Does melatonin improve the yield attributes of field-droughted banana under Egyptian semi-arid conditions?

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Abstract: Drought is regarded as one of the environmental constraints threatening agriculture worldwide. Melatonin is a pleiotropic molecule prevalent in plants capable of promoting plant endogenous resilience to many environmental challenges including drought. Banana is an important staple food consumed in developing countries especially in Africa. In this research, we studied the role of melatonin in the growth of bananas subjected to drought under the Egyptian semi-arid conditions. To achieve this objective, a field experiment on banana (Musa spp., cv. Williams) mother plants and first ratoon was conducted on a private farm for two seasons - 2019 and 2020. Three irrigation treatments, 100, 90 and 80% irrigation water requirements (IWR) were used in conjunction with four concentrations of melatonin as a foliar spray (0 μmol, 40 μmol, 60 μmol, and 80 μmol) to determine the effect of both treatments on banana plant performance under drought. The results showed that there was a substantial difference between treatments, with the foliar application of melatonin at 80 μmol concentration improving most of the yield attributes, relative water content, total chlorophyll and proline with water deficit. However, the foliar application of the molecule lowered the biochemical characteristics mostly at 80% IWR under the Egyptian semi-arid conditions. Overall, there was a concentration-dependent response with regards to IWR for the two seasons 2019 and 2020.

Keywords: banana, deficit irrigation, evapotranspiration, melatonin, photosynthesis

INTRODUCTION

Water shortages and rising evapotranspiration requirements are severe difficulties for agriculture globally, endangering the future supply of many agricultural outputs as a result of the climate change dilemma. Fruit tree crops are particularly vulnerable to these environmental constraints since they are perennials, and growers must devise reasonable techniques to increase the water usage efficiency of their orchards. The banana plant (Musa sp.) belongs to the Musacea family and is regarded as one of the most important tropical crops that can only take a limited duration of water scarcity before dying; otherwise, it cannot withstand long periods of drought. Due to its nutritional benefits, the banana's role in exports has increased massively; therefore banana has become an important plantation for many farmers. However, chronic water shortages have a substantial impact on its output [SURENDAR et al. 2013b]. Thus, water shortage is one of the principal restrictive factors for cultivating Musa spp.

Although Egypt is one of the most drought-stressed countries, the total area cultivated by bananas expands yearly. The total cultivated area with bananas in Egypt is estimated at about 30,389 ha with a total annual production of 1,359,297 ton according to [FAOSTAT 2019]. Soil drought is one of the stark environmental stresses intimidating growers around the world generally and Egypt particularly. Drought stress can cause losses of 20–60% in yield compared to more humid areas of the region [ISLAM et al. 2019]. There are different strategies that banana plants could use to tolerate drought, one of these strategies is stomata closure, osmolyte buildup, and reactive oxygen species (ROS) scavenging mechanism [LI et al. 2018]. However, this strategy is not useful with a long drought period [EL NAMAS 2020]. There is, therefore, growing interest in the use of osmolytes like melatonin to promote drought resistance for many horticultural plants. Melatonin (N-acetyl-5-methoxytryptamine) is known as a pleiotropic endogenous growth regulator that is involved in many physiological processes, including photosynthesis, vegetative growth, roots growth and osmoregulation [ÇOLAK 2018; WANG et al. 2012]. Furthermore, melatonin can enhance the tolerance of plants against several environmental stresses, such as drought [ARNAO, HERNÁNDEZ-RUIZ 2014; HELALY et al. 2022. TAN et al. 2012]. Also, melatonin plays very important roles in many

MATERIALS AND METHODS

EXPERIMENT

This research work was conducted on banana plants (*Musa* spp., cv. Williams) basically produced through tissue culture technique and planted in a commercial farm located at Markz Badr (30°36' 36.5" N 30°45' 45.5" E), El Beheira, Egypt. The experimental work was carried out over two seasons 2019 (first ratoons) and 2020 (second ratoons). The study area has semiarid conditions, weather data, and the calculated reference crop evapotranspiration (*ETo*) were collected daily from the nearest meteorological stations from planting to harvesting during the 2019 and 2020 seasons. The FAO–Penman–Monteith equation (CROPWAT software, version 8) was used to calculate weather data and *ETo* as an average of two seasons, and the results are presented in Table 1 and 2. Soil and water were examined according to CHAPMAN and PRATT [1961] and ALLEN et al. [1998] at the beginning of the first season Table 3.

The irrigation source was deep groundwater well, and plants were drip irrigated after one month of growth till the harvest in three various treatments of different crop evapotranspiration (*ETc*) levels; 100 (control), 90, and 80% *IWR*, determined using the reference crop evapotranspiration (*ETo*) and crop coefficient

Table 1. Weather data of Markz Badr, El Beheira, Egypt (2019 and 2020)

Year	Minimum temperature	Maximum temperature	Humidity (%)	Wind (day^{-1})	Solar radiation (MJ·m^2·day^{-1})
	°C				
2019	14.1	31.8	54	216	24.5
2020	17.5	34.6	56	190	26.3

Source: own elaboration.

plant cells, like improving the antioxidant abilities against ROS like H_2O_2 as well as endogenous hormones ABA content, thus causing an increase of plant inner immunity against several stresses [AYYAZ et al. 2021; BANO et al. 2021; SHAHZADI et al. 2021]. In addition, melatonin can activate changes in gene expression of multiple physiological processes [FLETA-SORIANO et al. 2017]. Consequently, it is pertinent to study the role of the small molecule in plants especially when they are growing under natural conditions in the field. The use of melatonin in the soil and its foliar application revealed that there was an improvement in the drought tolerance of plants [YE et al. 2016]. However, the mechanism of tolerance is poorly understood with most of the experiments done under controlled conditions, such as in greenhouses or using a limited number of plants. There are few studies that have been published on the use of melatonin to improve the development of drought-stressed banana plants in the open field [EL NAMAS et al. 2020]. There has been no reported usage of melatonin to increase the mechanism of drought resistance and production in banana plants to date. As a reason, this study is regarded as the first in this open field. Thus, the purpose of this study was to assess the effects of foliar application of melatonin at various dosages on yield and several biochemical parameters in response to drought banana plants cultivated in the open field under Egyptian semi-arid conditions.

