Research Article

SOHAG JOURNAL OF SCIENCES

https://doi.org/10.21608/sjsci.2024.264172.1176

Effects of Salicylic Acid, Melatonin, and Mycorrhizal Fungi on the Growth and Physiological Responses of Wheat Under Varying Water Irrigation Stress Levels

Khalaf Ali Fayez[1], Fatma Abdel-Monsef Abdo[2] and Hosna Mohamed Sabra[2, *]

[1] *Botany and Microbiology Department, Faculty of Science, Sohag University, Sohag 82524, Egypt.*
[2] *Crop Physiology Department, Field Crop Research Institute, Agricultural Research Center, Giza 12619, Egypt.*
[*]Email: *abodoh80@yahoo.com*

Received: 27th January 2024**, Revised:** 26th February 2024, **Accepted:** 29th March 2024
Published online: 22nd April 2024

Abstract: The current study was carried out in Shandaweel Agricultural Research Station in Sohag governorate, Egypt, during the two growing seasons 2017/2018 and 2018/2019. The purpose of this research is to investigate the influence of melatonin(30 ppm), salicylic acid (200 ppm), mycorrhizal fungi inoculation (250 spores), and the combination of melatonin (30 ppm) + mycorrhizal fungi inoculation (250 spores), and salicylic acid (200 ppm) + mycorrhizal fungi inoculation (250 spores) treatments on growth parameters and physiological activitiesof wheat (*Triticum aestivum* L cv. Shandaweel1), which subjected to three water irrigation levels (5476 ($I_{100\%}$), 4380 ($I_{80\%}$) and 3285 ($I_{60\%}$) m^3 ha^{-1} compared to the control (untreated plants). The results showed that the decreasing irrigation water amount from 5476 m^3 ha^{-1} ($I_{100\%}$) to 3285 m^3 ha^{-1} ($I_{60\%}$) caused a significant decrease of relative water content, membrane stability index, leaf area duration, crop growth rate, relative growth rate and net assimilation rate of wheat in both seasons, respectively. According to our findings, the use of melatonin, salicylic acid, and mycorrhizal fungal treatments singly or in combination reduced the deleterious effects of water stress on all of the aforementioned parameters.
Keywords: Growth parameters, Melatonin, Mycorrhizal fungi, Salicylic acid, Water stress, Wheat.

1. Introduction

Wheat is considered one of the most important strategic cereals for human nutrition. Egypt's wheat planted area was 1.44 million hectares, producing 9.38 million metric tons in 2020/2021 growing season**[1]**.Drought is a notable factor that limits growth and development of plant as salt stress**[2]**. Water stress severely decreased the yield of wheat **[3,4]**. Egypt's water scarcity has surpassed the 1000 m^3/capita/year mark. In this context, the water scarcity in Egypt will be down to a level 500 m^3/capita/year due to the expected population predictions for 2025 [5]. As a result, limiting the amount of irrigation water used for agriculture will contribute to solving this problem and optimize the beneficial effects of the existing irrigation water to satisfy the rising food demands of the growing population. In the meantime, irrigation with less water than the optimum crop requirements are a water-saving method**[6]**.Water scarcity has a negative impact on all stages of plant growth, but it is most noticeable during the reproductive stage as well as duringthe grain filling period, which results in adecrease in grains and grain size in cereal crops such as wheat**[4,7]**.Moreover, water stress affects various physiological and metabolic changes, which inhibits the growth and development of plants**[8,9]**. In this respect, wheat productivity and quality has been reduced in the arid and semiarid areas **[10]**. Water stress applied to wheat plants at various phases of development lowered the percentage of membrane stability index and leaf relative water content **[11,12]**.

Applying plant growth regulators exogenously is thought to be a potential strategy for increasing crop resilience to water stress.Melatonin (N-acetyl-5-methoxytryptamine) is a low molecular weight chemical with an indole ring structure. It is present in living organisms from bacteria to mammals **[13,14]**.Melatonin plays a significant role in various types of stress resistance **[15]**. Exogenous melatonin administration improved various physiological functions, including root development, seed germination, photosynthesis, blooming, leaf withering, and fruit maturity**[16-18]**.Also, several studies have been reported that melatonin mediates plant response to various environmental stressors conditions, which include drought, salt, inadequate nutrients, toxicity of heavy metals, cold, heat, and UV-B irradiation**[19-22].**

Salicylic Acid (SA, 2-hydroxybenzoic acid) is an essential signaling molecule that modulates plant responses to environmental stresses **[4,23-26]**. Recently, SA regulatedmany of plant physiological processes such as: growth, development, flowering, stomata closure, photosynthesis, transpiration and ions transport **[27]**. Previous studies demonstrated that exogenous salicylic acid might alleviate the negative effects of salt and drought stress in wheat **[28,29]**.Useful plant-microbe interaction to improve crop yield and quality is a long-term strategy for achieving environmentally friendly agricultural production**[30]**.Due to the growing world population and scarecenessof arable water for agriculture, the increasing productivity and quality of crops is necessary in the future**[31]**. Mycorrhiza is a symbiotic relationship between arbuscular mycorrhizal fungi (AMF) and plants. Over 80% of terrestrial plants form a symbiotic connection with arbuscular mycorrhizal (AM) fungus [32, 33]. Therefore, the field experiments were conducted to investigate the effect of application of salicylic acid, melatonin and mycorrhizal fungi inoculation, individually or in combinations, on growth parameters and physiological activity of wheat crop under stress of different irrigationwater levels.

