Plant Physiology and Biochemistry 139 (2019) 578–586

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy

Research article

Omeprazole alleviates water stress in peppermint and modulates the expression of menthol biosynthesis genes

Hosam O. Elansary[a,b,c,*], Tarek K. Zin El-Abedin[d]

[a] Plant Production Department, College of Food and Agriculture Sciences, King Saud University, P.O. Box 2460, Riyadh 11451, Saudi Arabia
[b] Floriculture, Ornamental Horticulture and Garden Design Department, Faculty of Agriculture (El-Shatby), Alexandria University, Alexandria, Egypt
[c] Department of Geography, Environmental Management and Energy Studies, University of Johannesburg, APK Campus, 2006, South Africa
[d] Department of Agricultural Engineering, College of Food and Agriculture Sciences, King Saud University, Riyadh, Saudi Arabia

A R T I C L E I N F O

Keywords:
Peppermint
Water stress
Omeprazole
Menthol biosynthesis
Antioxidants
Gene expression

A B S T R A C T

Water stress is a worldwide agricultural challenge that limits crop growth and quality. Chemical compounds that promote tolerance to water stress, such as omeprazole showed recently promising results. The present study investigates the effect of weekly drenching applications of 0, 10, 50, 100, or 200 μM omeprazole on *Mentha piperita* (peppermint) subjected to water stress by watering at 100%, 70%, and 50% of container substrate capacity for 7 weeks in an experiment that spanned two seasons. Peppermint that received higher doses of omeprazole showed increased plant height, leaf number, leaf area, and dry weight under normal and water stress conditions. The amounts of chlorophyll and proline in the leaves as well as gas exchange increased in omeprazole-treated plants relative to the control plants. Omeprazole treatment also resulted in increased activity of the enzymes catalase and ascorbate peroxidase, reduced accumulation of the reactive oxygen species hydrogen peroxide, increase in the essential oil ratio, and improvement in essential oil composition. Omeprazole-treated plants showed higher ratios of menthol and menthone composition relative to the control plants. The changes in essential oil composition were associated with increased expression of genes associated with the menthol biosynthesis pathway. These findings indicate that omeprazole can ameliorate water stress in peppermint by increasing vegetative and root growth; increasing chlorophyll amount, photosynthetic rate, and gas exchange; reducing water loss by boosting leaf water potential and relative water content; increasing proline content; and modulating the gene expression of secondary metabolites.

1. Introduction

Water stress is a worldwide agricultural challenge that limits crop growth and development by reducing vegetative growth, flowering and fruiting through decreased gas exchange, inhibited photosynthesis, metabolic alterations, and molecular regulation (Argyrokastritis et al., 2015; Osakabe et al., 2014; Zhong et al., 2019). Plants have developed several mechanisms to overcome water stress, including modulation of membrane transport (Babak et al., 2013), increased expression of drought-responsive gene (Fard et al., 2017), early response of proteins localized to membranes (Maathuis, 2013), alterations in photosynthetic metabolism, accumulation of sugar and proline (Al-Ghamdi and Elansary, 2018; Velázquez-Márquez et al., 2015), reduction in gas exchange (Dias et al., 2018; Elansary, 2015), and accumulation of antioxidants that control the build-up of reactive oxygen species (Chandra Rai et al., 2012; Elansary et al., 2017). Exogenous stress tolerance elicitors have been used in agriculture, including seaweed extracts (Elansary et al., 2016a; Salvi et al., 2019), trinexapac ethyl (Elansary, 2017; Sheikh Mohammadi et al., 2017), and omeprazole, recently introduced as a saline stress ameliorant (Rouphael et al., 2018).

Omeprazole is a synthetic molecule (345 Da) and a member of the group of pharmacological proton pump retardants, which play an essential role in controlling stomach acid production in humans by suppressing the proton pump in the stomach (Shin and Sachs, 2008). It is classified as a proton pump inhibitor (PPI) belonging to the benzimidazoles, which control the movement of H+/K + -ATPases across membranes in the gastric system. It was recently introduced as a salt stress ameliorant and vegetative growth stimulant in tomato (Rouphael et al., 2018; Van Oosten et al., 2017). It achieves these effects in plants by stimulating cutin biosynthesis, phenolic and carotenoid accumulation, and antioxidant build-up, leading to ion homeostasis and salt stress tolerance (Rouphael et al., 2018). It also has hormone-like

* Corresponding author. Plant Production Department, College of Food and Agriculture Sciences, King Saud University, P.O. Box 2460, Riyadh 11451, Saudi Arabia
E-mail address: helansary@ksu.edu.sa (H.O. Elansary).

https://doi.org/10.1016/j.plaphy.2019.04.018
Received 12 March 2019; Received in revised form 11 April 2019; Accepted 11 April 2019
Available online 17 April 2019
0981-9428/

functions that modulate natural hormone-signaling networks (Van Oosten et al., 2017). Recent work on salt stress tolerance in basil (*Ocimum basilicum*) indicated that omeprazole applications might have different effects on different genotypes and might function through the interaction of complex mechanisms (Cirillo et al., 2019). Nevertheless, no studies have explored the effects of omeprazole as a water stress ameliorant in plants.

Mentha piperita L. (peppermint) belongs to the family Lamiaceae, which contains several species that have economic value. *M. piperita* is the most popular of these and is used in drinks, including tea; for cooking; in cosmetics; and in the food industries as a flavoring material. Traditionally, the plant is used to treat gastric flatulence, vomiting, nausea, anorexia, indigestion, and ulcerative colitis (Iscan et al., 2002). The essential oil obtained from the plants has a long history of medicinal use and wide applications in the pharmacological industries (Grigoleit and Grigoleit, 2005). Peppermint tolerates light to moderate water stress that modulates the composition profiles of phenolic and essential oils (Elansary, 2017; Figueroa-Perez et al., 2014). More severe water stress may shift the essential oil profile by molecular regulation of specific known genes (Rahimi et al., 2017). The effects of omeprazole application on this plant have not been studied before.

The purpose of the present investigation was to elucidate the morphological, physiological, and molecular responses of *M. piperita* plants to omeprazole application under water stress conditions. Omeprazole has shown obvious value as a saline stress ameliorant in other crops. The effects of omeprazole on peppermint may be associated with physiological parameters such as gas exchange, amounts of proline and chlorophyll, leaf water potential, and essential oil profile. The detection of the molecular mechanism of new elicitors such as omeprazole during water stress on model medicinal plants such as peppermint will add to our minimal knowledge about these elicitors.

2. Material and methods

2.1. Plant material and treatments

Young *Mentha piperita* L. plants were obtained in January 2017 and January 2018 from a local nursery in Alexandria, Egypt, and vouchered at the Faculty of Agriculture, Alexandria University. The plants were repotted in 14-cm PVC pots containing a soil mixture of peat:perlite (2:1), then maintained under controlled greenhouse conditions. Compound fertilizer (Crystalon®, NPK, 19:19:19, Chema Industries, Cairo, Egypt) was added to the growing soil at 2 g L^{-1}. The photosynthetic active radiation was around 1000 µmol $m^{-2.}$ s^{-1} and the daily temperature was between 15 and 28 °C. A full container substrate field capacity (CSC) was obtained by drip irrigation at 38–50 mL/plant/day during the experiment. The CSC or field capacity was determined using the gravimetric method by saturating the soil, then leaving the pots to drain for 60 min. The water loss was quantified, and CSC was calculated as the difference between the supplied and lost water volumes. The plants were then subjected to 100%, 70%, and 50% CSC and/or weekly substrate drenching applications of 0, 10, 50, 100, or 200 µM omeprazole (Globe Pharmaceuticals, Cairo, Egypt) for 7 weeks. Untreated plants were used as a control. The experiment was repeated in three blocks (repetitions) and each treatment was represented by five replicates. The total number of plants per block was 75 [3 (water levels of 100%, 70%, and 50% CSC) × 5 (doses of omeprazole) × 5 (replicates) = 75] in a split plot experimental design.