Table 2. Reference crop evapotranspiration of banana (*ETo*) in Markz Badr, El Beheira, Egypt as average of the 2019 and 2020 seasons

Month	ETo (mm·day^{-1})
January	2.3
February	2.9
March	4.2
April	5.7
May	6.7
June	7.2
July	6.9
August	6.3
September	5.3
October	4.2
November	3.0
December	2.5

Source: own elaboration.

Table 3. Soil and water analysis of the experimental farm

Parameter	Value in soil (0–40 cm)	Value in water
pH	8.28	6.88
Sand (%)	88.0	–
Silt (%)	7.0	–
Clay (%)	5.0	–
Total dissolved salts (ppm)	704	250
$CaCO_3$ (%)	3.4	–
Ca^{2+} (meq·$(100\ g)^{-1}$)	4.5	1.0
Mg^{2+} (meq·$(100\ g)^{-1}$)	2.5	0.8
Na^+ (meq·$(100\ g)^{-1}$)	14.3	1.49
K^+ (meq·$(100\ g)^{-1}$)	0.4	0.45
Cl^- (meq·$(100\ g)^{-1}$)	3.2	0.0
SO_4^{2-} (meq·$(100\ g)^{-1}$)	3.5	0.8
CO_3^{-} (meq·$(100\ g)^{-1}$)	0	–
HCO_3^{-} (meq·$(100\ g)^{-1}$)	5.2	1.0

Source: own study.

factor (K_c) of banana (Tabs. 1, 2), as suggested by Allen et al. [1998], using the following equation:

$$ETc = ETo \cdot K_c \qquad (1)$$

Irrigation water requirement (*IWR*) of the trees was calculated using the theoretical irrigation rate ($m^3 \cdot ha^{-1} \cdot season^{-1}$) during the whole seasons from late April to January according to the following equation:

$$IWR = A \cdot ETc \cdot Ii \cdot K_r \cdot Ea \cdot 1000 \cdot (1 - LR) \qquad (2)$$

where: *IWR* = irrigation water requirement ($m^3 \cdot ha^{-1}$ per irrigation), *A* = crop area (ha), *ETo* = reference evapotranspiration (mm·day^{-1}), K_c = crop coefficient, K_r = reduction factor [Keller, Karmeli 1975], *Ii* = irrigation interval (day), *Ea* = irrigation efficiency, 85%, *LR* = leaching requirement = 10% of the total water amount delivered to the treatment.

$$Kr = (0.10 + GC) \leq 1 \qquad (3)$$

where: *GC* = the ground cover.

The leaching requirements (*LR*) were estimated according to the Equation (4):

$$LR = EC_w / 2EC_{emax} \qquad (4)$$

where: EC_w = the electrical conductivity of the irrigation water (dS·m^{-1}), $2EC_{e\ max}$ = the maximum electrical conductivity of the soil saturation extract for a given crop (see the table shown according to Keller and Bliesner [1990].

Based on the soil moisture content and climate conditions, a number of irrigation times differed among the three treatments, with a frequency of 1–5 irrigation times per week. The three treatments' soil water concentration was obtained weekly with such a soil tensiometer from the Model64xx series (Spectrum Technologies Inc., Aurora, IL, USA). The control treatment (100% *IWR*) used two lateral lines of irrigation pipes (one on each side of the trees row) with 10 drippers per tree (8 $dm^3 \cdot h^{-1} \cdot dripper^{-1}$), whereas the 90 and 80% *IWR* treatments used 9 and 8 drippers, respectively.

BANANA PLANATION

Mother plants were grown at distance 3×3m apart, the first and second ratoons of "Williams" banana plants were carefully selected similar in size and vigor and free from any disease symptoms. All the experimental plants received the traditional and regular fertilisation program as recommended by Murray [1960].

MELATONIN FOLIAR APPLICATION TREATMENTS

Melatonin was purchased from Sigma-Aldrich, Germany with newly prepared solutions foliar sprayed in four concentrations (0, 40, 60 and 80 µmol·dm^{-3} water). Tween 20 was added as a wetting agent at 0.05%, the foliar application was as a surfactant done until dropping and full coverage of the plant. Banana plants were sprayed three times. At the 14th-leaf-stage of plant growth, banana plants were foliarly sprayed three times in both seasons. The application was done in the early morning in mist form when the foliar stomata were open.

VEGETATIVE GROWTH CHARACTERISTICS

Pseudostem height (cm) (from the soil surface up to the petiole of the last emerged leaf) and leaves/plant were evaluated after the emergence of the inflorescence. Leaf area was taken from the third upper leaf in the descending leaves from the top of the plant after bunch shooting (mid of Sept.) using the formula of (*LA* = 0.86*L·W*, where *LA* = leaf area and *LW* = length width) [Saini 2001].