2. Materials and methods

At Shandaweel Agricultural Research Station in Sohag Governorate, Egypt, a field experiment was carried out over two consecutive growing seasons in 2017/2018 and 2018/2019. This study was planned to investigate the influence of melatonin, salicylic acid, and mycorrhizal fungi individually and in combination treatments on some physiological and growth parameters of wheat cultivar (L. cv. Shandaweel 1) under three irrigation water levels. The soil texture at the experimental site was clay loam, and the pH was 7.4. The available N, P, and K concentrations in the cultivated layer (0-30 cm) were 54, 15, and 310 ppm, respectively.The average annual temperature andrainfall are 23.5°Cand 1mm, respectively. The experiment was laid out ina strip plot designwith threereplications. The three irrigationwater levels $I_{100\%}$(5476),$I_{80\%}$(4380) and$I_{60\%}$(3285) m^3 ha^{-1}occupied the horizontal plots, whilethe vertical plots were devoted to the six treatments i.e., T1: control (water), T2: melatonin (30 ppm), T3: salicylic acid (200 ppm), T4: mycorrhizal fungi, T5: salicylic acid (200 ppm) + mycorrhizal fungi and T6: melatonin (30 ppm) + mycorrhizal fungi. The sowing method was drill. The plot area was 8.4 m^2 (2.40×3.5 m), with 12 rows spaced 20 cm apart and 3.5 m long.Sowing took place on 25 November in the two growing seasons. Salicylic acid (200 ppm) and melatonin (30 ppm) were given foliarly twice, 45 and 65 days after sowing. At sowing, 250 spores of mycorrhizal fungi were inoculated with wheat grains.A local strain of Glomus macrocarpum was graciously acquired from the plant production department, Faculty of Agriculture (Saba Basha).Allother cultural practices were carried out as recommended.

2.1. The studied traits:

2.1.1. Physiological Parameters

1- Leaf relative water content (RWC %)

It wascalculated at mid-grain filling according to Pask et al [34].
RWC% = [FW - DW] / [TW - DW] × 100.
where, the FW, DW and TW are the fresh leaf weight, dry leaf weight and turgid leaf weight, respectively.

2- Membrane stability index (MSI %)

It was calculated at mid-grain filling according to Sairam et al.[35].
MSI% = 1- $[(C_1/C_2)]$ x 100
Where, the C_1 and C_2 are the electric conductivity at 45 and 100 ºC, respectively.

2.1.2. Growth parameters

Two quadratesamples of ground area covered for each plot were taken at 90 (t_1) and 110 (t_2) days after sowing (DAS) to estimate the growth parameters as follow.

Leaf area duration (LAD - day)
It was calculated according to Hunt [36].
$LAD = (LAI_1 + LAI_2) \times (t_2–t_1) \times ½$
where, the LAI_1 and LAI_2 are the leaf area index at time t_1 and t_2, respectively.

Crop growth rate (CGR - g m-2 day-1)
It was calculated according toWatson [37].
$CGR = [(W_2-W_1)/ (t_2-t_1)]\times1/A g\ m^{-2}\ day^{-1}$
where, the W_1 and W_2 are the total dry weight per m^2 (g) at time t_1 and t_2, respectively and the A is the ground area covered by the m^2.

Relative Growth Rate (RGR g g-1 day-1):
It was calculated according to Blackman [38].
$RGR = (\log_e W_2 – \log_e W_1) /(t_2 - t_1)\ g\ g^{-1}\ day^{-1}$
where, the W_1 and W_2 are the total dry weights per m^2 (g) at time t_1 and t_2, respectively.

Net assimilation rate (NAR - g m-2 day-1)
It was calculated according to Watson [37].
$NAR = (W_2 - W_1) (\log_e L_2–\log_e L_1)/(t_2 – t_1) (L_2 – L_1) g\ m^{-2}\ day^{-1}$.
where, the L_1 and L_2 are the Leaf area (m^2) of land area at time t_1 and t_2, while W_1 and W_2 are the total dry weights per m^2 (g) at time t_1 and t_2, respectively.

2.2. Statistical analysis:

All collected data during the two growing seasons were subjected to appropriate statistical analysis in a strip plot design.The means of treatments were compared using least significant difference test (L.S.D) at $p< 0.05$ and <0.01 of probability as reported bySteel and Torrie[39].

3. Results and Discussion

3.1. Analysis of variance

Separate analyses of variance for each of the studied traits in each of the two growing seasons are presented in Tables1 and 2. Data showed highly significant differences between the three irrigation water levels as well as between the six applied treatments i.e., control (untreated plants),melatonin (ME),salicylic acid (SA), mycorrhizal fungi (MF),salicylic acid + mycorrhizal fungi(SA + MF) and melatonin + mycorrhizal fungi (ME + MF)on relative water content (RWC%), membrane stability index (MSI%), leaf area duration (LAD), crop growth rate (CGR), relative growth rate (RGR) and net assimilation rate (NAR) in both seasons.The interaction effect between the three irrigation levels and the applied treatments (control, ME, SA, MF, SA + MF and ME + MF) was significant for those above-mentioned traits, except net assimilation rate in 2017/2018 season (Tables 1 and 2).

3.2. Physiological Parameters

3.2.1. Effect of irrigation water levels

Water stress is a notable factor that affects the physiological processes of wheat.The results illustrated in Table 3 indicated that the highest values of relative water content (RWC %) and membrane stability index (MSI %) traits

Table 1. Analysis of variance for the studied traits in wheat growing season 2017/2018.

S.O.V	D. F.	Traits					
		RWC	MSI	LAD	CGR	RGR	NAR
Rep	3	3.91	8.86	14.92	3.21	1.09	0.02
Irrigation (I)	2	642.75**	373.92**	1681.70**	184.82**	7.35**	0.45**
Error a	6	1.79	1.30	4.83	1.09	0.07	0.01
Treatments (T)	5	68.14**	101.63**	1024.60**	79.88**	2.48**	0.31**
Error b	15	2.66	4.28	16.37	1.27	2.73	0.01
I×T	10	8.79*	12.48*	46.87*	4.65*	3.91*	0.01^{ns}
Error c	30	3.76	5.53	17.48	2.09	2.68	0.01

RWC: relative water content, MSI: membrane stability index, LAD: leaf area duration (day), CGR: crop growth rate ($g\ m^{-2}\ day^{-1}$), RGR: relative growth rate ($g\ g^{-1}\ day^{-1}$) and NAR: net assimilation rate ($g\ m^{-2}\ day^{-1}$).
*, ** and ns refer to significant at 0.05, 0.01 and non-significant, respectively.