2.2. Morphological measurements

Plant heights (cm), leaf number (leaf $plant^{-1}$), leaf area (cm^2 $plant^{-1}$), plant dry weight (g), and root dry weights (g) were determined at the end of each season (after 7 weeks of treatment). The leaf area was determined using a digital area meter. The plant fresh weight was determined by weighing the vegetative and root parts after cleaning the remaining soil particles. The dry weight was determined by drying the plants in an oven (35 °C) until they reached constant weight.

2.3. Physiological measurements

Stomatal conductance (*gs*), net photosynthetic rate (*A*), and transpiration rate (*E*) were measured using a photosynthesis system analyzer (Bioscientific Ltd., Hoddesdon, UK) at the end of the experiment in clear and sunny conditions. Leaf midday water potential and leaf midday relative water contents were measured following Elansary and Salem (2015). Leaf proline content was calculated (Elansary and Yessoufou, 2015) and the total chlorophyll content was quantified (Moran and Porath, 1980). Catalase (CAT) activity, ascorbate peroxidase (APX) activity, amount of hydrogen peroxide (H_2O_2) in the leaves, and free ascorbate were quantified in frozen leaf tissues (Elansary et al., 2017).

2.4. Essential oil and gas chromatography/mass spectrometry (GC/MS)

The dried leaves were hydro-distilled for 1 h in a Clevenger apparatus, and the essential oil (EO) ratio was determined per treatment. The obtained EOs were exposed to anhydrous sodium sulfate and then stored at 4 °C. A Thermo Scientific Trace GC Ultra machine was used, coupled with MS (ISQ) and a TG-1MS column (narrow bore, length 30 m × 0.32 mm ID, 0.25-µm film thickness). The carrier gas was helium, and the temperature was gradually increased from 45 °C to 165 °C (4 °C min^{-1}) and then 280 °C (15 °C min^{-1}). The injection volume was 2 µL in splitless mode flow of 1 mL/min, splitless time 3 min and split flow 10 mL/min. The same column and program were used for the flame ionization detector (FID) analyses. A homologous series of *n*-alkanes (C_{10}-C_{36}) standard (Sigma-Aldrich, Berlin, Germany) was used to identify the compounds by the retention time and indices, coupled with MS program (NIST ver. 2.0) and WILEY libraries. Selected references from the literature were also used for comparison (Elansary and Ashmawy, 2013; Elansary et al., 2016b).

2.5. Menthol biosynthesis and antioxidant genes

To study menthol biosynthesis–related gene expression in *M. piperita*, several genes involved in menthol and menthofuran biosynthesis during stress conditions were investigated, including menthofuran synthase (*Mfs*), limon-3-hydroxylase (*L3oh*), limonene synthase (*LS*), *trans*-isopiperitenol dehydrogenase (*Ipd*), menthol dehydrogenase (*Mdeh*), isopiperitenone reductase (*Ipr*), and pulegone reductase (*Pr*) (Rahimi et al., 2017). *Mentha spicata* actin (KM044035.1) was used as a reference gene to normalize the transcription levels.

2.6. Statistical analyses

Watering levels represented the main plot and omeprazole was the subplot in a split plot experimental design. Experiments were repeated twice in two successive seasons of 2017 and 2018. The two season showed comparable data with no significant differences, then the data was merged. The least significant differences (LSD) were determined for all means in the ANOVA test (SPSS, PASW Ver. 21).

3. Results

3.1. Morphological measurements

Under normal watering conditions (100% CSC), the plants treated with omeprazole showed increases in height compared with the control plants. The highest increase was the treated of 100 and 200 µM omeprazole, as shown in Fig. 1. Plant height was limited under 70% and 50% CSC, but this effect was remarkably reduced following treatment

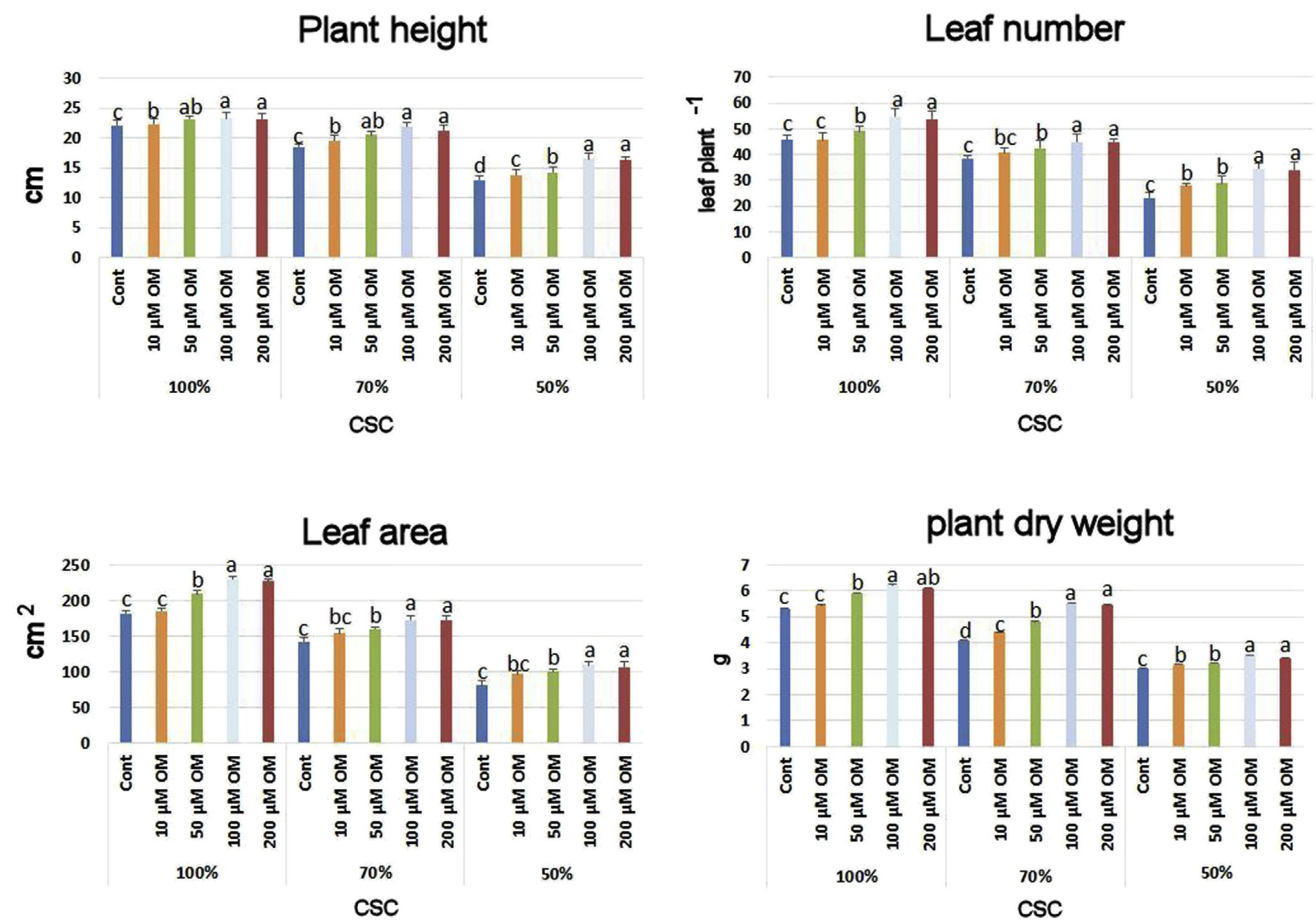

Fig. 1. Effects on plant height, leaf number, leaf area, and plant dry weight of watering levels of 100%, 70%, and 50% container substrate field capacity (CSC) and omeprazole applications at 10, 50, 100, and 200 µM. Means followed by different lowercase letters indicate significant differences between treatments based on LSD test ($p = 0.05$).