BUNCH CHARACTERISTICS

The harvesting stage occurred when the bunch started to reach full maturation, when fruit became fully developed (75% maturity ≈ 80–90 days after the opening of the first hand). Afterwards, bunch weight (kg), number of hands per bunch, hand weight (kg) and finger length (cm) were calculated.

PHOTOSYNTHETIC PIGMENTS AND WATER RELATIONS CHARACTERISTICS

The total Chl (a + b) was calculated according to Wintermans and de Mots [1965] by measuring absorbance at 649 and 665 nm.

Electrolyte leakage. Electrolyte leakage (*EL*, %) was determined according to the methodology of Saini [2001] by cutting ten discs of freshly expanded leaves (0.5 cm diameter) from completely expanded leaves. The leaf discs were cleaned three times with deionised water to remove dust before being placed in sealed tubes containing 10 cm^3 of deionised water and agitated for 30 min prior to being kept at room temperature for 24 h. An electrical conductivity meter was used to determine the solution's initial electrical conductivity (EC_1). The samples were placed in an 80°C water bath for twenty minutes to release all

endogenous electrolytes before being cooled to 25°C. Further-more, the electrical conductivity (EC_2) of the material was analysed and EL calculated as follows:

$$EL = (EC_1/EC_2) \cdot 100\% \qquad (5)$$

Relative water content (RWC). Leaf relative water content (RWC) was estimated according to the method described by SAINI [2001], performed by incubating 0.2 g of fresh leaf sample in 50 cm^3 of distilled water for 4 h. Then the turgid weights of leaf samples were measured. The leaf samples were oven-dried at 60°C for 48 h for dry weight determination. The RWC was calculated using the following equation:

$$RWC = [(FW - DW)/(TW - DW)] \cdot 100\% \qquad (6)$$

where: FW, DW, and TW are fresh, dry and turgid weights.

BIOCHEMICAL MEASUREMENTS

Proline concentration. Proline accumulation was determined using the ninhydrin reagent method described by BATES *et al.* [1973]. The absorbance of the solution with toluene was determined at 520 nm, using a spectrophotometer (Model UV-120-20, Japan).

Lipid peroxidation (Malondialdehyde, MDA). The determination of lipid peroxidation (MDA), 100 mg of fresh leaf tissue was extracted in 1% trichloroacetic acid (TCA), and the extract was centrifuged for ten minutes at 10,000·g. A 1.0 cm^3 sample of the supernatant was mixed with 4 cm^3 of thiobarbituric acid (0.5%, TBA) and heated for 30 min at 95°C. The samples were cooled in an ice bath then subjected to centrifugation at 5000·g for 5 min. The absorbance of the supernatant was read at 532 and 600 nm [HEATH, PACKER 1968].

Hydrogen peroxide (H_2O_2). The measurement was done by 0.1 g of the leaf was extracted in 5 cm^3 of 0.1% trichloroacetic acid (TCA) and centrifuged at 12,000 rpm for 15 min. The supernatant was then blended with 0.5 cm^3 of 10 mM phosphate buffer (pH 7.0) and 1 cm^3 of 1 M potassium iodide (KI). The absorbance was measured at 390 nm, and the H_2O_2 content was calculated by drawing a standard calibration curve with a fixed concentration of H_2O_2 [HEATH, PACKER 1968].

Abscisic acid (ABA) content. Leaf abscisic acid was extracted and determined according to the method of UTHAIBUTRA and GEMMA [1991].

## EXPERIMENTAL DESIGN AND STATISTICAL ANALYSIS

The experiment used a split-plot design, with three irrigation levels in the main plot and four melatonin concentrations in the sub-main plot. Five replicates were used, and a total of 60 experimental plants were used. CoStat Statistics Software (ver. 4.20) was used to analyse the data [CoStat 1990]. A two-way analysis of variance was performed, and means were compared using Duncan's multiple range tests (DMRT) at $p = 0.05$. In the figures, standard error bars were also added for mean comparisons [DUNCAN 1955]. Where the years 2019 and 2020 are significantly different from each other, the symbol (*) is included whilst non-significance between the years is represented as "ns".