Table 2. Analysis of variance for the studied traits in wheat growing season 2018/2019.

S.O.V	D. F.	Traits					
		RWC	MSI	LAD	CGR	RGR	NAR
Rep	3	9.43	2.73	19.47	1.095	8.76	0.005
Irrigation (I)	2	514.72**	312.94**	2257.04**	169.76**	8.68**	0.39**
Error a	6	4.62	1.51	5.08	0.96	0.06	0.002
Treatments (T)	5	90.01**	155.55**	1285.94**	123.51**	3.45**	0.45**
Error b	15	4.45	2.23	11.31	1.01	1.23	0.002
I×T	10	6.67*	12.38*	43.31*	4.02*	2.41*	0.01*
Error c	30	2.89	4.96	14.2	1.85	1.08	0.003

RWC: relative water content, MSI: membrane stability index, LAD: leaf area duration (day), CGR: crop growth rate ($g\ m^{-2}\ day^{-1}$), RGR: relative growth rate ($g\ g^{-1}\ day^{-1}$) and NAR: net assimilation rate ($g\ m^{-2}\ day^{-1}$).
*, ** refer to significant at 0.05, 0.01, respectively.

were obtained under 100%of irrigation water amount ($I_{100\%}$), while the lowest values were recorded under 60%of irrigation water amount ($I_{60\%}$). Reducing irrigation water from5476 $m^3\ ha^{-1}$($I_{100\%}$) to 4380$m^3\ ha^{-1}$ ($I_{80\%}$) significantly reduced RWC%by 3.59 and 2.27%as well as MSI% by 2.99 and 3.92% in 2017/2018 and 2018/2019 seasons, respectively. On the other hand, decreasing irrigation water amount from 5476 $m^3\ ha^{-1}$ ($I_{100\%}$) to 3285$m^3\ ha^{-1}$ ($I_{60\%}$) significantly reduced RWC% by 10.31 and 10.6% and MSI% by 8.32 and 9.00% in 2017/2018 and 2018/2019 seasons, respectively. The results stated that the reduction in RWC% and MSI% increased in response to decreasing irrigation water amount. Reduced irrigation water resulted in lower RWC% and MSI% **[11-12, 40]**.Moreover**,** Azmat et al.**[41]**found that the drought severely decreased the water status of wheat seedlings depicted by reduced RWC than that of the optimum irrigated plants. Drought stress impacts the integrity of the cell membrane, as evidenced by the reduced value of MSI%**[42]**.

3.2.2. Effect of Melatonin, salicylic acid, and mycorrhizal fungi treatments

Data in Table 4 showed that all treatments of melatonin (ME), salicylic acid (SA) and mycorrhizal fungi (MF) and the combination of ME + MF and SA + MF significantly increased RWC% and MSI% traits as compared to the control (untreated plants).The highest mean values of RWC% (87.95 and 88.66%) and MSI% (87.04 and 87.50%) were recorded in wheat plants treated with SA + MF in 2017/2018 and 2018/2019 seasons, respectively. However, SA + MF treatment was statistically similar with that of ME + MF treatment in 2017/2018 season and with SA treatment in 2018/2019 season for RWC%, while it was statistically similar with ME + MF treatment in the second season for MSI%. In contrast, the lowest mean values of RWC% (80.32 and 82.15%) and MSI (77.17 and 79.67%) were noted with the untreated plants in 2017/2018 and 2018/2019 seasons, respectively. Data presented in Figure 1 and 2 showed that application of ME, SA, MF, SA + MF and ME + MF treatments significantly increased RWC (3.61 and 3.68 %), (5.48 and 6.43 %), (3.90 and 5.48 %), (9.50 and 7.92 %) and (8.11 and 7.12 %), and MSI by (4.21 and 2.94 %), (7.72 and 5.36 %), (5.74 and 3.58 %), (12.79 and 9.83 %) and (11.07 and 8.46 %) compared to the control in 2017/2018 and 2018/2019 seasons, respectively. These results reported that treated wheat plantsbysalicylic acid, melatonin and mycorrhizal fungi as well as their combinations could regulate RWC%as well as MSI% under water stress conditions.These results are consistent with those obtained by Sofy **[11]**.In this context, exogenous application of SA promotes the buildup of Ca^{+2}, which helps maintain membrane stability **[43]**.RWC% in wheat plants treated with

AMF was substantially higher [44]. Cui et al. [45] found that melatonin affects water balance and cell turgor in wheat seedlings in response to drought stress. Furthermore, melatonin's unique role in epidermal cell proliferation may aid to reduce plant water loss. Therefore, treated wheat plants by SA + MF and ME + MF could improvewheat productivity, particularly in dryland areas.

Table 3. Means of relative water content (RWC) and membrane stability index (MSI) traits under the three irrigation water levels in the two growing seasons 2017/2018 and 2018/2019.

Treatments	Traits			
	RWC (%)		MSI (%)	
	2017/18	2018/19	2017/18	2018/19
$I_{100\%}$	88.52	90.30	85.74	87.44
$I_{80\%}$	85.34	88.25	83.18	84.01
$I_{60\%}$	79.39	80.49	78.61	79.57
LSD at 0.05	**1.52**	**0.95**	**0.87**	**0.81**

Table 4. Means of relative water content (RWC) and membrane stability index (MSI) affected by melatonin (ME), salicylic acid (SA) and mycorrhizal fungi (MF) treatments in the two growing seasons 2017/018 and 2018/019.

