Journal of Horticultural Research 2021, vol. 29(1): 9–22
DOI: 10.2478/johr-2021-0003

CHITOSAN IMPROVES MORPHOLOGICAL AND PHYSIOLOGICAL ATTRIBUTES OF GRAPEVINES UNDER DEFICIT IRRIGATION CONDITIONS

Hoda Ali KHALIL[1]*, Rasha M. BADR ELDIN[2]
[1]Department of Pomology
[2]Department of Soil and Water Sciences
Faculty of Agriculture (EL-Shatby), Alexandria University, Alexandria, Egypt

Received: July 2020; Accepted: February 2021

ABSTRACT

This study aimed to estimate the morphological and physiological effects of chitosan foliar spray and/or three irrigation levels of 100%, 60%, and 40% of field capacity on grapevines grown in plastic containers to simulate water shortage conditions. The results showed that water irrigation deficit significantly reduced leaf area, trunk cross-sectional area, plant dry weight, root dry weight, relative chlorophyll content, leaf total carbohydrates, catalase activity, leaf midday water potential (ψ), relative water content (RWC), and crop evapotranspiration (ET_c), but increased the proline content. Under well-watered condition, foliar-applied chitosan, in particular, 5 and 10 $g \cdot dm^{-3}$ increased plant growth and biomass production compared with untreated plants. Also, chitosan sprays during deficit irrigation conditions significantly improved plant tolerance to water deficit by enhancing the morphological and physiological parameters of grapevines. The results of this work suggest the opportunity to grow grapevines under deficit irrigation conditions using chitosan foliar spray. Increased plant biomass and root weight, and the positive impacts of chitosan as antitranspirant on increased ψ, RWC, and decreased ET_c play the main role in drought stress avoidance mechanisms in grapevines raised under moderate deficit irrigation conditions.

Key words: drought, evapotranspiration, proline, relative water content, leaf water potential

INTRODUCTION

Water deficit is considered the most common abiotic factor that limits plant growth and productivity of fruit trees in many areas of the world, especially in arid and semi-arid regions. Approximately one-third of the cultivated area of the world suffers from chronically insufficient supplies of water (Massacci et al. 2008). It has been reported that 70% of the total water consumption in the world occurs in the agricultural sector (FAO 2015), and the need for water is increasing in other sectors such as domestic consumption and industry. This will add more pressure to water availability for horticulture production. A periodical reduction in the yields of rain-fed crops due to drought and the continuing global climate change may increase the severity of the problem (IPCC 2013). Therefore, it is important to use wise irrigation such as deficit irrigation and to improve drought tolerance in horticultural crops. Water deficit can stimulate production of reactive oxygen species (ROS) in plants, which can cause damage to plants by oxidation of DNA, lipids, and proteins. Plants grown under drought stress are capable of introducing morphological modifications to cope with water scarcity, including reduction of leaf area, shoot elongation, and shoot-to-root ratio, as well as increasing root growth (Toscano et al. 2014; Khalil & El-Ansary 2015, 2020).

*Corresponding author:
e-mail: hodaagri@hotmail.com; hoda.khalil@alexu.edu.eg

Some plants can modify their structures that help them survive under drought conditions, such as the thickness of the upper and lower epidermis (Ennajeh et al. 2010) and thick cuticle (De Micco & Aronne 2012). They also established physiological strategies involved in leaf water potential maintenance, stomatal conductance control, and osmotic adjustment during drought (Karimi et al. 2012; Khalil 2015). It is widely known that the deliberate withholding of irrigation water by the deficit irrigation technique and the use of drought-adapted trees to cope with water shortage can be effective management strategies for controlling crop water use. Deficit irrigation is a useful production technique for fruit crops that can control their growth and improve fruit quality (Khalil & El-Ansary 2015). This is done by adding water at a rate or amount lower than evapotranspiration. Deficit irrigation at any growth stage could pose negative impacts on physiological, morphological, and biochemical processes in plants, which could include decreased stem elongation, leaf area, root size, and depth, and changes in stomatal formation and plant-water relations with reduced water use efficiency and crop production (Li et al. 2009).

Elicitation is an effective strategy to induce drought tolerance and physiological changes in plants (Baenas et al. 2014). Chitosan is one of the most common elicitors inducing cell defense reactions in plants (Shibuya & Minami 2001). Chitosan is a natural polysaccharide derived from a low-acetyl form of chitin, mainly composed of glucosamine and N-acetyl glucosamine and is commercially produced from crab shells, shrimp shells, lobster and squid, and filamentous fungi (Kumaresapillai et al. 2011). Previous studies have shown that chitosan can stimulate plant growth indices (Farouk et al. 2008), increase yields, induce plant resistance to bacterial, fungal, and viral infections, and reduce transpiration (Karimi et al. 2012). Moreover, chitosan-treated plants may be less susceptible to stress induced by adverse conditions, such as low or high temperature, salinity, and drought (Lizárraga-Paulín et al. 2011; Pongprayoon et al. 2013). Chitosan has decreased transpiration in pepper plants, and hence, reduction of water loss while maintaining biomass and yield. This result suggests that chitosan can effectively counteract transpiration, protecting water in drought conditions (Bittelli et al. 2001). Foliar application of chitosan may induce stomatal closure. However, the mechanisms of action of chitosan on plant growth remain unclear. Yang et al. (2009) reported that pretreatment of leaves of apple seedlings with chitosan solution (100 $mg \cdot dm^{-3}$) prior to drought stress significantly enhances leaf membrane stability and antioxidant enzyme activities. Górnik et al. (2008) found that chitosan significantly enhanced rooting of the cuttings, increased the number of new canes and their length as well as the number of nodes and chlorophyll content in the leaves developed on grapevines grown under drought stress conditions. El-Kenawy (2017) described morphological and physiological responses, such as the increase in shoot length, leaf surface area, and total chlorophyll content in grapevines subjected to chitosan. However, studies on the interaction effects of foliar application of chitosan and drought stress on grapevine growth are still lacking.