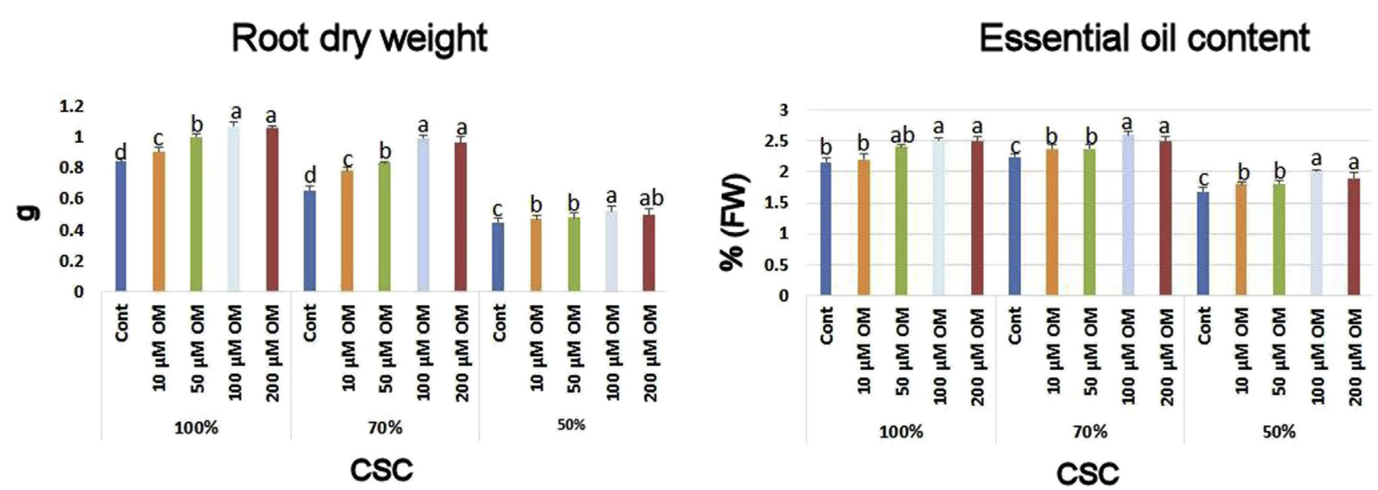

Fig. 2. Effects on root dry weight and percentages of essential oils of watering levels of 100%, 70%, and 50% container substrate field capacity (CSC) and omeprazole applications at 10, 50, 100, and 200 µM.

with 100 and 200 µM omeprazole. Leaf number, leaf area, and plant dry weight followed a comparable pattern to plant height. There were reductions in leaf number, leaf area, and plant dry weight in plants subjected to 70% and 50% CSC (Fig. 1). These reductions were much lower in plants subjected to high doses of omeprazole. The reduction in plant dry weight were due to a reduction in root dry weight, and these reductions were also much lower in plants treated with omeprazole (Fig. 2).

3.2. Physiological measurements

Omeprazole applications increased the *A* under 100%, 70%, and

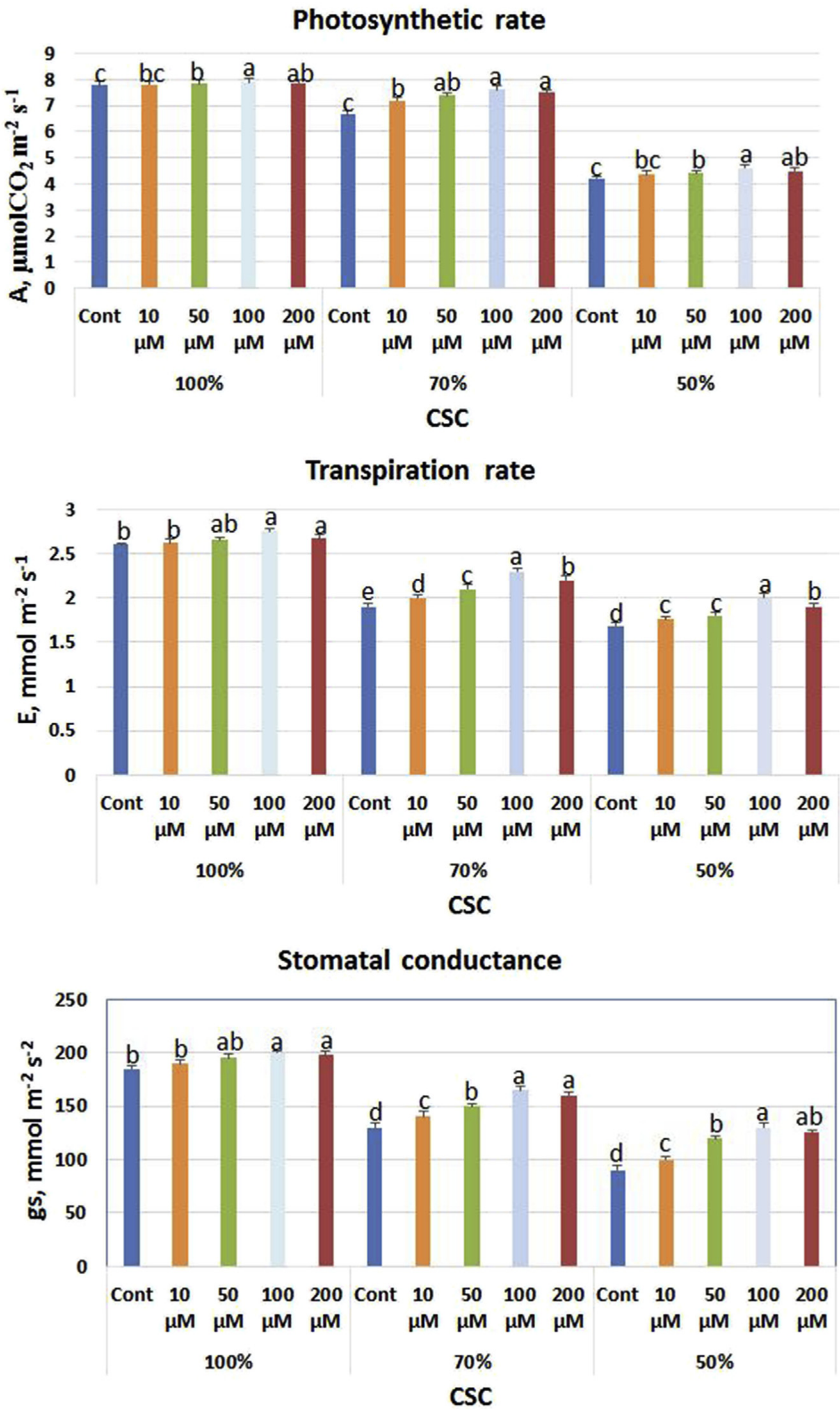

Fig. 3. Effects on photosynthetic rate, transpiration rate, and stomatal conductance of watering levels of 100%, 70%, and 50% container substrate field capacity (CSC) and omeprazole applications at 10, 50, 100, and 200 µM.