Treatments	Traits			
	RWC (%)		MSI (%)	
	2017/18	2018/19	2017/18	2018/19
Control	80.32	82.15	77.17	79.67
ME	83.22	85.17	80.42	82.01
SA	84.72	87.43	83.13	83.94
MF	83.45	86.65	81.60	82.52
SA+MF	87.95	88.66	87.04	87.50
ME+MF	86.83	88.00	85.71	86.41
LSD at 0.05	**1.84**	**1.42**	**1.30**	**1.80**

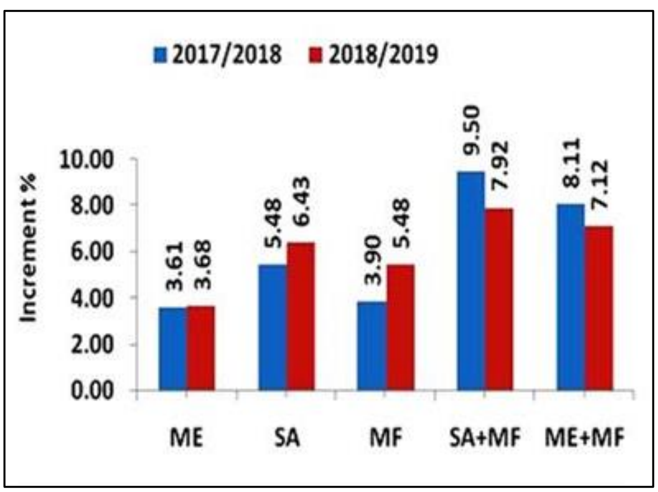

Fig.1. Increments of relative water content (RWC) under effect of ME, SA, MF, SA+MF and ME+MF compared to the control in 2017/018 and 2018/019 seasons.

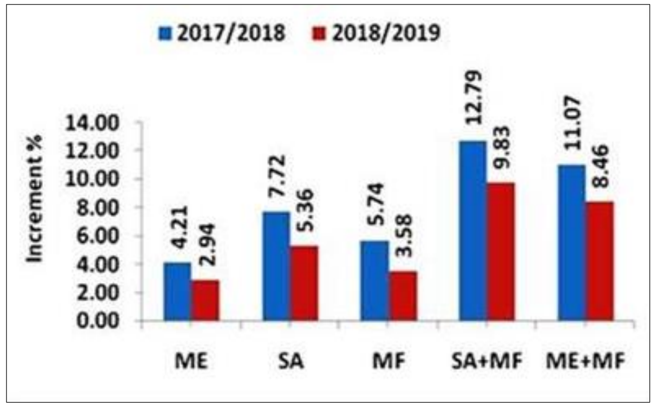

Fig.2. Increments of membrane stability index (MSI), under effect of ME, SA, MF, SA+MF and ME+MF compared to the control in 2017/018 and 2018/019 seasons.

3.2.3. Effect of Interaction

The mean values of RWC% and MSI% for the interaction effect between the three irrigation levels and the applied treatments, namely control, ME, SA, MF, SA + MF and ME + MF are presented in Table 5. The highest mean values of RWC% (91.52 and 92.89%) were recorded under the combination of $I_{100\%}$irrigation level and ME + MF treatment in 2017/2018 season andSA + MF treatment in 2018/2019 season. While the lowest mean values of RWC% (73.13 and 79.30%) were obtained under the combination of $I_{60\%}$ irrigation level and control treatment in the first and second seasons, respectively. Regarding to membrane stability index, SA + MF treatment of wheat under $I_{100\%}$ irrigation water level had the highest values of MSI% (90.02 and 90.73%), while the lowest values of MSI% were recorded under the combination effect of low water irrigation level ($I_{60\%}$)and control plants in the first and secondseasons, respectively. Moreover, SA + MF treatment or ME + MF treatment significantly increased RWC and MSI compared to the control treatment and gave the highest values under all the three levels of irrigationwater.These results showed that treating wheat plants with SA + MF or ME + MF treatments significantly reduced the negative effects of irrigation water deficit on RWC% and MSI%. These findings are like to results reported by other workers, which demonstrating that SA, ME, and MF, and their combination treatments of wheat plants are very efficient in mitigating the harmful effects of environmental stresses including water stress [11, 41, 46-47].

3.3. Growth parameters:

3.3.1. Effect of Irrigationwater levels

Results in Table 6 showed a significant effect in response to various water irrigation levels for wheat growth parameters in both seasons. The highest values of leaf area duration (LAD), crop growth rate (CGR), relative growth rate (RGR) and net assimilation rate (NAR) traits were recorded under 100% of irrigation water amount (5476 m^3 ha^{-1}), while the lowest values were obtained under 60% of irrigation water amount (3285 m^3 ha^{-1}). As compared to 100% of irrigation water level, application of 80% of irrigation water level significantly decreased (LAD), (CGR), (RGR) and (NAR) by 8.47, 10.35, 10.00 and 5.79% in the first season and by 7.43, 8.46, 5.00 and 5.38% in the second season, respectively. While application of 60% of irrigation water level decreased (LAD), (CGR), (RGR) and (NAR) by 14.35, 22,61, 20.00 and 13.16% in the first season and by12.47, 21.74, 15.00 and 13.23 in the second season, respectively. Under effect of drought condition, almost plant growth parameters significantly decreased comparing with that well water irrigated level ($I_{100\%}$), these results may be due to a decrease in soil moisture that affects the movement of nutrient in the soil **[48]**.Optimum water irrigation application produces a positive effect on crop growth rate was reported **[49]**.