Grapevine (*Vitis vinifera* L.) is one of the most ancient and widely cultivated fruit trees grown in a temperate and semi-arid climate, where it may be afforded consecutive cycles of water deficit and rewatering either through rainfall or irrigation. Vines are considered drought-tolerant plants, characterized by diverse stomatal behaviors and hydraulics, depending on the cultivar and can be classified based on their water potential as tolerant to water limitations (Martorell et al. 2015). Grapevine is able to perform physiological drought avoidance mechanisms, such as effective stomatal management of transpiration and xylem embolus, and the potential for osmotic adjustment. Therefore, the aim of this study was to determine the morphological and physiological response of young grapevines to various doses of foliar applied chitosan under deficit irrigation conditions. The morphological indices chosen were leaf area, trunk cross-sectional area, plant dry weight, and root dry weight. Physiological stress-associated characteristics are leaf midday water potential (ψ), relative water content (RWC), crop evapotranspiration rate (ET_c), chlorophyll content, proline content, leaf total carbohydrates, and catalase activity.

MATERIAL AND METHODS

Plant materials and growing conditions

Experiments were conducted in the glasshouse of the Soil Science Department, Alexandria University, Egypt. Own-rooted one-year-old ‘Crimson’ grapes (*Vitis vinifera* L.) obtained from the nurseries of the Faculty of Agriculture farm were singly transplanted into 5 liter pots. The average high and low temperatures recorded during the experimental period were 33.4 and 20.5 °C day/night and the photoperiod varied from 9.3 to 10.8 hours. Relative humidity of air ranged from 64 to 69%. The growing substrate was sandy soil that contained 0.4% organic matter, 10 $meq \cdot dm^{-3}$ Na, 3 $meq \cdot dm^{-3}$ K, 9.4 $meq \cdot dm^{-3}$ Ca, 2.8 $meq \cdot dm^{-3}$ Mg, and a pH of 7.35 according to a soil test performed. Soil field capacity and wilting point were 0.1 and 0.03 $gm^3 \cdot gm^{-3}$, respectively. The soil field capacity and wilting point were determined directly at the four soil samples at 0.1 and 15 bar, respectively, using the pressure plate apparatus as described by Israelsen and Hansen (1962). Before the commencement of the treatments, the plants were irrigated twice a week with tap water. In addition, Ezzogreen Compy blue fertilizer (4% iron, 4% manganese, 4% zinc, 0.5% copper, and 0.5% magnesium per dm^3) was added to each plant as a foliar spray at a rate of 0.75 mL per dm^3 in mid-May. The plants were irrigated for full field capacity prior to the initiation of experimental treatments for 2 months. During the period of adaptation, all plants seemed vigorous, healthy, and well established. The experimental treatments were begun on the July 30 and August 8, and were terminated after 120 and 112 days, in the 2017 and 2018 seasons, respectively.

Treatments

Grapevines were subjected to the three irrigation levels of 100%, 60%, and 40% of field capacity for four months, along with 3 doses of weekly foliar applications of chitosan (low molecular weight, 40 kDa, from crab shells) at rates of 1, 5, and 10 $g \cdot dm^{-3}$. Chitosan obtained from the commercial product Chitosan Powder, manufactured by Chitosan Egypt. Chitosan was dissolved in 5% acetic acid and diluted with distilled water to the required concentrations. 100 mL of this solution per plant was sprayed at the dew point using a hand sprayer. In untreated control plants, chitosan was replaced with an equivalent volume of distilled water. Irrigation levels assumed well-irrigated conditions (100%), moderate deficit irrigation (60%), and severe deficit irrigation (40%). During the time of the treatment period, the amount of water required to attain the pot field capacity exhibited the consumption of water during the prior days. The irrigation level reached 100%, 60%, and 40% of the soil field capacity by adding 800, 480, and 320 mL of water for each pot, respectively, at the beginning of the experiment. Thereafter, the volume of water added to each pot was estimated by weighting the pots of each treatment periodically at weekly intervals. The plants were distributed on four blocks, and each treatment was represented by four replicates with a total number of 192 plants in each experimental season. The plant pots were placed on the tables and spaced 50 cm between plant pots and 50 cm between the rows.

Measurements

At the termination of the experiment, the data were collected in November 2017 and 2018 after four months of deficit irrigation. Relative chlorophyll content was determined according to Yadava (1986), using a Minolta SPAD chlorophyll meter (SPAD-502 plus, Konica Minolta Sensing, Japan). The field portable, hand device measured the relative chlorophyll content using dual-wavelength optical absorbance (620 nm and 940 nm wavelength). The results were expressed as SPAD units. The measurements were performed on fully expanded mature leaves with an area of 30–115 cm^2, in the middle of the canopy. The trunk circumference of each plant was measured using a Vernier caliper, and the trunk cross-sectional area (cm^2) was calculated. The plants were then lifted from the pots, and the leaves, stems, and roots of each plant were separated, washed with tap water, and then with distilled water. The total leaf area (cm^2 per plant) was measured using a planimeter. The leaf, stem, and root tissues of each plant were then oven-dried at 70 °C until reaching a constant weight and plant dry weight (g per plant) and root dry weight (g per plant) of each plant was recorded.