50% CSC, compared with control plants, as shown in Fig. 3. The *E* increased in omeprazole-treated plants compared with control plants under 70% and 50% CSC. However, only high doses of omeprazole resulted in increases in the *E*. The *gs* increased in plants subjected to omeprazole compared with control (under water stress). However, under normal irrigation, the increase in the *gs* was visible only in plants that received high doses of omeprazole. Leaf water potential increased in plants subjected to high omeprazole doses, compared with control (under normal and stress conditions) (Fig. 4). The relative water content increased in plants subjected to omeprazole, compared with the control, and the highest increases were in the groups that received 100 and 200 µM. Proline composition increased significantly in plants that received omeprazole under stress conditions, and the chlorophyll composition increased in plants that received 100 µM, compared with the control treatment, as shown in Fig. 5. The activity level of antioxidant enzymes (CAT and APX) significantly increased in plants subjected to omeprazole treatment under normal and water stress conditions, compared with the control, as shown in Fig. 6. The reactive oxygen species (ROS), measured as the amount of H_2O_2 in the leaves, was reduced in plants that received omeprazole treatment, compared with the control group, under water stress conditions. Free ascorbate increased in plants that received omeprazole treatments, compared

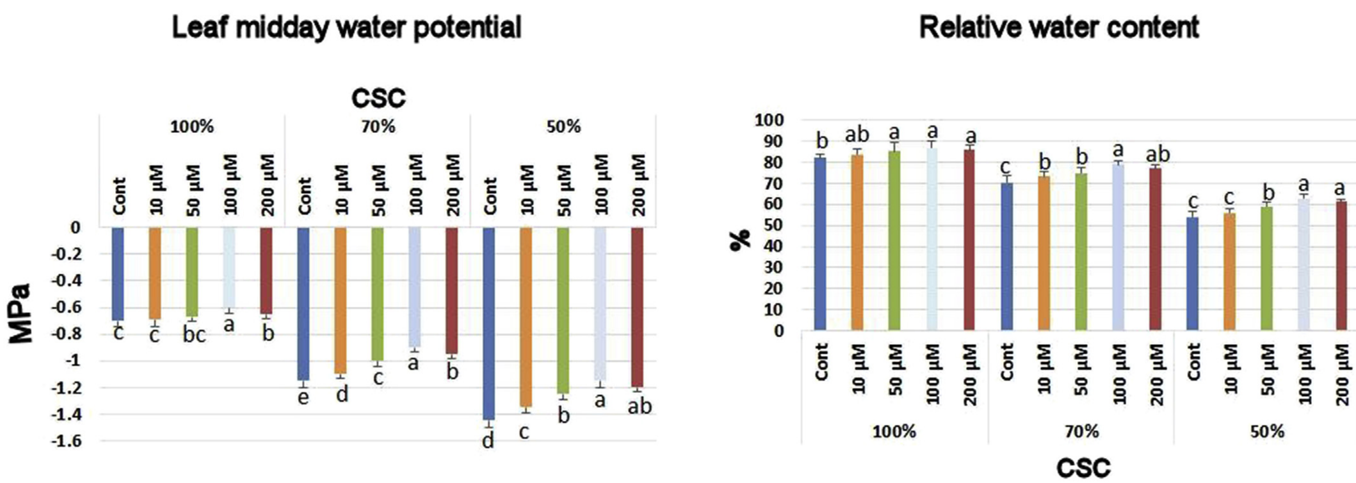

Fig. 4. Effects on leaf midday water potential and relative water content of watering levels of 100%, 70%, and 50% container substrate field capacity (CSC) and omeprazole applications at 10, 50, 100, and 200 μM.

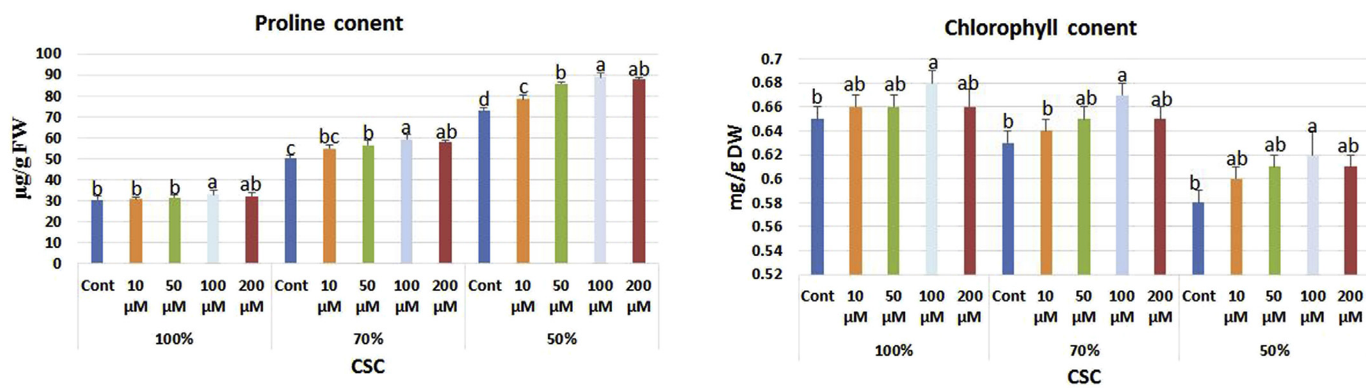

Fig. 5. Effects on amounts of proline and chlorophyll of watering levels of 100%, 70%, and 50% container substrate field capacity (CSC) and omeprazole applications at 10, 50, 100, and 200 μM.

with the control, under water stress conditions. The EO ratio increased in omeprazole-treated plants, compared with the control, under normal watering and stress conditions, as shown in Fig. 7. Major EOs were 1-menthone, L-menthol, menthofuran, pulegone, caryophyllene, and germacrene-D (Table 1). There were increases in the amounts of 1-menthone and L-menthol in omeprazole-treated plants, compared with the control group. These increases were associated with decreased amounts of menthofuran and pulegone. Under water stress conditions, 1-menthone and L-menthol decreased in control plants. The application of omeprazole increased the amounts of 1-menthon, L-menthol, caryophyllene, and germacrene-D and reduced the amounts of pulegone and menthofuran compared with the control.

3.3. Gene expression

There were increases in the expression of *Mfs*, *Ipd*, and *Ipr* in omeprazole-treated plants compared with the control plants under normal and water stress conditions, as shown in Figs. 8 and 9. *LS* and *L3oh* expression increased under 70% CSC but decreased under 50% CSC. The largest increases were found in mint treated with high doses of omeprazole. There were significant increases in the expression of *Mdeh* in omeprazole-treated plants under stress conditions. However, only a slight increase in *Mdeh* expression was found in plants that received high doses of omeprazole under normal conditions, compared with the control. Significant increases were detected in the expression of *Pr* in omeprazole-treated plants compared with control. The largest increase was found in plants treated with 100 μM omeprazole.