RESULTS

VEGETATIVE GROWTH CHARACTERISTICS
Pseudostem height

Data presented in Figure 1a showed the combination effect between drought stress and melatonin foliar application treatments on banana pseudostem height under semi-arid conditions. In 2019, results illustrated that pseudostem height decreased with decreasing water values 90 and 80% IWR compared to 100% IWR treatment, but the foliar application of melatonin enhanced significantly pseudostem height at 100 and 90% IWR. The best performance for plant height (225 cm) occurred in the presence of 80 µmol melatonin without drought stress. A similar trend was noticed in the other season 2020, respectively.
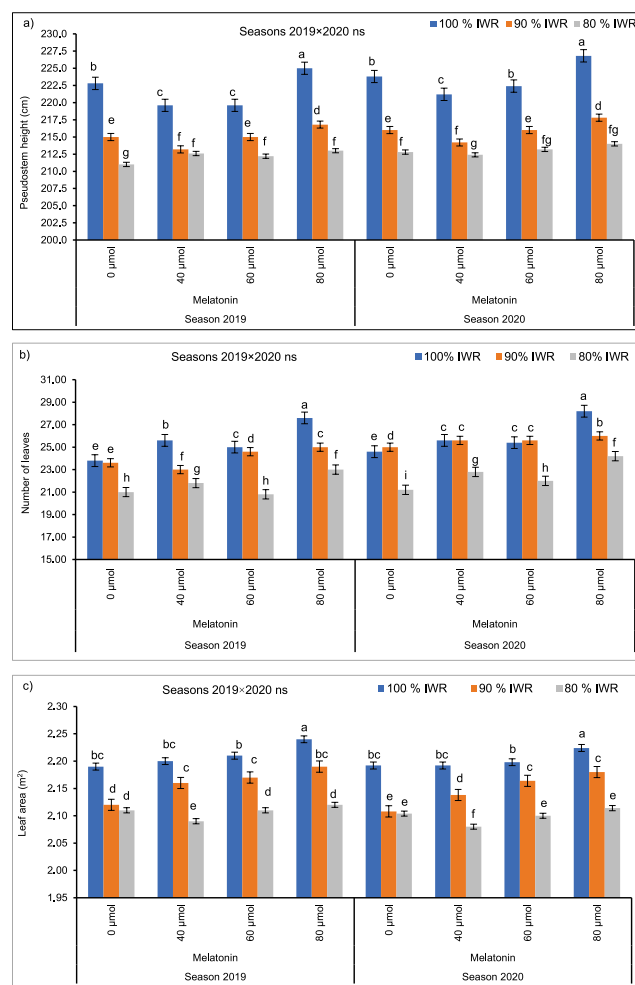
Fig. 1. The interaction effect of drought stress and melatonin concentrations in semi-arid conditions during the seasons 2019 and 2020 on: a) banana plant pseudostem height (cm), b) number of leaves per plant, c) leaf area (m^2); standard error bars and Duncan's multiple range test (DMRT) were applied for mean comparisons within the same year ($N = 5$); data with the different letters represent significant difference at $p < 0.05$; 2019×2020[ns] (ns = non-significance at 0.05); source: own study.

Number of leaves per plant

In season 2019 the results show that the number of leaves improved after melatonin foliar application and declined with increased water stress in the absence of melatonin. The highest

number of leaves was obtained by the combination of 80 μmol melatonin and the 100% *IWR* irrigation treatment (average = 27.6 leaf), while 80% *IWR* combined with 0 μmol of melatonin had the lowest number of leaves (average = 21.0 leaf). In season 2020, the results show that 80 μmol melatonin's treatments compensated for the water shortage, and the plant produced the highest number of leaves (average = 25.6 leaf) as shown in Figure 1b.

Leaf area

The data in Figure 1c significantly illustrated the effect of the combination between irrigation water quantities and melatonin foliar application. Thus, the outcomes show that leaf growth is affected negatively by the reduction of irrigation water quantities from 100 to 90 and 80% *IWR* without melatonin application. Conversely, the foliar application of melatonin enhanced significantly leaf area development and enlargement at 90% *IWR* for both seasons. This was observed particularly when melatonin concentration increased from 40 to 80 μmol, whilst the highest values were obtained with the interaction of irrigation treatments at 100% *IWR* and foliar application of melatonin at 80 μmol, followed by 90% *IWR* and 60 μmol. However, the lowest values were recorded by the combination of irrigation treatment at 80% *IWR* and foliar application of melatonin at 60 and 40 μmol in both seasons compared to control treatment at 100% *IWR*.

BUNCH CHARACTERISTICS

Bunch weight

Bunch weight was generally higher in season 2020 relative to season 2019. The obtained results also show that drought stress significantly compromises the bunch weight when irrigation water was decreased from 100 to 90 and 80% *IWR* in both seasons. The effect of the combination between melatonin and drought stress presented in Figure 2a illustrated that irrigation treatment of 100% *IWR* irrigation treatment produced the highest yield when it was combined with (0 μmol melatonin) in 2019 and 80 μmol melatonin in 2020 (25 and 25.6 kg), respectively. Also, we can notice that the treatment of 80% *IWR* produced the lowest bunch weight values without melatonin in both seasons of the experiment (19.2 and 20.6). Nonetheless, in the presence of melatonin, the obtained yield increased in all irrigation treatments, particularly at 80% *IWR* compared to other concentrations in the two growing seasons 2019 and 2020.

Number of hands per bunch

In accordance with the data in Figure 2b there was no consistent effect of melatonin foliar spray with regards to the number of hands per bunch in the two seasons. However, there was a general decline with *IWR* across the studied concentrations.