To determine the daily crop evapotranspiration rate (ET_c), four replicates of each treatment were irrigated with enough water and left to dry for 2 hours, then each replicate was weighed, re-irrigated every 24 hours and the daily differences in weight expressed the daily ET_c. At the end of the experiment, estimations of leaf water potential (ψ) and relative water content (RWC) were performed at midday using fully expanded leaves from the middle part of the shoots of these plants. RWC was measured in fully opened leaves. The leaves were cleaned, and their fresh weights (FW) were determined. The turgid weight (TW) of the leaves was determined after floating in distilled water in a covered Petri dish for 24 hours at 4 °C. Thereafter, the leaves were oven dried at 70 °C to a constant weight and their dry weights (DW) were determined. RWC was calculated using the following formula (Smart & Bingham 1974): RWC (%) = (FW − DW)/(TW − DW) × 100 was used for RWC calculation. The midday ψ estimations were done one day before irrigation. Five leaves were taken from the middle part of the shoot and their ψ was measured immediately using a pressure chamber (Model PMS 1505D-EXP, USA). At the end of the experiment, total carbohydrates, proline content, and catalase activity were determined in samples of dry materials taken from the entire leaves of the plant in each replicate. Total carbohydrates (TC) were determined in 0.5 g of dry materials of the leaves, according to the method of Nelson-Somogyi as described by Thimmaiah (2004). Leaf proline content was determined spectrophotometrically at 520 nm according to the methodology of Bates et al. (1973). This was done as follows: 0.1 g from the dry ground leaf materials was homogenized with 10 mL aqueous sulfosalicylic acid (3%) and then filtered, and 2 mL of the filtrate stands to react with 2 mL glacial acid and 2 mL acid-ninhydrin for 1 hours at 100 °C. The reaction was terminated in an ice bath. The reaction mixture was extracted with 4 mL toluene and mixed for 15–20 seconds. The chromophore containing toluene was aspirated from the aqueous phase and the absorbance was read. Catalase (CAT) enzyme activity was determined in frozen leaf samples according to Kar and Mishra (1976), analyzed according to KMnO4 titration method and expressed as µmol H_2O_2 reduced per g FW per min (µmol $H_2O_2 \cdot g\ FW^{-1} \cdot min^{-1}$).

Statistical analysis

Statistical analysis was performed for a factorial experiment with a completely randomized block design with four biological replicates. The factors were three irrigation levels (100%, 60%, and 40% F.C.) and four chitosan treatments (1, 5 and 10 $g \cdot dm^{-3}$, and control). Statistical analysis was done by ANOVA, F-test, and LSD procedures available within the SAS software package (version 9.13, 2008).

RESULTS

Leaf area, trunk cross-sectional area, and dry weights

The effect of irrigation with different soil moisture levels 100%, 60%, and 40% of the field capacity as well as the effect of chitosan sprays was significant for leaf area, trunk cross-sectional area, plant dry weight, and root dry weight (Fig. 1). A significant interaction was found between irrigation levels and chitosan doses on leaf area, trunk cross-sectional area, plant dry weight, and root dry weight.

Decreasing the irrigation level from 100% to 40% resulted in a significant reduction in leaf area, trunk cross-sectional area, plant dry weight, and root dry weight (Fig. 1). This reduction was evident during both experimental seasons. For example, in the 2017, the leaf area reduced from 89.9 to 59.1 cm^2 per plant when the irrigation level decreased from 100% to 40%, and the trunk cross-sectional area reduced from 11.9 to 8.4 cm^2. Sprays with chitosan of 1, 5, and 10 $g \cdot dm^{-3}$ significantly increased the leaf area, trunk cross-sectional area, plant dry weight, and root dry weight compared with the control plants in both experimental seasons. Considering the interaction effect between drought and chitosan treatments in Figure 1, it was noticed that under deficit irrigation (60% and 40% irrigation level), the highest leaf area, trunk cross-sectional area, plant dry weight, and root dry weight values were obtained with 5 and 10 $g \cdot dm^{-3}$ chitosan.

For example, in the 2017 season, at the 40% irrigation level × 10 g·dm^{-3} chitosan treatment, the trunk cross-sectional area increased from 7.5 (1 g·dm^{-3}) to 10.1 cm^2, and plant dry weight increased from 68.3 (1 g·dm^{-3}) to 91.9 g per plant. Significant differences were found in most morphological indices when the chitosan spray doses increased from 1 to 5 or 10 g·dm^{-3} in plants subjected to lower irrigation levels of 60% and 40%. However, in most cases, there were no significant differences between the chitosan sprays at 5 and 10 g·dm^{-3} (Fig. 1). Moreover, in both seasons, the results indicated that the highest leaf area, trunk cross-sectional area, plant dry weight, and root dry weight values obtained at spraying 5 and 10 g·dm^{-3} chitosan under 100% irrigation level, and the lowest values were obtained with control and spraying of 1 g·dm^{-3} chitosan under 40% irrigation level.