3.3.2. Effect of Melatonin, salicylic acid, and mycorrhizal fungi treatments

Results presented in Table 7 revealed that the melatonin, salicylic acid and mycorrhizal fungi treatments differed significantly for growth parameters in the two growin seasons. The highest mean values of(LAD), (CGR), (RGR) and (NAR) were obtained from plants that treated with SA + MF in both seasons and were significant than other treatments, except ME + MF treatment for crop growth rate in both seasons. Application of ME, SA, MF, SA+MF and ME+MF treatments led to increase of leaf area duration by (6.52 and 7.20%), (11.78 and 10.91%) , (3.66 and 3.29%), (23.99 and 21.97%) and (18.92 and 15.93%), crop growth rate by (7.87 and 9.20%), (23.54 and 18.56%), (23.66 and 17.93%), (44.22 and 35.18%) and (48.15% and32.78%),relative growth rate by (13.33 and 12.5 %), (20.00 and 18.75 %), (20.00 and 18.75%), (33.33 and 25.00 %) and (26.67 and 25.00 %), and net assimilation rate by (9.93 and 8.52 %),(18.54 and 14.06%), (13.52 and 8.11 %), (33.77 and 26.20 %) and (31.13 and 20.36%) as compared by control treatment in the first and second seasons, respectively (Fig 3,4,5 and 6). On the other hand, the lowest mean values of the traits mentioned above were obtained with the control treatment in both seasons.Exogenous application of SA improved the overall dry and fresh weight of wheat plants under stress conditions**[50]**. This rise in wheat dry weight under water stress in response to SA treatment shows the activation of antioxidant reactions, which protect the plant from harm. **[23, 51-52]**. Melatonin improved wheat drought tolerance by reducing photosynthetic inhibition and oxidative damage under drought stress.**[4, 53]**.

3.3.3. Effect of Interaction

The interaction of irrigation water levels with melatonin, salicylic acid, and mycorrhizal fungal treatments (Table 8) was significant for all growth indices except net assimilation rate in the first season.The combination of $I_{100\%}$irrigationwater level and SA + MF treatment recorded the highest mean values (LAD), (CGR), (RGR) and (NAR) in 2017/2018 and 2018/2019 seasons, except forCGR in the second season.Furthermore, there are no significant changes in growth parameters between the SA + MF and ME + MF treatments at the $I_{100\%}$ irrigation water level in both seasons, except for leaf area duration.In contrast, the $I_{60\%}$ irrigation water level and control treatment produced the lowest mean values of (LAD), (CGR), (RGR) and (NAR) in both seasons.Helgi and Rolfe **[54]**found that spraying SA increased LAD, CGR and NAR, which they attributed to its role in promoting and influencing cell proliferation, development, and differentiation, as well as improving plant growth parameters. The increase in growth of wheat plants infected with mycorrhizal fungi than that of non-inoculated plants was probably indirectly attributable to mycorrhizal enhancement of phosphorus uptake, which enhances photosynthesis **[55]**.

4. Conclusion

Farmers in arid and semi-arid locations frequently utilize less water than is required to irrigate their crops, resulting in a significant drop in crop output. Our results indicated that treated wheat plants by melatonin (ME), salicylic acid (SA), mycorrhizae fungi (MF) and their combinationscan effectively improve the tolerance of wheat to water stress through their function in increasing relative water content, membrane stability index, leaf area duration, crop growth rate, relative growth rate and net assimilation rate.The combination application of SA + MF or ME + MF was the most effective treatment in mitigating the detrimental effects of water stress. Using 80% of irrigation water and combining SA + MF or ME + MF could be a beneficial strategy in water-stressed areas for increasing wheat productivity and water usage efficiency.

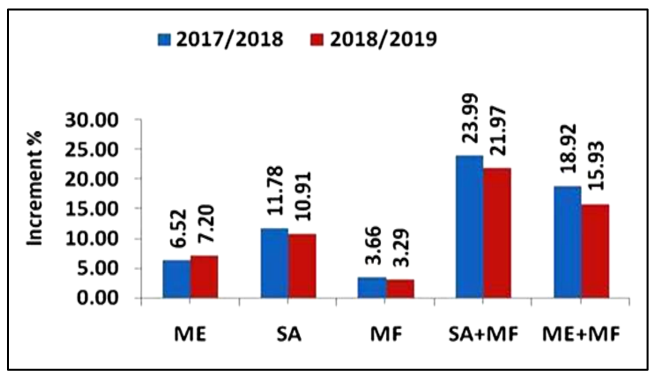

Fig. 3. Increments of leaf area duration (day) under effect of ME, SA, MF, SA+MF and ME+MF compared to the control in 2017/018 and 2018/019 seasons.

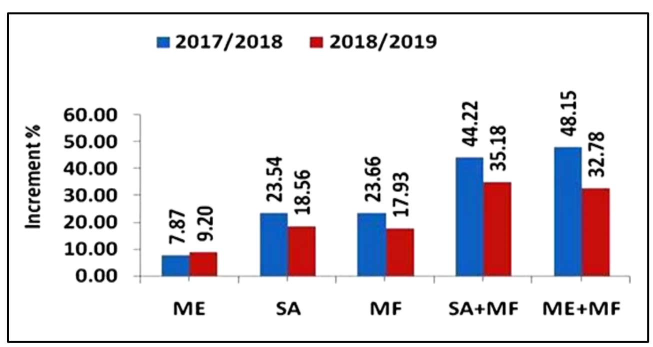

Fig. 4. Increments of crop growth rate (g m-2 day-1) under effect of ME, SA, MF, SA+MF and ME+MF compared to the control in 2017/018 and 2017/018 and 2018/019 seasons.