Hand weight

The illustrated data in Figure 2c revealed that water shortage from 100 to 80% *IWR* affected the hand weight negatively by decreasing the available water for the banana plant. In contrast, melatonin foliar application did not show a clear trend on the hand weight. Although, melatonin treatment in combination with irrigation treatment at 100% *IWR* (control), achieved significantly (p < 0.05) the highest hand weight among other treatments in both seasons 2019 and 2020 (2.54 and 2.76). However, the lowest hand weight
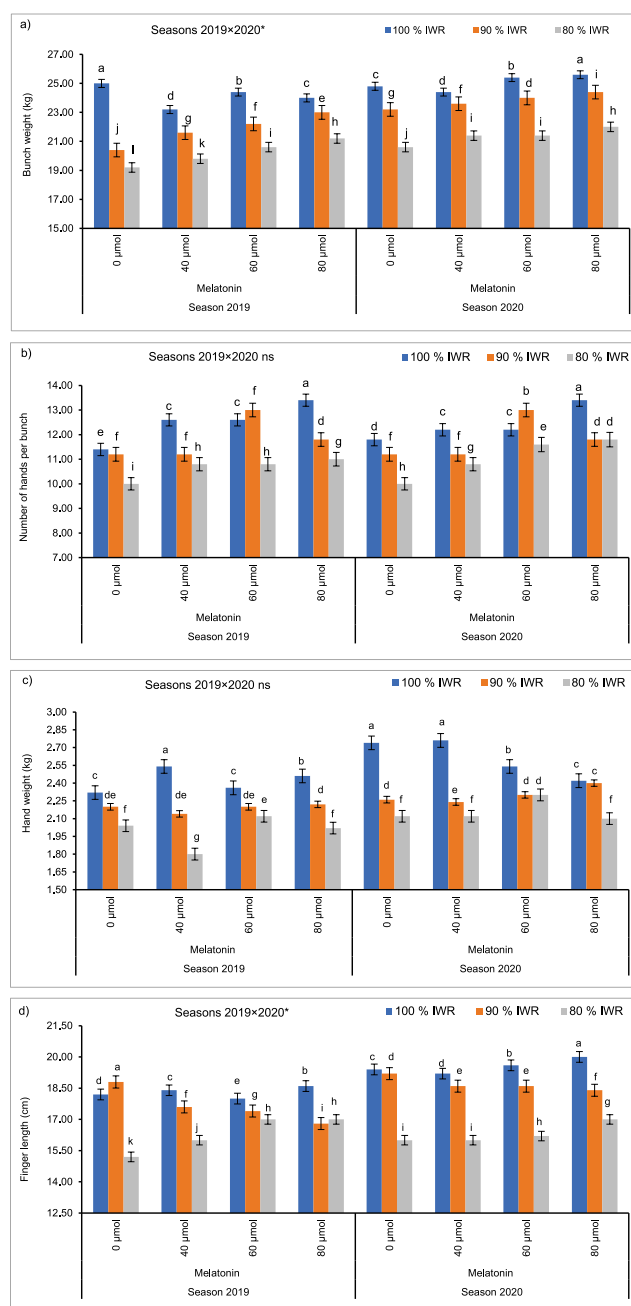
Fig. 2. The interaction effect between drought stress and melatonin concentrations under semi-arid conditions during seasons 2019 and 2020 on: a) bunch weight, b) number of hands per bunch, c) hand weight, d) finger length; standard error bars and Duncan's multiple range test (DMRT) were applied for mean comparisons within the same year (N = 5); data with the different letters represent significant difference at p < 0.05; 2019×2020[ns] (ns = non-significance at 0.05, while 2019 X 2020* (* = significance at 0.05) in both bunch weight and finger length; source: own study

values were recorded by irrigation treatment at 80% *IWR* in the experimental seasons 2019 and 2020.

Finger length

Finger length is one of the most common measurements to judge banana bunch quality. Thus, improving the finger length of banana fruit is the main concern for farmers and researchers as it directly implies its effect on the end product. The obtained data

presented in Figure 2d, clearly showed that the finger length of banana fruit was generally higher at 100 and 90% *IWR* in season 2020 relative to season 2019. The result also showed that finger length was affected adversely by decreasing water from 100 to 90 and 80% *IWR* in both seasons 2019 and 2020. While, it increased with foliar application of melatonin particularly at 80 μmol and 80% *IWR* compared with control (0 μmol melatonin) treatments and no water deficit at 100% *IWR*.

PHOTOSYNTHETIC PIGMENTS AND WATER RELATIONS CHARACTERISTICS

Total chlorophyll

Data presented in Figure 3a showed that water stress affected the total chlorophyll content (in mg·$(100 \text{ g})^{-1}$ fw) of banana leaves negatively during both seasons. Furthermore, Chl concentration declined when the leaves were subjected to drought stress treatments (90 and 80% *IWR*) compared to control 100% *IWR* in the absence of melatonin foliar application during both seasons 2019 and 2020. In contrast, different concentrations 40, 60 and 80 μmol of melatonin reduced significantly ($p \leq 0.05$) the harmful

effect of decreasing irrigation water from 100 to 80% *IWR* with elevated total chlorophyll values relative to control particularly at 80 μmol of melatonin over the two seasons 2019 and 2020.

Relative water content

Relative water content (*RWC*) is one of the indicator keys for measuring plant tolerance to water stress and could be considered an indicator for plant water status under water stress. The obtained data shown in Figure 3b indicated that relative water content decreased markedly when water levels declined from 100 *IWR* to 80% *IWR* in the two seasons 2019 and 2020. The lowest values at 78% were achieved by irrigation treatments of 80% *IWR* in absence of melatonin during the two seasons 2019 and 2020. The highest values (86.8%) were recorded by the combined effect between irrigation treatment at 100% *IWR* and foliar application of melatonin at 80 μmol in both seasons.