Chlorophyll content, proline content, leaf total carbohydrates, and catalase activity

Irrigation levels and chitosan spray had significant effects on the relative chlorophyll and proline contents, leaf total carbohydrates, and catalase activity (Fig. 2). Increasing irrigation deficiency decreased the relative chlorophyll content, leaf total carbohydrates, catalase activity, and increased the proline content. For example, in 2017, decreasing irrigation levels from 100% to 40% significantly reduced the relative chlorophyll content from 27.4 to 17.9 SPAD; leaf total carbohydrates from 15.4 to 11.1 mg per 100 g DW; and catalase activity from 12.9 to 9.1 µmol H_2O_2 per g FW per min, while increased the proline content from 58.9 to 69.3 mg per 100 g DW. Chitosan spray significantly increased the relative chlorophyll content, leaf total carbohydrates, leaf catalase activity, and decreased leaf proline content in both seasons. In the leaf tissues of grapevines sprayed with 10 g·dm^{-3} chitosan, the proline content values reached as much as 54.5 and 54.3 mg per 100 g DW in 2017 and 2018, respectively (Fig. 2). As for the interaction effect between irrigation levels and chitosan treatments, the data of the present study showed that deficit irrigation coupled with chitosan sprays at 5 and 10 g·dm^{-3} significantly increased the relative chlorophyll content, leaf total carbohydrates, and catalase activity, but reduced the proline content compared to 1 g·dm^{-3} and control. For example, in the 2018 season, chitosan-treated plants with 10 g·dm^{-3} chitosan and water to 40% irrigation level showed a significant increase in the relative chlorophyll content from 16.1 (1 g·dm^{-3}) to 21.1 SPAD. This increase in the relative chlorophyll content coincided with an increase in total leaf carbohydrates from 10.5 to 15.1 mg per 100 g DW, and catalase activity from 6.9 to 12.1 µmol H_2O_2 per g FW. However, the proline content was reduced from 77.9 (1 g·dm^{-3}) to 56.2 mg per 100 g DW. With an increased concentration of chitosan spray from 1 to 5 or 10 g · dm^{-3}, the relative chlorophyll content, leaf total carbohydrates and catalase activity increased significantly, in most cases there was a decrease in proline content below 60% and 40% irrigation levels. The 5 and 10 g·dm^{-3} chitosan-treated plants did not show significant differences ($p \leq 0.05$) under control, moderate, and severe deficit irrigation conditions in most cases (Fig. 2).

Leaf midday water potential, relative water content, and crop evapotranspiration rate

At the end of the experiment, irrigation levels, chitosan sprays, and their interactions had significant effects on leaf midday water potential (ψ), relative water content (RWC), and daily crop evapotranspiration rate (ET_c) (Figs. 3–5). Decreasing irrigation levels from 100% to 40% in control plants significantly reduced ψ, RWC, and ET_c. Chitosan sprays increased ψ, RWC, and decreased ET_c under 100%, 60% and 40% irrigation levels compared to the control plants in both experimental seasons. Chitosan sprays at 5 and 10 g·dm^{-3} significantly increased ψ, RWC, and decreased ET_c compared to 1 g·dm^{-3} under 60% and 40% irrigation levels. Differences were also significant for ψ in the second season and for RWC in both seasons with the 10 g·dm^{-3} chitosan treatment (Figs. 3–5). In the first season, increasing chitosan doses from 1 to 5 g·dm^{-3} increased RWC, but these increases were not significant at the 100% irrigation level.

