993). It appears that ABA has a chemical indicator that sends signals from the roots to the vegetative part to activate the mechanics related to water loss control, particularly the closure of stomata (Sauter *et al*., 2001), as well as drought stress is also believed to lead to accumulate of ABA due to close stomata and the exit of K ions outside guard cells (Kiani *et al*., 2008; Al-Hamdani 2014). Table (4) results also indicated that external treatment with different concentrations of melatonin 0, 50, 100, 150 and 200 ppm had significant effect on reducing ABA content and a decrease of 58.97%, 66.05%, 94.88% and 87.88% compared to the treatment of control of all previous and respective concentrations. The decline in the treatment of melatonin may be due to the fact that melatonin during dry periods regulates the biosynthesis of the q. cis-epoxycarotenoid dioxy genase (NIED) responsible for the synthesis of ABA acid (Shi and Cha, 2014; Li *et al*.; 2015). At the same time, melatonin regulates copies of the gene involved in the deterioration of the ABA, (MJcYP707A1) and this response is accompanied by an antioxidant response and H_2O_2 root scavenge (Li *et al*., 2015). The results of the same table indicated that the interaction between drought stress and melatonin was significant, and it was noted that the lowest content was when treated with irrigation every 5 days and the concentration of melatonin 150 ppm, which was valued at 61.04 mg/ml. The highest value of ABA content was when treated with irrigation every 15 days and the concentration of melatonin zero ppm, which was 57.08 mg/ml.

Table 4. The melatonin effect on the rate of abscisic acid content of the mung plant exposed to drought stress

Melatonin concentration	Irrigation interval (day)			Mean Melatonin (Mean ±S. E)
	5 (Control)	10	15	
	Abscisic acid content (mg/ml) (Mean ±S. E)			
0	71.04±0.20	74.62±0.85	57.08±1.15	67.58±5.35
50	22.02±1.07	28.15±1.04	33.03±1.21	27.73±3.19
100	11.36±0.16	11.52±0.21	28.12±0.94	17.00±5.56
150	1.04±0.23	1.14±0.21	8.21±0.30	3.46±2.37
200	2.97±0.03	4.91±0.48	16.69±0.33	8.19±4.29
Mean irrigation	21.69±12.88	24.07±13.46	28.63±8.33	
L.S.D (0.05)	Melatonin= 1.20	Irrigation= 0.93	Interaction=2.08	-

3.5. The effect of melatonin on the auxin IAA hormone (µg/gm)

The results showed significant differences in drought stress in the rate of auxin content, as it was observed when the drought period increased from 5 to 10 days, the rate of auxin content increased from 111.93 to 132.47 mg/ml and an increase of 18.35% compared to control plants (Table 5). The reason for the increasing may be that the plants redirect their growth through morphological, physiological and chemical responses that reduce or reduce stress exposure, known as stress escape to survive by modifying some of their morphological and physiological features and in particular the collaborative work of two plant hormones auxin and cytokinin with stress-induced free radical signals (Bielach *et al*., 2017). It has been noted that overexpression of auxin synthesis genes shows resistance to drought and oxidation (Cha *et al*., 2015; Ke *et al*., 2015), and those high levels of auxin are increased by increasing of gene expression (Tryptophan-2-monooxygenase), which in turn drought tolerance increases through its positive impact on antioxidant activities (Shi and Cha, 2014). It has also been noted that in drought-resistant patterns, the increase in auxin is associated with a severe reduction in the roots of H_2O_2 and O_2^- (Kim *et al*., 2013; Park *et al*., 2013; Shi and Cha, 2014). When the drought period increased to 15 days, the auxin content rate decreased to 30.30 (µg/gm) and decreased by 72.93% compared to control treatment. The decline may be due to the decreasing in different growth criteria caused by drought, as auxin is produced in the tops of the stems and young leaves (Went *et al*., 1928), which has led to decrease in production, and this decreasing in auxin is an initial response to temperature rise (Bielach *et al*., 2017). Table (5) results also indicated that the external treatment of various melatonin concentrations 0, 50, 100, 150 and 200 ppm led to significant increasing in the content of auxin 153.17%, 866.26% and 846.71% compared to the control treatment and for all the melatonin concentrations mentioned above, respectively. This increase may be due to the fact that melatonin is an important regulator of plant hormone gene expression similar to auxin capable of stimulating the growth of buds and roots (Arnao and Hernandez-Ruize, 2017) as melatonin increases the internal content of auxin (Chen *et al*., 2009). The results also showed that the interaction between drought stress and melatonin have significant effects, giving the highest auxin content when treated with irrigation every 10 days with the concentration of melatonin 150 ppm, which was valued at 238.05 (µg/gm). The lowest content of auxin was when treated with irrigation every 15 days and the concentration of melatonin zero ppm, which was 11.82 (µg/gm).

Table 5. The effect of melatonin on the auxin IAA hormone (µg/gm) of the mung plant exposed to drought stress

Melatonin concentration	Irrigation interval (day)			Mean Melatonin (Mean±S.E)
	5 (Control)	10	15	
	Root length (cm) (Mean±S.E)			
0	20.77±0.13	17.11±0.78	11.82±0.51	16.57±2.60
50	46.48±0.66	61.27±0.56	18.11±0.46	41.95±12.66
100	97.55±0.27	117.41±1.38	32.11±0.53	82.33±25.79
150	204.83±2.13	238.05±12.77	37.44±1.20	160.11±62.08
200	190.00±1.45	228.52±1.92	52.07±0.98	156.87±53.56
Mean irrigation	111.93±37.10	132.47±44.15	30.30±7.13	-
L.S.D (0.05)	Melatonin= 5.59	Irrigation= 4.33	Interaction=9.68	

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