Electrolyte leakage

Electrolyte leakage (*EL*) is considered a vital analysis to investigate the plant response to water shortage, in order to measure membrane damage. As presented in Figure 3c a progressive increase in *EL* was noticed with an increase in the drought stress from 100 to 80% *IWR*. Irrigation control treatment at 100% *IWR* recorded a lower *EL* in the absence of melatonin foliar application during two seasons – 2019 and 2020 (14.4 and 15.8%) whilst irrigation treatment at 80% *IWR* which recorded the highest values in the absence of melatonin (22.2 and 21.2). On the other hand, a noticeable deterioration in *EL* occurred when the concentration of melatonin foliar application increased during the two seasons.
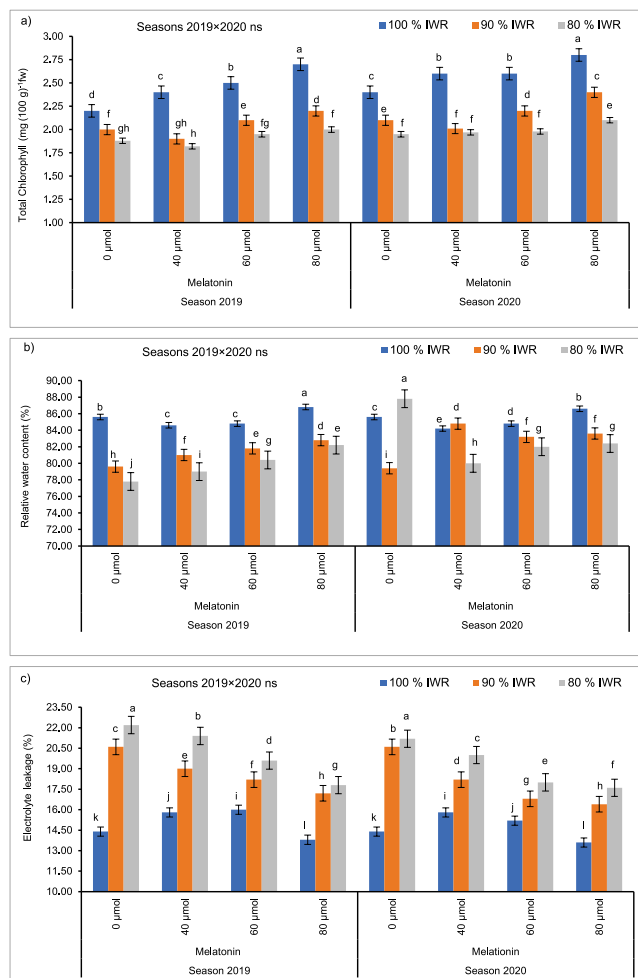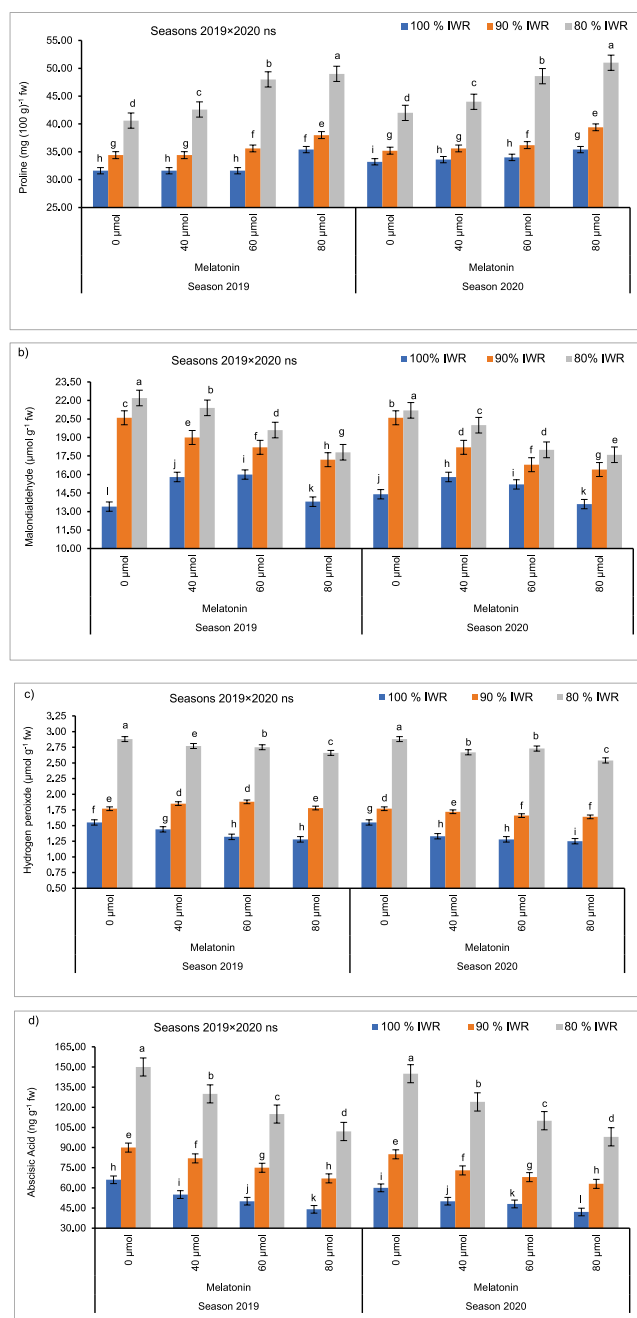
BIOCHEMICAL MEASUREMENTS

Proline

The effect of melatonin application on the proline content of banana plants exposed to different irrigation water quantities is presented in Figure 4a. The illustrated data show a remarkable correlation between drought stresses and proline content with decreasing water regimes from 100 to 80% *IWR* either with or without melatonin foliar application. This could be an inner mechanism by banana plants to cope with a drought stress injury. Nonetheless, foliating banana plants with different concentrations of melatonin significantly ($p \leq 0.05$) increased their proline accumulation. Hence, proline content increased consecutively with water deficit especially at 80% *IWR* over the two seasons.

Lipid peroxidation malondialdehyde (MDA)

Drought stress can destroy plant cellular membranes of banana plants and this could be shown in the formation of malondialdehyde content as a result of lipid peroxidation. Accordingly, data in Figure 4b showed that the decrease in irrigation water regimes from 100 to 80% *IWR* significantly enhanced the formation of malondialdehyde content in the leaves of banana plants in both seasons 2019 and 2020. As for the interaction effects between water stress treatments and melatonin foliar application, it could be seen that at 80% *IWR* treatment in combination with foliar application of melatonin, there was a significant increase ($p < 0.05$) in MDA formed relative to control. However, a rise in melatonin concentration applied decreased MDA formation in the banana plants.