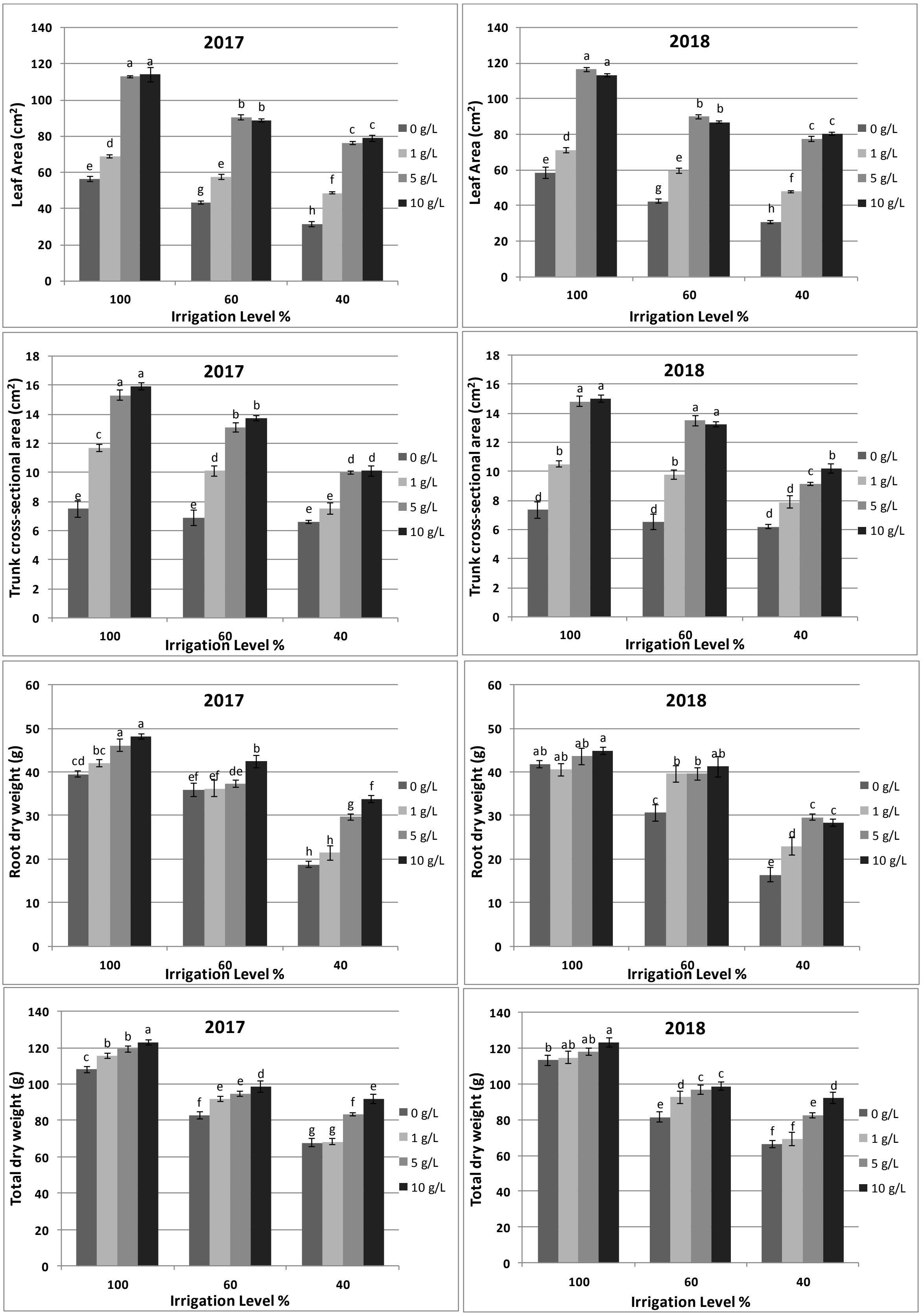


Figure 1. Effect of foliar application of chitosan and irrigation levels on leaf area, trunk cross-sectional area (TCA), total dry weight and root dry weight of grapevines ‘Crimson’. Means followed by different lowercase letters indicate significant differences between treatments based on LSD test (p = 0.05)

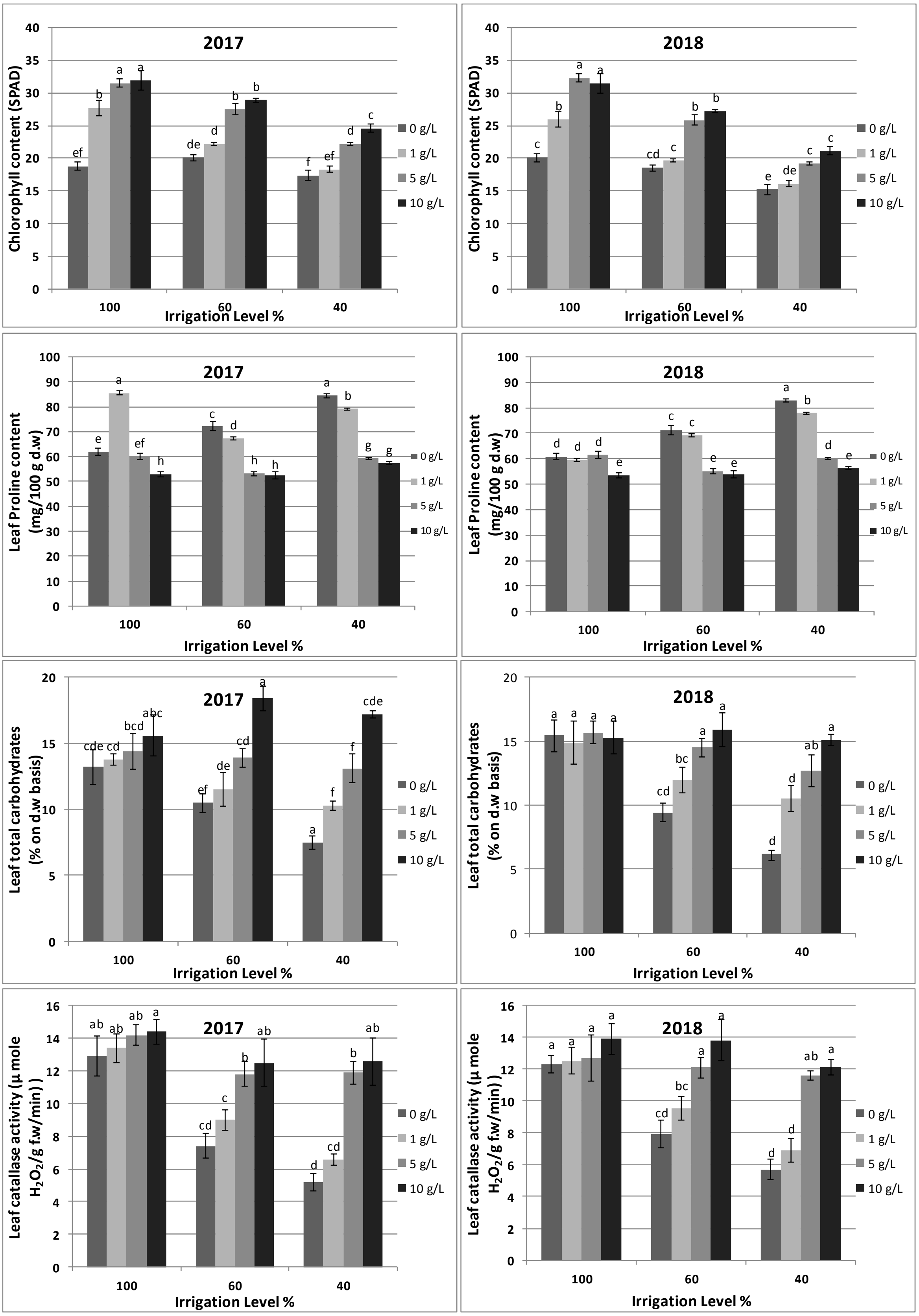


Figure 2. Effect of foliar application of chitosan and irrigation levels on relative chlorophyll content, proline content, leaf total carbohydrates, and leaf catalase activity. Means followed by different lowercase letters indicate significant differences between treatments based on LSD test (p = 0.05)