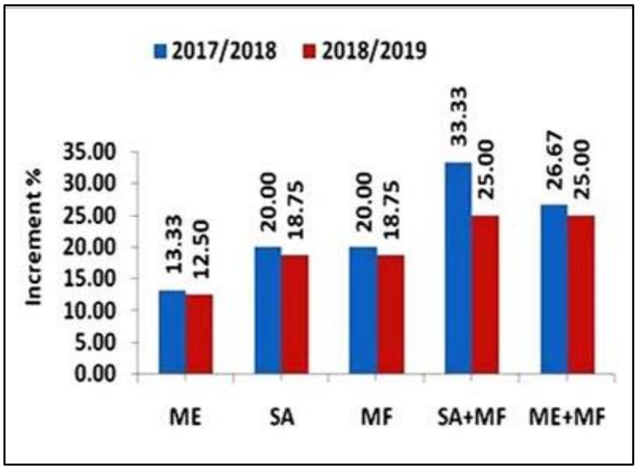

Fig. 5. Increments of relative growth rate (g g^{-1} day^{-1}) under effect of ME, SA, MF, SA+MF and ME+MF compared to the control in 2017/018 and 2018/019 seasons.

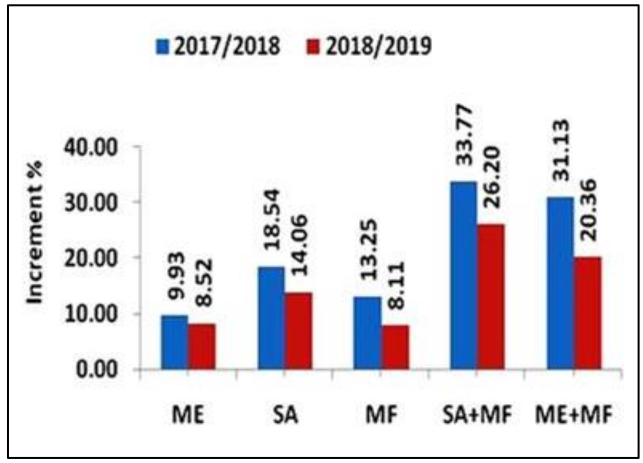

Fig. 6. Increments of net assimilation rate (g m^{-2} day^{-1}) under effect of ME, SA, MF, SA+MF and ME+MF compared to the control in 2017/018 and 2018/019 seasons.

Table 5. Means of relative water content (RWC) and membrane stability index (MSI) traits affected by the interaction between the irrigation water levels and melatonin (ME), Salicylic acid (SA) and Mycorrhizal fungi (MF) treatments in the two growing seasons 2017/018 and 2018/019.

Traits	RWC (%)			MSI (%)		
2017/2018						
Treatments	$I_{100\%}$	$I_{80\%}$	$I_{60\%}$	$I_{100\%}$	$I_{80\%}$	$I_{60\%}$
Control	84.42	83.41	73.13	82.66	78.28	70.58
ME	86.49	84.01	79.18	83.69	81.69	75.88
SA	89.40	84.87	79.90	85.16	84.10	80.14
MF	87.90	84.71	77.75	84.89	83.58	76.34
SA+MF	91.38	88.53	83.94	90.02	86.21	84.88
ME+MF	91.52	86.50	82.47	88.04	85.22	83.86
F test	*			*		
LSD at 0.05	**2.74**			**2.65**		
2018/2019						
Treatments	$I_{100\%}$	$I_{80\%}$	$I_{60\%}$	$I_{100\%}$	$I_{80\%}$	$I_{60\%}$
Control	85.64	81.50	79.30	85.16	81.28	72.58
ME	88.80	86.84	79.87	85.94	82.71	77.38
SA	91.74	89.75	80.82	87.16	84.37	80.29
MF	90.43	89.39	80.14	86.64	83.75	77.19
SA+MF	92.89	91.40	81.69	90.73	86.39	85.38
ME+MF	92.30	90.60	81.10	89.04	85.57	84.61
F test	*			*		
LSD at 0.05	**2.49**			**2.98**		

Table 6. Means of growth parameters under the three irrigation water levels in the two growing seasons 2017/018 and 2018/019.

Treatments	Traits							
	LAD		CGR		RGR		NAR	
	2017/ 18	2018/ 19	2017/18	2018/19	2017/ 18	2018/19	2017/ 18	2018/19
$I_{100\%}$	134.41	133.46	23.48	25.30	0.020	0.020	1.90	2.06
$I_{80\%}$	123.02	123.54	21.05	23.16	0.018	0.019	1.79	1.95
$I_{60\%}$	115.12	116.82	18.17	19.80	0.016	0.017	1.65	1.79
LSD at 0.05	**1.59**	**1.55**	**0.694**	**0.74**	**0.0005**	**0.0006**	**0.034**	**0.06**

LAD, leaf area duration (day); CGR, crop growth rate (g m^{-2} day^{-1}); RGR, relative growth rate (g g^{-1} day^{-1}); and NAR, net assimilation rate (g m^{-2} day^{-1}).

Table 7. Means of growth parameters affected by melatonin (ME), salicylic acid (SA) and mycorrhizal fungi (MF) treatments in the two growing seasons 2017/018 and 2018/019.

Treatments	Traits							
	LAD		CGR		RGR		NAR	
	2017/18	2018/19	2017/18	2018/19	2017/18	2017/18	2018/19	2017/18
Control	112.07	113.40	16.78	19.13	0.015	0.016	1.51	1.71
ME	119.38	121.57	18.10	20.89	0.017	0.018	1.66	1.86
SA	125.27	125.77	20.73	22.68	0.018	0.019	1.79	1.96
MF	116.17	117.13	20.75	22.56	0.018	0.019	1.71	1.85
SA+MF	138.95	138.31	24.20	25.86	0.020	0.020	2.02	2.16
ME+MF	133.27	131.47	24.86	25.40	0.019	0.020	1.98	2.06
LSD at 0.05	**2.93**	**3.52**	**0.873**	**0.98**	**0.001**	**0.001**	**0.041**	**0.087**

LAD, leaf area duration (day); CGR, crop growth rate (g m-2 day-1); RGR: relative growth rate (g g-1 day-1) and NAR, net assimilation rate (g m-2 day-1).