Fig. 3. The interaction effect between drought stress and melatonin concentrations under semi-arid conditions during seasons 2019 and 2020 on banana: a) total chlorophyll, b) relative water content (*RWC*, %), c) electrolyte leakage (*EL*, %); standard error bars and Duncan's multiple range test (DMRT) were applied for mean comparisons within the same year (*N* =5); data with the different letters represent significant difference at $p < 0.05$; 2019×2020[ns] (ns = non-significance at 0.05); source: own study.

Fig. 4. The interaction effect between drought stress and melatonin concentrations under semi-arid conditions during seasons 2019 and 2020 on: a) banana proline, b) malondialdehyde, c) hydrogen peroxide, d) abscisic acid; standard error bars and Duncan's multiple range test (DMRT) were applied for mean comparisons within the same year ($N = 5$); data with the different letters represent significant difference at $p < 0.05$; 2019×2020[ns] (ns = non-significance at 0.05); source: own study

Hydrogen peroxide (H_2O_2)

Compared to the control treatment of 100% *IWR*, water deficit treatments of 90 and 80% *IWR* without foliar spray of melatonin significantly increased the reactive oxygen species ROS (H_2O_2) content in leaves in both seasons 2019 and 2020 (Fig. 4c). Water deficit enhanced anion (H_2O_2) accumulation in leaves, while melatonin foliar application treatment under water deficit stress significantly reduced ROS (H_2O_2) content in the leaves especially at 80 µmol compared to 100% *IWR* in both growth seasons 2019 and 2020.

Abscisic acid (ABA)

We investigated the potential involvement of melatonin in drought and its link to abscisic acid (in ng·g^{-1} fw). The obtained results shown in Figure 4d revealed that under normal conditions, melatonin did not prevent abscisic acid accumulation in banana plant leaves grown under semi-arid conditions. The obtained data showed that with decreasing water regimes ABA accumulated in both seasons 2019 and 2020. The irrigation treatments 80% *IWR* recorded the highest ABA values compared with 100% *IWR*, while irrigation treatment at 90% *IWR* came in between. However, a rise in melatonin concentration applied decreased ABA accumulation significantly ($p < 0.05$) in the banana plants.

DISCUSSION

Banana plant is considered as a plant sensitive to water shortage [Zhang *et al.* 2015]. As a result, the banana plant is vulnerable to soil moisture stress, which has a negative impact on its development and productivity [Surendar *et al.* 2013]. Water deficit regimes have been employed to address water shortage, which has lately become a global issue [Ashraf *et al.* 2011].

Exogenous melatonin serves as an antioxidant and/or growth regulator, as well as a probable signaling molecule [Ayyaz *et al.* 2021]. The activity pathways involved in drought stress tolerance are not well defined [Garg *et al.* 2002]. Several studies have demonstrated the beneficial impact of melatonin in alleviating the adverse effects of drought stress, for instance, the exogenous melatonin can promote drought tolerance in tomatoes [Liu *et al.* 2015] and maize [Ye *et al.* 2016]. Our research was conducted over two seasons (2019 and 2020) to study plant performance amidst water stress in semi-arid regions. The impact of foliar melatonin administration on several morphological (plant height, number of leaves, leaf area, bunch weight, number of hands per bunch, hand weight, finger length), physiological (total chloro-phyll, relative water content) and biochemical (electrolyte concentration, proline, MDA, H_2O_2, ABA) character-istics were described in this study.

The findings of our work highlight the importance of melatonin in enhancing plant performance [Surendar *et al.* 2013a] or, at the very least, reducing the fatality rate of meristematic tissues [Kallarackal *et al.* 1990] by interacting with auxin (growth hormone and the most important growth regulator during plant growth and development) [Sharma, Zheng 2019] in water-deficient treatments. Numerous traits enhanced by melatonin treatment mostly at 80 µmol foliar application include plant height, leaf number, bunch weight, number of hands per bunch, finger length, total chlorophyll and relative water content (*RWC*). However, those traits were negatively affected by water stress when irrigation water was lowered from 100 to 80% *IWR*, according to Hernández-Ruiz *et al.* [2005]. A high and positive correlation was noted between leaf growth expansion and water stress that led to a significant decrease in precise leaf area in this study.

An increase in vegetative growth development as deter-mined by the number of leaves per plant and leaf area in bananas affected by melatonin foliar treatment was observed in both seasons 2019 and 2020. This might be linked to an increase in the efficiency of carbon assimilation and total chlorophyll formation

[Afreen *et al.* 2006; Ballester *et al.* 2018; Sarropoulou *et al.* 2012]. This is contrary to what occurs when there is no melatonin foliar spray. Reduced watering from 100 to 40% *ETc* resulted in a slight decrease in the yield of Crimson seedless grapevine [Ahmad *et al.* 2021; Thomas, Turner 2001]. While modest quantities of melatonin enhanced vegetative growth and development in *Glycyrrhiza uralensis* and *Prunus avium*. This could be linked to a beneficial impact on the photosynthetic rate, which resulted in normal vegetative growth [Bolat *et al.* 2014; Zaky *et al.* 2018].