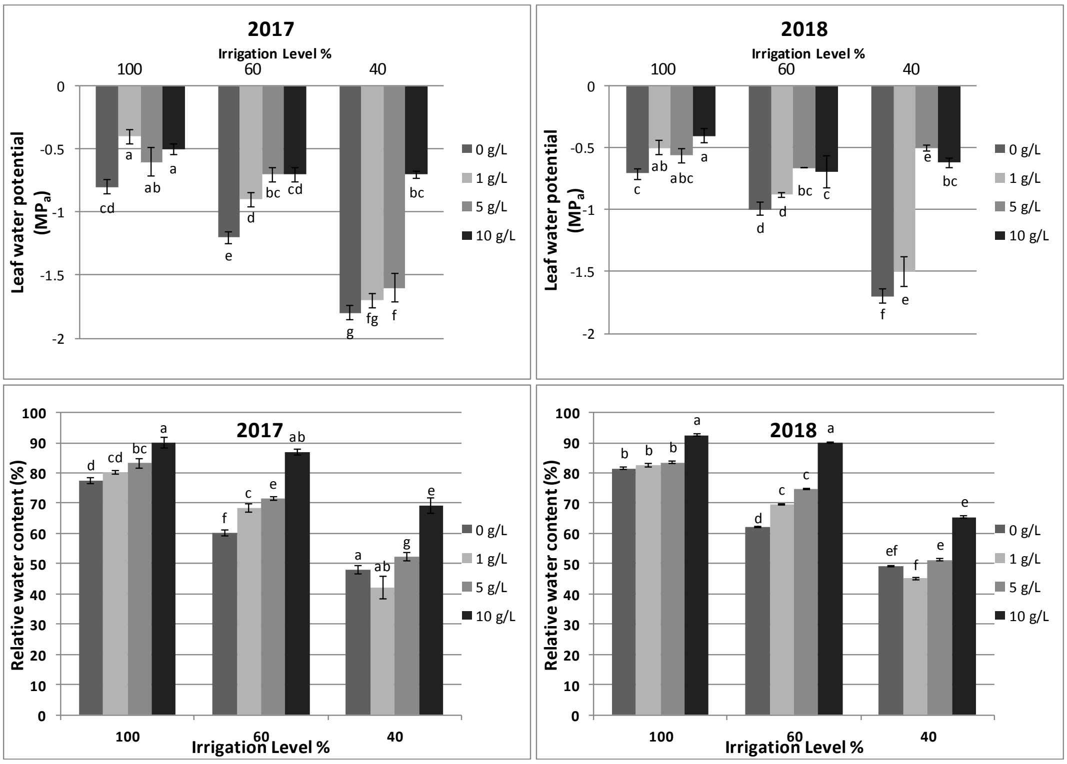
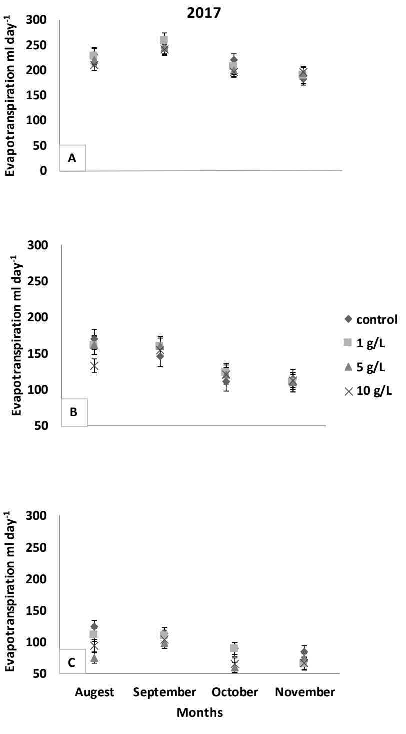


Figure 3. Effect of foliar application of chitosan and irrigation levels on leaf water potential and relative water content. Means followed by different lowercase letters indicate significant differences between treatments based on LSD test (p = 0.05)