4. Discussion

Water stress causes reduced vegetative growth, affecting plant height, leaf number and area, and plant dry weight, which is the natural response of plants to stress conditions and is caused by restricted cell proliferation and expansion (Bechtold and Field, 2018; Wang et al., 2018). Previous investigations revealed that peppermint shows reduced vegetative growth under water stress and is sensitive even to moderate water stress (Zade et al., 2019). In the present study, we noted reductions in the root dry weight in plants subjected to lower CSC, suggesting that peppermint is indeed sensitive to water stress. The roots mediate the uptake of water from soil; under stress conditions, this function is regulated by several pathways, such as hydrotropism and natural growth regulators, including ABA and auxins (Dietrich, 2018).

Peppermint plants subjected to omeprazole were taller under all conditions, compared with the control. This result appears to confirm previous work on tomato during saline conditions (Rouphael et al., 2018; Van Oosten et al., 2017) and suggests that omeprazole ameliorated water stress and augmented the growth of treated plants, serving as a water stress adaptation mechanism. The effect of omeprazole on root dry weight during stress conditions supports the conclusion that omeprazole interacts with ABA and auxins (Van Oosten et al., 2017). The dose of 100 μM was associated with the best vegetative growth. In a previous study of tomato, 1 μM omeprazole stimulated vegetative growth (increasing the fresh and dry weight of shoots and roots), but higher doses (≥ 10 μM) inhibited growth (Van Oosten et al., 2017). In another study, 10 or 100 μM omeprazole increased shoot dry weight in tomato subjected to saline stress (Rouphael et al., 2018). In basil, the

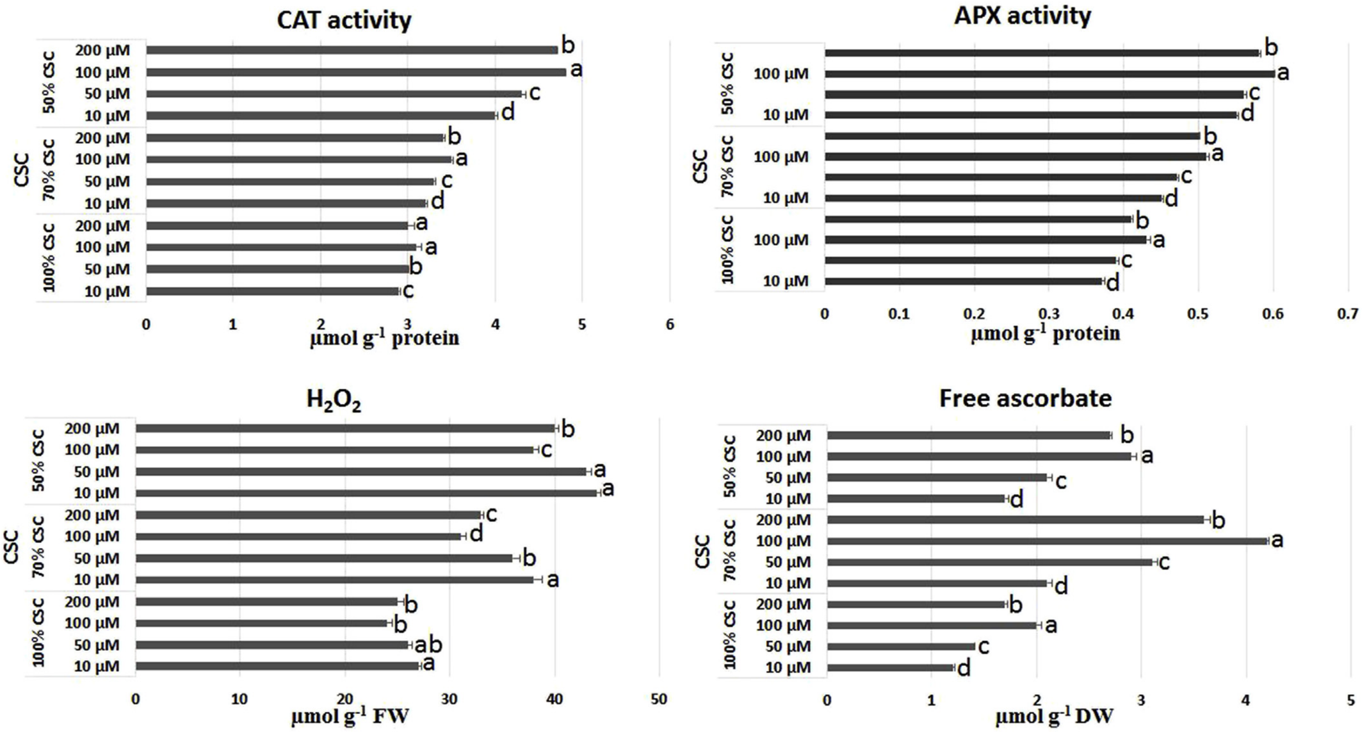

Fig. 6. Effects on catalase (CAT, µmol g^{-1} protein); ascorbate peroxidase (APX, µmol g^{-1} protein); reactive oxygen species, represented by hydrogen peroxide (H_2O_2, µmol g^{-1} FW); and free ascorbate (µmol g^{-1} DW) of watering levels of 100%, 70%, and 50% container substrate field capacity (CSC) and omeprazole applications at 10, 50, 100, and 200 µM. Means followed by different lowercase letters indicate significant differences between treatments based on LSD test ($p = 0.05$).

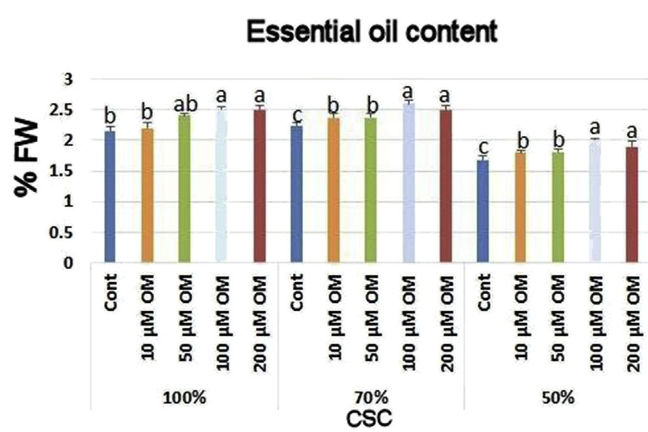

Fig. 7. Effects on percentage of essential oils of watering levels of 100%, 70%, and 50% container substrate field capacity (CSC) and omeprazole applications at 10, 50, 100, and 200 µM.

increase/decrease in plant height, stem diameter, and leaf area in response to omeprazole treatment was cultivar dependent (Cirillo et al., 2019). The present study indicates that the physiological response to omeprazole during water stress is dose dependent.

A reduction in *gs* is a strong indicator of water stress in crops. In the present study, there were increases in the photosynthetic and *E* and the *gs* in plants that received omeprazole under normal and water stress conditions, compared with the control group. These increases were associated with increased chlorophyll composition. They may be attributed to increased expression of the key photosynthetic genes found in tomato plants (Van Oosten et al., 2017). The increase in the photosynthetic content and rates reflect increases in the *E* and *gs*. In a previous study of mint, reduction in the leaf water potential and relative water content were indicators of stress conditions (Elansary, 2017). In another study, meadowsweet subjected to water stress and treated with omeprazole showed increased leaf water potential and content, evidence of water stress tolerance (Stanton and Mickelbart, 2014). The reduction of water loss and maintenance of leaf water potential after omeprazole treatment reflects increased water uptake due to enhanced root growth and overall vegetative performance.