Table 8. Means of growth parameters affected by the interaction between the irrigation water levels, and melatonin (ME), Salicylic acid (SA) and Mycorrhizal fungi (MF) treatments in the two growing seasons 2017/018 and 2018/019.

Treatments	Traits							
	LAD		CGR		RGR		NAR	
	2017/18	2018/19	2017/18	2018/19	2017/18	2018/19	2017/18	2018/19
$I_{100\%}$× Control	124.69	125.62	19.88	22.38	0.018	0.020	1.67	1.835
$I_{100\%}$× ME	128.89	128.94	20.79	23.29	0.019	0.020	1.78	1.975
$I_{100\%}$× SA	132.20	131.89	22.48	23.60	0.020	0.021	1.86	2.060
$I_{100\%}$× MF	129.20	129.43	21.81	24.23	0.019	0.019	1.80	1.965
$I_{100\%}$× SA+MF	151.67	149.44	28.28	28.66	0.021	0.022	2.17	2.300
$I_{100\%}$× ME+MF	139.83	135.45	27.66	29.66	0.021	0.021	2.13	2.240
$I_{80\%}$× Control	108.83	110.23	16.64	19.53	0.015	0.016	1.50	1.745
$I_{80\%}$× ME	120.86	121.33	17.91	21.29	0.017	0.018	1.70	1.895
$I_{80\%}$× SA	124.01	124.90	20.84	23.59	0.019	0.019	1.83	1.983
$I_{80\%}$× MF	110.93	112.30	21.43	22.93	0.018	0.019	1.76	1.905
$I_{80\%}$× SA+MF	138.02	138.17	24.12	26.39	0.020	0.020	1.98	2.140
$I_{80\%}$× ME+MF	135.45	134.31	25.39	25.22	0.019	0.019	1.96	2.043
$I_{60\%}$× Control	102.69	104.35	13.82	15.47	0.012	0.013	1.37	1.563
$I_{60\%}$× ME	108.39	114.44	15.59	18.09	0.015	0.016	1.50	1.710
$I_{60\%}$×SA	119.60	120.52	18.86	20.86	0.017	0.018	1.67	1.823
$I_{60\%}$× MF	108.39	109.66	19.02	20.52	0.016	0.017	1.56	1.688
$I_{60\%}$× SA+MF	127.16	127.31	20.21	22.52	0.018	0.019	1.91	2.048
$I_{60\%}$× ME+MF	124.53	124.66	21.52	21.31	0.017	0.018	1.85	1.908
LSD at 0.05	**4.86**	**5.51**	**1.69**	**1.82**	**0.0015**	**0.0019**	**0.070**	**0.123**

LAD, leaf area duration (day); CGR, crop growth rate (g m^{-2} day^{-1}); RGR, relative growth rate (g g^{-1} day^{-1}); and NAR, net assimilation rate (g m^{-2} day^{-1})

CRediT authorship contribution statement:

Supervision and Conceptualization, K.A. Fayez and F.AM. Abdo; Methodology, investigation and data analysis, H.M. Sabra; Data duration and writing the manuscript, H.M. Sabra, K.A. Fayez and F.AM. Abdo; Revised the manuscript, K. A. Fayez; All authors have read and agreed to the published version of the manuscript.