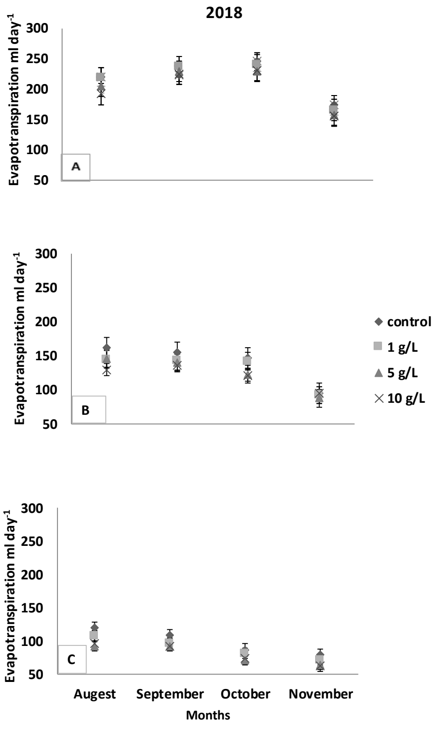


Figure 4. The effect of irrigation levels and chitosan treatments on the evapotranspiration (mL per day) during four months of drought and chitosan treatments of grapevines in 2017. Values are mean ± S.E. (n = 16). Irrigation levels: A = 100% of field capacity, B = 60% of field capacity, C = 40% of field capacity

Figure 5. The effect of irrigation levels and chitosan treatments on the evapotranspiration (mL per day) during four months of drought and chitosan treatments of grapevines in 2018. Values are mean ± S.E. (n = 16). Irrigation levels: A = 100% of field capacity, B = 60% of field capacity, C = 40% of field capacity

DISCUSSION

The results of the present study show that irrigation levels alone and jointly with chitosan application had significant effects on most studied morphological indices (Fig. 1). There was a reduction in leaf area following decreased irrigation levels in control plants, which is in line with the previous investigations on grapes (Gómez-del-Campo et al. 2002; Buesa et al. 2017). The reduction in leaf area is considered as one of the adaptation mechanisms in plants to avoid drought stress conditions by minimizing the evapotranspiration rate and reducing water consumption (Toscano et al. 2014). The reduction in plant trunk cross-sectional area following decreasing irrigation levels is expected as a result of drought stress and lessen growth rate (Boland et al. 2000) and is also a morphological adaptation of plants to water stress (Bañon et al. 2006). A reduction in grapevine dry weight was found in this investigation following decreasing irrigation levels. This result has been shown in previous studies on the grapevines (Tehrani et al. 2016; Conesa et al. 2016). In contrast to our results, increased root dry weight under moderate deficit irrigation is considered as a defense mechanism to cope with drought conditions and induce water uptake in some plants (Toscano et al. 2014) and could be attributed to the accumulation of photoassimilates in the roots more than in the shoots. The redistribution of dry matter in favor of the root at the expense of the shoot is considered the plant’s demand to keep enough surface leaf area under drought stress (Conesa et al. 2016). Drought stress reduced the plant vegetative growth, which appears to be the result of disrupted plant water relations in specific turgor potential (Hussain et al. 2009). The application of chitosan at 1, 5, and 10 $g \cdot dm^{-3}$ improved all the growth indices under well-watered and drought conditions and resulted in a significant increase in vegetative growth features compared to the untreated control plants. Malekpoor et al. (2016) found that foliar-applied chitosan increased plant growth (common bean and basil) under stressed or non-stressed conditions. Moreover, Ait Barka et al. (2004) reported that chitogel, a derivative of chitosan, improved the vegetative growth of grapevine plantlets. The increase in total dry weight due to chitosan application may be due to its effects in stimulating the photosynthetic process (Khan et al. 2002). Moreover, the stimulating effect of chitosan on plant growth under drought conditions might be explained by increasing nutrient and water uptake by adjusting cell osmotic pressure and reducing free radicals by stimulating antioxidant activity (Guan et al. 2009). Physiological determinations of grapevine leaves responding to drought have revealed that chlorophyll content decreased, which in turn inhibited the photosynthetic activity. Similar to the results of this study, the reduction in chlorophyll content (SPAD values) has been reported in grapevines in response to drought stress (Haider et al. 2017). This could be due to the destruction of the pigment-protein complexes, which defend the photosynthetic machinery (Lai et al. 2007), or to a reduction of specific enzymes such as HemA glutamyl-tRNA reductase 1 and magnesium chelatase H subunit, which are important for the construction of photosynthetic pigments (Murkute et al. 2006). The increase in chlorophyll content following chitosan application supports the previous suggestion that chitosan enhances the photosynthesis process by increasing photosynthetic pigments (Dzung et al. 2011). The proline level in grapevines increased substantially as drought stress intensified. Proline accumulation is considered as one of the initial responses of many plants to drought stress. It is well known that proline accumulation reduces the water potential of plant tissues, particularly in leaves, thereby enabling them to restrict water loss and/or continue to absorb water from soil under drought stress conditions. In contrast, foliar spraying of chitosan in both seasons showed a significant decrease in the proline content under normal or stressed conditions. The effect of chitosan on the reduction of the proline content was at least partly due to its role in reducing the water potential of plant tissues. The reduction in total carbohydrate content induced by water deficit treatments may be due to their inhibitory effect on photosynthetic pigment concentrations (Fig. 2) as well as the decrease in photosynthetic rate (Jie et al. 2010).

Moreover, under drought conditions, the breakdown of polysaccharides caused an accumulation of osmolytes such as soluble sugars, which helped the plants to maintain the cell turgor (Nazarli et al. 2011), and this is considered as an adaptive mechanism to drought stress conditions. The effects of chitosan, especially at 10 $g\cdot dm^{-3}$, on increasing total carbohydrate content were confirmed by Farouk et al. (2008) on cucumber. The influence of chitosan on alleviating drought stress effects on photosynthetic pigments leads to stimulate photosynthetic activity and carbohydrate accumulation (Farouk et al. 2008).