Table 1
Essential oils main constitutes (%) as affected by different watering levels of 100, 70 and 50% CSC and omeprazole applications at 10, 50, 100 and 200 µM.

	RI	100% CSC					70% CSC					50% CSC				
		Cont.	10 µM	50 µM	100 µM	200 µM	Cont.	10 µM	50 µM	100 µM	200 µM	Cont.	10 µM	50 µM	100 µM	200 µM
1-menthone	1153	29c	30c	32b	34.7a	32.8b	21.8c	23.7b	24.8b	27.4a	26.4 ab	18.6d	21.2c	24.7b	27.9a	26.1a
Menthofuran	1163	9.5a	9b	7.5c	5.5e	6.4d	21.46a	19.5b	16.5c	12.9e	14.9d	24.54a	21.9b	17.8c	13.5e	14.8d
L-menthol	1172	31.3c	32.4bc	33b	35.6a	34.7a	25.3c	26.9b	27.5b	29.2a	28.5 ab	24.67c	25.7c	27.6b	29.7a	28.1 ab
Pulegone	1237	5.4a	4.7b	3.3c	2d	2d	6.7bc	6.4c	7.2a	6.5c	6.8b	7.94a	7.6b	7.4bc	6.5d	7c
Caryophyllene	1425	6.1c	6.5bc	6.7b	6.8a	6.7b	5.2c	5.4bc	5.6b	5.9a	5.8a	4.2c	4.4b	4.6b	5a	4.8a
Germacrene-D	1500	6.2d	6.4c	6.4c	6.7a	6.6b	5.6c	5.7b	5.7b	5.9a	5.8 ab	5.3d	5.5cd	5.6c	5.9a	5.8 ab

RI: Retention Index
Different letters among values within a row and watering level indicate least significant differences at $p \leq 5\%$.

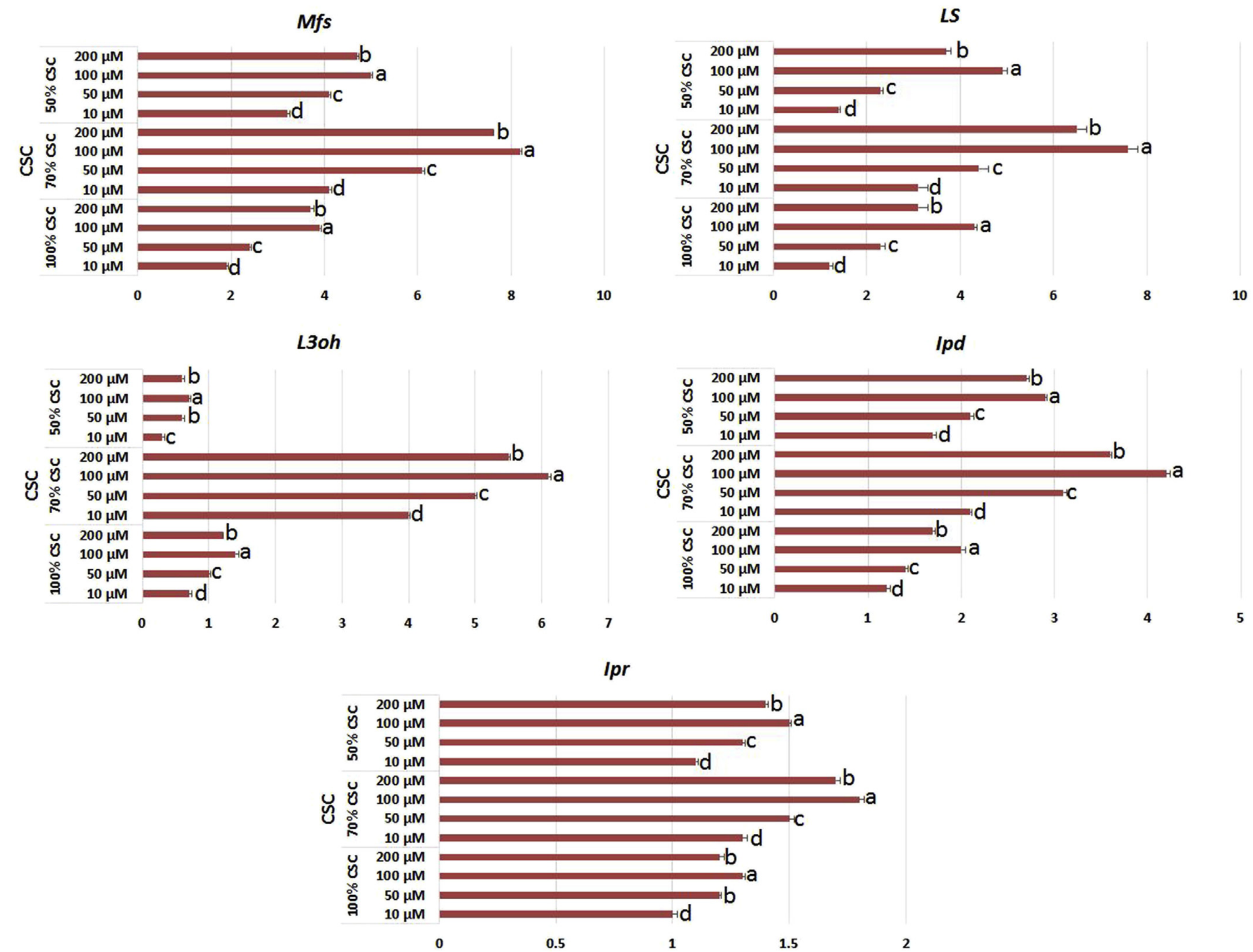

Fig. 8. Effects on the expression of the genes *Mfs, LS,L3oh, pd, and Ipr*I of watering levels of 100%, 70%, and 50% container substrate field capacity (CSC) and omeprazole applications at 10, 50, 100, and 200 µM.

Proline is a non-protein amino acid that accumulates in stressed plants. Plants treated with omeprazole showed significantly higher ratios of proline. Proline is an osmolyte that maintains osmotic leaf potential during water stress and maintains turgor. High production of proline under water stress could be an adaptation to stress; this amino acids functions as a scavenger that supplies osmolytes and energy to stressed tissues and helps the plant to tolerate stress.