Data availability statement

The data used to support the findings of this study are available from the corresponding author upon request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] Economic Affairs Sector, Annual report for 2021 Egypt: Ministry of Agricultural and Land reclamation, 2021.
[2] W. Y. Song, Z. B. Zhang, H. B. Shao, X. L. Guo, H. X. Cao, H. B.Zhao, X. J. Hu,.*International Journal of BiologicalSciences*, 4(2008)116.
[3] K. Khadka, M.N. Raizada, A. Navabi,*Frontal Plant Science*, 11(2020) 1149.
[4]K. A.Fayez, F. A. M. Abdo, H. M. Sabra,*Sohag Jornal of Sciences*,8(2023) 65-74.
[5] FAO,(2016). AQUASTAT Country Profile-Egypt. http://www.fao.org/3/i9729en/I9729EN
[6] L. S. Pereira, T. Oweis, A. Zairi, *Agricultural water management*, 57(2002)175-206.
[7] K. Maghsoudi, Y. Emam, M. Ashraf, M. J. Arvin, *Crop and Pasture Science*, 70(2019)36-43.
[8] U. Bechtold, C. A. Penfold, D. J. Jenkins, R. Legaie, J. D. Moore, T. Lawson, P. M. Mullineaux, *The Plant Cell*, 28(2016) 345-366.
[9] B. Heinemann, T. M. Hildebrandt, *Journal of Experimental Botany*, 72(2021) 4634-4645.
[10] M. Tester, P. Langridge, *Science*, 327(2010) 818-822.
[11] M. R. Sofy, *Journal of Plant Research*, 5(2015) 136-56.
[12] A. G. Abd El-Rady, G. M. M. Soliman, Y. S. I. Koubisy, *Journal of Plant Production*, 11(2020) 907-920.
[13] R. Hardeland, D. P. Cardinali, V. Srinivasan, D. W. Spence, G. M. Brown, S. R. Pandi-Perumal, *Progress in neurobiology*, 93(2011) 350-384.
[14]D. X. Tan, R. Hardeland, L. C. Manchester, A. Korkmaz, S. Ma, S. Rosales-Corral, R. J. Reiter, *Journal of experimental botany*, 63(2012) 577-597.
[15] M. Luo, D. Wang, P. Delaplace, Y. Pan, Y. Zhou, W. Tang, K. Chen, J. Chen, Z. Xu, Y. Ma, M. Chen,*Plant Physiology and Biochemistry*,202(2023) 107974.
[16] K. Back, X. Tan, R. J. Reiter,*Journal of Pineal Research*, 61(2016) 426-437.
[17] J. Fan, Y. Xie, Z. Zhang, L. Chen,*International Journal of Molecular Sciences*, 19(2018) 1528.
[18] M. B. Arnao, J. Hernández-Ruiz, *Trends in Plant Science*, 24(2019) 38-48.
[19] A. Sharma, B. Zheng,*Plants*, 8(2019) 190.
[20] S. K. Bose, P. Howlader, *A review. Environmental and experimental botany*, 176(2020)104063.
[21] M. Moustafa-Farag, A. Mahmoud, M. B. Arnao, M. S. Sheteiwy, M. Dafea, M. Soltan, S. Ai,*Antioxidants*, 9(2020) 809.
[22] C. Sun, L. Liu, L. Wang, B. Li, C. Jin, X. Lin,*Journal of Integrative Plant Biology*, 63(2021)126-145.
[23] F. M. Shakirova, A. R. Sakhabutdinova, M. V. Bezrukova, R. A. Fatkhutdinova, D. R. Fatkhutdinova, *Plant science*, 164(2003) 317-322.
[24] K. A. Fayez, S. A. Bazaid, *Journal of the Saudi Society of Agricultural Sciences*, 13(2014) 45-55.
[25] K. H. Alamer, K. A. Fayez, *Physiology and Molecular Biology of Plants*, 26(2020) 1361-1373.
[26] K. Fayez, D. Radwan, A. Mohamed, A. Abdelrahman, *Journal of Environmental Studies*, 6(2011) 55-61.
[27] N. Kaznina, N. Repkina, A. Ignatenko, Y. Batova, E. Kholoptseva, *Vegetos*,*36* (2023) 1-10.
[28] M. Waseem, H. Athar, M. Ashraf,*Pakistan Journal of Botany*, 38(2006) 1127-1136.
[29] M. Arfan, H. R. Athar, M. Ashraf, *Journal of plant physiology*, 164(2007) 685-694.
[30] M. Asadi, F. Rasouli, T. Amini, M. B. Hassanpouraghdam, S. Souri, S. Skrovankova, S. Ercisli,*Agronomy*, 12(2022) 1943.
[31] S. Maitra, A. Hossain, M. Brestic, M. Skalicky, P. Ondrisik, H. Gitari, M. Sairam, *Agronomy*, 11(2021) 343.
[32] B. Wang, Y. L. Qiu, *Mycorrhiza*,16(2006) 299-363.
[33] E. H. Lee, J. K. Eo, K. H. Ka, A. H. Eom, *Mycobiology*,41(2013) 121-125.
[34] A. Pask, J. Pietragalla, D. Mullan, M. P. Reynolds, Physiological Breedung II: a field guide to wheat phenotyping, *Cimmyt*, 2012.
[35] R. K. Sairam, P. S. Deshmukh, D. S. Shukla, *Journal of Agronomy and Crop Science*, 178(1997) 171-178.
[36] R. Hunt,*Journal of Agronomy*, 56(1978) 240-241
[37] D. J. Watson,*Advances in agronomy*, 4(1952) 101-145.
[38] V. H. Blackman, *Annals of botany*, 33(1919) 353-360.
[39] R. G. D. Steel, J. H. Torrie,Principles and procedures of statistics, a biometrical approach2nd McGraw Hill Book Co., Singapore,1984.
[40] A. M. Morsy, I. M. Abd El-Hameed, *Egyptian Journal of Agronomy*, 34(2012) 227-247.
[41] A. Azmat, H. Yasmin, M. N. Hassan, A. Nosheen, R. Naz, M. Sajjad, M. N. Akhtar, *Peer J*, 8(2020) e9960.
[42] S. Tas, B. Tas, *World Journal of Agricultural. Science*, 3(2007), 178-183.
[43] N. A. Khan, S. Syeed, A. Masood, R. Nazar, N. Iqbal, *International Journal of Plant Biology*, 1(2010) e1.
[44] A.F. Mohammed, *Novel Research in Microbiology Journal*, 4(2020) 992-1004.
[45] G. Cui, F. Sun, X. Gao, K. Xie, C. Zhang, S. Liu, Y. Xi, *Planta*, 248(2018) 69–87
[46] A. A. Khalafallah, H. H. Abo-Ghalia, *Journal of Applied Sciences Research*, 4(2008)559-569.
[47] M. A. El Tayeb, N. L. Ahmed, *American-Eurasian Journal of Agronomy*, 3(2010)1-7.
[48] T. S. Abdelmoneim, T. A. Moussa, O. A. Almaghrabi, I. Abdelbagi,*Life Science Journal*, 11(2014) 255-263.
[49] R.K. Thakuria, H. Singh, T. Singh, Annals *of Agricultural Research*, 25(2004) 433-438.
[50] B. Singh, K. Usha, *Plant growth regulation*, 39(2003) 137-141.
[51] S.E.A. Khodary, *International Journal of Agriculture Biological*, 6(2004) 5-8.
[52] M.A. El Tayeb, *Plant Growth Regulation*, 45(2005) 215– 224.
[53] J. Ye, S. Wang, X. Deng, L. Yin, B. Xiong, X. Wang, Acta *physiologiae plantarum*, 38(2016) 48.
[54] Ö.S. Helgi, A. Rolfe, The physiology of flowering plants. Cambridge University Press Plant Physiology, 2005.
[55] G. N. Al-Karaki, A. Al-Raddad, *Mycorrhiza*, 7(1997) 83-88.