Multiple studies have previously shown that drought or salt conditions have a negative impact on the overall chlorophyll content of plant leaves [Islam *et al.* 2020]. Similarly, in our investigation, we found that drought stress significantly reduced the overall chlorophyll content of banana plant leaves. This might be due to chlorophyll production inhibition caused by chlorophyllase activation degradation [Zahedi *et al.* 2021]. According to our findings, foliar application of melatonin mitigated the negative detrimental effects of water stress over the two seasons. This may be due to the protection of photosynthetic pigments against degradation which prevented the activation of chlorophyllase degradation [Kabiri *et al.* 2018]. In terms of the physiological repercussions of cellular water deficit; *RWC* is designated for measuring plant water status. It was used to explore the balance between the quantity of water received by plants and the amount lost via transpiration. Banana plants can save their internal water status by lowering radiation load and closing stomata [Yin *et al.* 2013]. Furthermore, *RWC* is regarded as a significant measure of plant survival efficiency and leaf water status. Drought stress lowered *RWC*, but melatonin treatment mitigated the negative effect of water stress. This was supported by previous research, which found that leaf *RWC* was clearly greater in all melatonin-treated maize seedlings under drought stress [Zhang *et al.* 2014]. Leaf *RWC* declined as a result of water scarcity. Furthermore, the impact of water stress grew as the water shortage increased, and melatonin therapy decreased the negative effect of water stress [Liang *et al.* 2019].

The malondialdehyde (MDA) levels indicator is proportional to the degree of water stress damage [Laxa *et al.* 2019]. Our MDA data demonstrated that a lack of water considerably increased the level of MDA formation. Furthermore, melatonin therapy did not inhibit the accumulation of MDA with water stress even though the values declined with a rise in concentration. MDA levels were greater in drought-stressed kiwifruit seedlings that did not receive melatonin, and lower in seedlings that got melatonin pretreatment in other investigations [Xia *et al.* 2018]. The explanation underlying water stress devastating impact is the massive creation of reactive oxygen species (ROS) and malondialdehyde (MDA) over cell tolerance abilities [Arnao, Hernández-Ruiz 2007]. The percentage of electrolyte leakage (*EL*) was calculated to quantify the effect of water scarcity on membrane permeability. The reaction to drought stress and melatonin administration was comparable to MDA findings. Our findings are consistent with prior research, which found that *EL* percent content of figs rose with water scarcity [Zhang *et al.* 2015]. The effect of a water deficit on apple and quince increased the *EL* [Gholami *et al.* 2012]. One of the key osmoprotectants that help plants resist oxidative damage under abiotic stress conditions is proline endogenous concentration [Moustafa-Farag *et al.* 2020]. The current experiment shows that water stress increased proline accumulation. Furthermore, melatonin treatments did not inhibit the rise in proline concentration in water-stressed banana plants. These findings are consistent with those of several earlier types of research [Nawaz *et al.* 2020].

Hydrogen peroxide (H_2O_2) is generated by the cellular breakdown and is a marker of a plant's ability to scavenge ROS under various environmental conditions, whereas melatonin is a very efficient reactive oxygen species (ROS) scavenger. The findings of our study showed that H_2O_2 production increased under water stress with varied treatments from 90 to 80% *IWR* compared to control treatments at 100% *IWR*, but melatonin foliar application lowered H_2O_2 generation. These findings are consistent with previous research on many plants, such as in horticultural crops [Tiwari *et al.* 2020] and mung bean [Bano *et al.* 2021]. The decrease of H_2O_2 caused by melatonin foliar spray might be linked to the ABA content via the instruction of ABA catabolism and biosynthesis genes [Zaky *et al.* 2018].

High ABA concentration in water-stressed banana plants has been demonstrated to result in a reduced vegetative growth rate when compared to a 100% *IWR* control treatment. This might be due to ethylene synthesis, which impairs cellulase and polygalacturonase activity [Li *et al.* 2015].

Recently, much emphasis has been placed on the utilisation of melatonin as a bioactive plant hormone in water-stress circumstances in many plant species. Melatonin regulates the amounts of reactive oxygen and nitrogen species in the plant and alters the molecular resistance to increase plant tolerance to water stress [Zhang *et al.* 2014]. It is in charge of promoting physiochemical responses to various environmental situations in many plant systems [Li *et al.* 2012]. It also improves drought tolerance in plants by increasing ROS scavenging. Melatonin-mediated activation of antioxidant defense response in horticultural crops also stimulates the up-regulation of numerous stress-responsive genes to deal with most abiotic stressors and pathogen infections. Overexpression of Arabidopsis class 1 phytoglobin (AtPgb1) alleviated the growth delay caused by PEG-induced drought stress by lowering ROS and consequent programmed cell death (PCD) associated with protein folding in the endoplasmic reticulum (ER) [Chaves *et al.* 2003; Jafari, Shahsavar 2021]. These melatonin roles are appropriate in our study.

Thus, the novelty of this work may be demonstrated in the fact that it is one of the few papers where a staple crop banana was studied in the field in response to drought. Also, the impacts of melatonin and its link to yield and biochemical parameters were demonstrated in detail. The current study's findings suggest that melatonin at a high concentration of 80 μmol has a beneficial function in alleviating the negative impacts of water stress when compared to well-irrigated conditions.

CONCLUSIONS

According to the current research work findings, melatonin administration might be an efficient technique for improving bananas' growth and yield under drought stress. Melatonin treatments, particularly at 80 μmol, promoted morphological growth metrics such as plant height, leaf number, bunch weight, number of hands per bunch and finger length when water was scarce. Furthermore, melatonin application improved the physiological parameters such as total chlorophyll and *RWC* and

enhanced proline concentration in water stress treatments compared to control. While *EL* percent, MDA, H_2O_2, and ABA generally reduced as a result of the cumulative effect of melatonin foliar application compared to control treatments in the two seasons – 2019 and 2020. However, additional research is needed in the future to demonstrate the molecular and more biochemical and anatomical processes that are responsible for several responses of bananas under drought in the field.

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