Water stress stimulates the production of ROS in plants, including hydrogen peroxide, which attacks and damages DNA and promotes cell death. One of the important mechanisms that enable plants to tolerate drought is the control of ROS during stress conditions through the production of antioxidant enzymes, such as CAT and APX. A previous study found that peppermint increased the production of these enzymes during water stress conditions. In the present study, the application of omeprazole increased the production of these enzymes and reduced the accumulation of hydrogen peroxide, which indicates that the

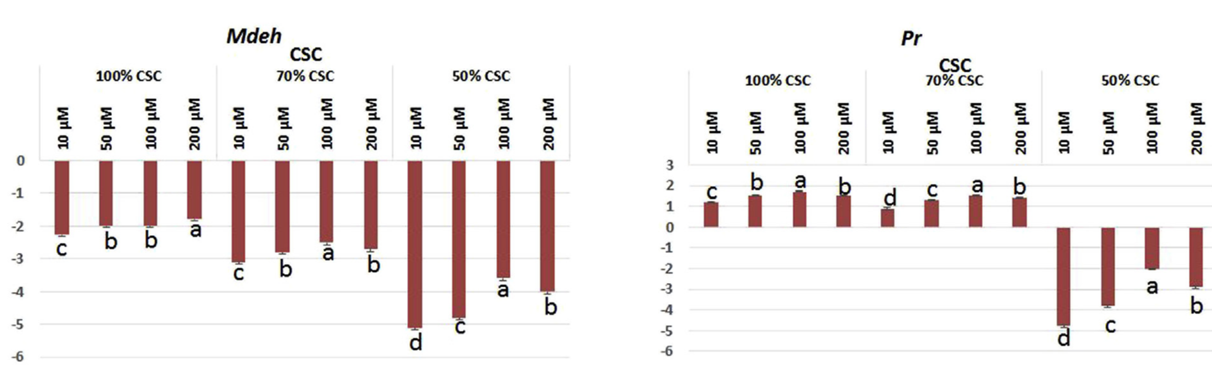

Fig. 9. Effects on the expression of the genes *Mdeh* and *Pr* of watering levels of 100%, 70%, and 50% container substrate field capacity (CSC) and omeprazole applications at 10, 50, 100, and 200 µM.

antioxidant mechanism for drought control was activated in peppermint as a response to omeprazole application.

The oil glands of *Mentha* sp. are mainly distributed on the leaf surface of the plants, and increased leaf area and number are associated with an increased oil ratio per plant. In the present study, there were increases in the essential oil ratio of omeprazole-treated plants compared with the control, a result that might be attributed to increased leaf area and leaf number of treated plants, as well as the improvement of the overall physiological status of treated plants.

The typical main composition of EOs in peppermint is 1-menthone, L-menthol, menthofuran, and pulegone, as found in the present study. The menthol terpenoid metabolism originates in the plastids of the secretory glandular structures of peppermint. There were increases in 1-menthone and L-menthol and decreases in menthofuran and pulegone in omeprazole-treated plants compared with the control plants. These changes in the composition of major EOs in peppermint are commonly associated with molecular expression of menthol and menthofuran biosynthesis genes. This finding indicates that omeprazole may have stimulatory effects on genes associated with menthol biosynthesis. In addition, the higher ratios of menthone and menthol are favored by the horticultural and pharmaceutical industries, while pulegone and its metabolite menthofuran have toxic effects. Interestingly, the peppermint EO profile is used to identify genuine peppermint EOs during production and market evaluation. This finding may have important economic applications for the agricultural and pharmaceutical industries.

There were increases in the expression of key genes associated with menthol biosynthesis, including *Mfs*, *LS*, *L3oh*, *Ipd*, *Ipr, Mdeh*, and *Pr* in omeprazole-treated plants, compared with control plants, under normal and water stress conditions. The increase in the expression of these genes is usually associated with modification of the composition of major EOs in peppermint. Previous investigation on peppermint revealed that water stress may increase the expression of *Ipd*, *Ipr*, and *Mfs* and decrease the expression of *Mdeh* and *Pr* (only at 25% field capacity). Results of the present study were comparable, but the most important finding was the stimulatory effect of omeprazole on these genes and the enhanced composition of EOs. In the menthol pathway of peppermint), limonene is formed by cyclization of geranyl diphosphate using *LS*. Then trans-isopiperitenol is formed by the hydroxylation of limonene using *L3OH*. Trans-isopiperitenol goes through three reactions (using *Ipd*, *Ipr*, and *Ipi*) to form pulegone. Pulegone is either converted to menthone/isomenthone using *Pr* or converted to menthofuran. Menthone is converted to menthol using *Mdeh*. In the present study, there was upregulation in the expression of *LS, L3oh, r*, and *Mdeh* in omeprazole-treated plants, which suggests that the menthol pathway was activated. This upregulation resulted in the accumulation of menthol and menthone.

Treatment with omeprazole increased morphological parameters, including plant height, leaf number, leaf area, and plant and root dry weights, by increasing gas exchange, chlorophyll composition, proline accumulation, leaf midday water potential, and relative water content. The physiological improvements were associated with an increased EO ratio and enhanced composition of the EOs. The essential oils function as secondary metabolites and were upregulated by the increased expression of the specific menthol and pulegone genes *Mfs*, *LS*, *L3oh*, *Ipd*, *Ipr*, *Mdeh*, and *Pr*. These results provide strong evidence that omeprazole has water stress–ameliorating effects on peppermint achieved by activating different water stress adaptation mechanisms, such as osmotic adjustment, enhanced gas exchange, and upregulation of menthol/menthofuran biosynthesis genes.

5. Conclusion

This is the first report on the effect of omeprazole as a water stress ameliorant and as an elicitor of genes associated with menthol/menthofuran pathway biosynthesis. Peppermint plants that received higher doses of omeprazole showed increased plant height, leaf number, leaf area, and dry weights under normal and water stress conditions. They also showed increases in the amounts of chlorophyll and proline in the leaves and gas exchange parameters compared with the control plants. Increased activity of CAT and APX and reduced accumulation of H_2O_2 were found in omeprazole-treated plants as antioxidative mechanisms to tolerate water stress. Omeprazole-treated plants also showed increases in the EO ratio, improved EO composition, and higher ratios of menthol and menthone composition compared with the control plants. The changes in EO composition were associated with increased expression of menthol biosynthesis pathway genes. These findings indicate that omeprazole ameliorates water stress in peppermint by increasing vegetative and root growth as an adaptation mechanism, increasing chlorophyll composition and the photosynthetic rate, increasing gas exchange, reducing water loss by increasing leaf water potential and relative water content, enhancing the amount of proline, and modulating the gene expression of secondary metabolites.

Author contribution

Hosam O. Elansary designed and performed the study and wrote the manuscript. Tarek K. Zin El-Abedin participated with Hosam O. Elansary in writing the final version of the manuscript and reproduction of the results.

Acknowledgments

The author extends appreciation to the Deanship of Scientific Research at King Saud University for funding this work through research group No. RG-1440-12.

References

Al-Ghamdi, A.A., Elansary, H.O., 2018. Synergetic effects of 5-aminolevulinic acid and Ascophyllum nodosum seaweed extracts on Asparagus phenolics and stress related genes under saline irrigation. Plant Physiol. Biochem. 129, 273–284.
Argyrokastritis, I.G., Papastylianou, P.T., Alexandris, S., 2015. Leaf water potential and crop water stress Index variation for full and deficit irrigated cotton in mediterranean conditions. Agric. Agric. Sci. Procedia 4, 463–470.
Babak, B., Satoshi, I., Miki, F., Yasunari, F., Hironori, T., Yuriko, O., Kazuko, Y.S., Masatomo, K., Kazuo, S., 2013. Characterization of the promoter region of an arabidopsis gene for 9-cis-Epoxycarotenoid dioxygenase involved in dehydration-inducible transcription. DNA Res. 20, 315–324.
Bechtold, U., Field, B., 2018. Molecular mechanisms controlling plant growth during abiotic stress. J. Exp. Bot. 69, 2753–2758.
Chandra Rai, A., Singh, M., Shah, K., 2012. Effect of water withdrawal on formation of free radical, proline accumulation and activities of antioxidant enzymes in ZAT12-transformed transgenic tomato plants. Plant Physiol. Biochem. 61, 108–114.
Cirillo, V., Van Oosten, M.J., Izzo, M., Maggio, A., 2019. Omeprazole treatment elicits contrasting responses to salt stress in two basil genotypes. Ann. Appl. Biol. 1–10.
Dias, C.S., Araujo, L., Alves Chaves, J.A., DaMatta, F.M., Rodrigues, F.A., 2018. Water relation, leaf gas exchange and chlorophyll a fluorescence imaging of soybean leaves infected with Colletotrichum truncatum. Plant Physiol. Biochem. 127, 119–128.
Dietrich, D., 2018. Hydrotropism: how roots search for water. J. Exp. Bot. 69, 2759–2771.
Elansary, H.O., Salem, M.Z.M., 2015. Morphological and physiological responses and drought resistance enhancement of ornamental shrubs by trinexapac-ethyl application. Sci. Hortic. 189, 1–11.
Elansary, H.O., 2015. Basil morphological and physiological performance under trinexapac-ethyl foliar sprays and prolonged irrigation intervals. Acta Physiol. Plant. 37.
Elansary, H.O., 2017. Green roof Petunia, Ageratum, and Mentha responses to water stress, seaweeds, and trinexapac-ethyl treatments. Acta Physiol. Plant. 39.
Elansary, H.O., Ashmawy, N.A., 2013. Essential oils of mint between benefits and hazards. J. Essent. Oil Bearing Plants 16, 429–438.
Elansary, H.O., Skalicka-Wozniak, K., King, I.W., 2016a. Enhancing stress growth traits as well as phytochemical and antioxidant contents of Spiraea and Pittosporum under seaweed extract treatments. Plant Physiol. Biochem. 105, 310–320.
Elansary, H.O., Yessoufou, K., 2015. Growth regulators and mowing heights enhance the morphological and physiological performance of Seaspray turfgrass during drought conditions. Acta Physiol. Plant. 37.
Elansary, H.O., Yessoufou, K., Abdel-Hamid, A.M.E., El-Esawi, M.A., Ali, H.M., Elshikh, M.S., 2017. Seaweed extracts enhance salam turfgrass performance during prolonged irrigation intervals and saline shock. Front. Plant Sci. 8.
Elansary, H.O., Yessoufou, K., Shokralla, S., Mahmoud, E.A., Skaicka-Wozniak, K., 2016b. Enhancing mint and basil oil composition and antibacterial activity using seaweed extracts. Ind. Crops Prod. 92, 50–56.

Fard, E.M., Bakhshi, B., Keshavarznia, R., Nikpay, N., Shahbazi, M., Salekdeh, G.H., 2017. Drought responsive microRNAs in two barley cultivars differing in their level of sensitivity to drought stress. Plant Physiol. Biochem. 118, 121–129.
Figueroa-Perez, M.G., Rocha-Guzman, N.E., Perez-Ramirez, I.F., Mercado-Silva, E., Reynoso-Camacho, R., 2014. Metabolite profile, antioxidant capacity, and inhibition of digestive enzymes in infusions of peppermint (Mentha piperita) grown under drought stress. J. Agric. Food Chem. 62, 12027–12033.
Grigoleit, H.G., Grigoleit, P., 2005. Pharmacology and preclinical pharmacokinetics of peppermint oil. Phytomedicine 12, 612–616.
Iscan, G., Kirimer, N., Kurkcuoglu, M., Baser, K.H.C., Demirci, F., 2002. Antimicrobial screening of Mentha piperita essential oils. J. Agric. Food Chem. 50, 3943–3946.
Maathuis, F.J.M., 2013. Sodium in plants: perception, signalling, and regulation of sodium fluxes. J. Exp. Bot. 65, 849–858.
Moran, R., Porath, D., 1980. Chlorophyll determination in intact tissues using < em > N,N < /em > -Dimethylformamide. Plant Physiol. 65, 478–479.
Osakabe, Y., Osakabe, K., Shinozaki, K., Tran, L.-S.P., 2014. Response of plants to water stress. Front. Plant Sci. 5 86-86.
Rahimi, Y., Taleei, A., Ranjbar, M., 2017. Changes in the expression of key genes involved in the biosynthesis of menthol and menthofuran in Mentha piperita L. under drought stress. Acta Physiol. Plant. 39.
Rouphael, Y., Raimondi, G., Lucini, L., Carillo, P., Kyriacou, M.C., Colla, G., Cirillo, V., Pannico, A., El-Nakhel, C., De Pascale, S., 2018. Physiological and metabolic responses triggered by omeprazole improve tomato plant tolerance to NaCl stress. Front. Plant Sci. 9.
Salvi, L., Brunetti, C., Cataldo, E., Niccolai, A., Centritto, M., Ferrini, F., Mattii, G.B., 2019. Effects of Ascophyllum nodosum extract on Vitis vinifera: consequences on plant physiology, grape quality and secondary metabolism. Plant Physiol. Biochem. 13-9, 21–32.
Sheikh Mohammadi, M.H., Etemadi, N., Arab, M.M., Aalifar, M., Arab, M., Pessarakli, M., 2017. Molecular and physiological responses of Iranian Perennial ryegrass as affected by Trinexapac ethyl, Paclobutrazol and Abscisic acid under drought stress. Plant Physiol. Biochem. 111, 129–143.
Shin, J.M., Sachs, G., 2008. Pharmacology of proton pump inhibitors. Curr. Gastroenterol. Rep. 10, 528–534.
Stanton, K.M., Mickelbart, M.V., 2014. Maintenance of water uptake and reduced water loss contribute to water stress tolerance of Spiraea alba Du Roi and Spiraea tomentosa L. Hortic. Res. Engl. 1.
Van Oosten, M.J., Silletti, S., Guida, G., Cirillo, V., Di Stasio, E., Carillo, P., Woodrow, P., Maggio, A., Raimondi, G., 2017. A Benzimidazole Proton Pump Inhibitor Increases Growth and Tolerance to Salt Stress in Tomato, vol. 8.
Velázquez-Márquez, S., Conde-Martínez, V., Trejo, C., Delgado-Alvarado, A., Carballo, A., Suárez, R., Mascorro, J.O., Trujillo, A.R., 2015. Effects of water deficit on radicle apex elongation and solute accumulation in Zea mays L. Plant Physiol. Biochem. 96, 29–37.
Wang, P.C., Zhao, Y., Li, Z.P., Hsu, C.C., Liu, X., Fu, L.W., Hou, Y.J., Du, Y., Xie, S.J., Zhang, C.G., Gao, J.H., Cao, M.J., Huang, X.S., Zhu, Y.F., Tang, K., Wang, X.G., Tao, W.A., Xiong, Y., Zhu, J.K., 2018. Reciprocal regulation of the TOR kinase and ABA receptor balances plant growth and stress response. Mol. Cell 69, 100.
Zade, N.S.E., Sadeghi, A., Moradi, P., 2019. Streptomyces strains alleviate water stress and increase peppermint (Mentha piperita) yield and essential oils. Plant Soil 434, 441–452.
Zhong, C., Jian, S.-F., Huang, J., Jin, Q.-Y., Cao, X.-C., 2019. Trade-off of within-leaf nitrogen allocation between photosynthetic nitrogen-use efficiency and water deficit stress acclimation in rice (Oryza sativa L.). Plant Physiol. Biochem. 135, 41–50.