In the current study, substantially lower CAT levels were detected in plants grown under drought stress than in those in well-watered conditions. These results are in agreement with the earlier reports indicating that drought stress decreased CAT activity in the leaves of pomegranate seedlings (Khalil 2015). Chitosan application to grapevines alleviated some of the negative impacts of drought and increased CAT activity (Fig. 2). This result supported the results of Guan et al. (2009), who indicated that the application of chitosan on maize plants significantly decreased lipid peroxidation, due to stimulation of some antioxidant enzymes.

The gradual reduction in ψ values following deficit water treatments found in this research is in agreement with those previously reported by Conesa et al. (2016) and Buesa et al. (2017) working on grapevines. A short period of mild water deficit may promote plants to reduce the leaf water potential substantially (Pérez-Pastor et al. 2014). Decreased leaf water potential acts as a hydraulic signal that triggers reduced leaf area expansion and partial closure of stomata (Shahnazari et al. 2007). Several reports on grapevines revealed that drought stress negatively affects leaf water potential due to stomatal closure and a decrease in stomatal conductance (Conesa et al. 2016; Tehrani et al. 2016). The reduction in stomatal conductance was strongly associated with a reduction in photosynthetic activity and leaf area, which are well known in plants grown under drought stress conditions (Medrano et al. 2002).

In this study, deficit irrigation treatments decreased RWC, while significantly higher RWC values were observed in chitosan-sprayed plants. The decrease in RWC values following water deficit treatments found in this study is consistent with those reported by Abdi et al. (2016). They agreed that with increasing moisture stress in the growing medium, a noticeable decrease in the RWC of the leaves was noticed. The results presented here showed that prolonged water deficit causes a decrease in RWC values, and these reductions may be lessened when spraying chitosan. Undoubtedly, this might be due to the role of chitosan in reducing plant transpiration. Bittelli et al. (2001) suggested that chitosan could be used as an effective anti-transpirant to reduce irrigation water use. The decrease in crop evapotranspiration (ET_c) following increased drought stress conditions of less irrigation water in fruit trees have been reported in several studies (Çamoğlu 2013). The decrease in the ET_c values coupled with an increase in leaf water potential values in most treatments was recorded. The results generally indicated that the ET_c obviously decreased as a result of chitosan application. The magnitude of this reduction at 40% field capacity reached as much as 29.3% and 23.9% for the 5 and 10 $g\cdot dm^{-3}$ chitosan treatments, respectively. The influence of chitosan in reducing ET_c during drought stress conditions could be attributed to the induction of stomatal closure (Bittelli et al. 2001).

CONCLUSION

Deficit irrigation, as expected, markedly reduced leaf area, trunk cross-sectional area, plant dry weight, and root dry weight, which proved biomass production and distribution. Water deficit decreased leaf midday water potential (ψ), relative water content (RWC), and crop evapotranspiration rate (ET_c), causing changes in plant water status. Chitosan application under all irrigation levels increased leaf area, trunk cross-sectional area, plant dry weight, and root dry weight, relative chlorophyll content, leaf total carbohydrates, catalase activity, and relative water content, but reduced the proline content. Reduction of plant biomass under drought stress and the positive impacts of chitosan as anti-transpiring agent play the main role in drought stress avoidance mechanisms in potted-vines grown under moderate and severe deficit irrigation conditions.

Acknowledgments

The authors would like to thank Project: Collective action and agricultural productivity in Egypt’s new lands (Nasr canal area) and STDF-IRD Joint Innovation Projects Fund No. 4652, for providing pressure chamber instrument (Model PMS 1505D-EXP USA) for the measurement of leaf water potential for this research (Project Egyptian PI: Dr. Diaa O. El-Ansary, Precision Agricultural Laboratory, Department of Pomology, Faculty of Agriculture, El-Shatby, University of Alexandria, Alexandria, Egypt). The authors would like to express their deepest gratitude and appreciation to Prof. Dr. Sherif M. Marei (s_marei@outlook.com), Professor of soil and water science, Faculty of Agriculture, University of Alexandria, Egypt, for editing the manuscript.

REFERENCES

Abdi S., Abbaspur N., Avestan S., Barker A.V. 2016. Physiological responses of two grapevine (*Vitis vinifera* L.) cultivars to Cycocel™ treatment during drought. Journal of Horticultural Science and Biotechnology 91(3): 211–219. DOI: 10.1080/14620316.2015.1123405.

Ait Barka E., Eullaffroy P., Clément C., Vernet G. 2004. Chitosan improves development, and protects *Vitis vinifera* L. against *Botrytis cinerea*. Plant Cell Reports 22: 608–614. DOI: 10.1007/s00299-003-0733-3.

Baenas N., García-Viguera C., Moreno D.A. 2014. Elicitation: a tool for enriching the bioactive composition of foods. Molecules 19: 13541–13563. DOI: 10.3390/molecules190913541.

Bañon S., Ochoa J., Franco J.A., Alarcón J.J., Sánchez-Blanco M.J. 2006. Hardening of oleander seedlings by deficit irrigation and low air humidity. Environmental and Experimental Botany 56: 36–43. DOI: 10.1016/j.envexpbot.2004.12.004.

Bates L.S., Waldren R.P., Teare I.D. 1973. Rapid determination of free proline for water-stress studies. Plant and Soil 39: 205–207. DOI: 10.1007/bf00018060.

Bittelli M., Flury M., Campbell G.S., Nichols E.J. 2001. Reduction of transpiration through foliar application of chitosan. Agricultural and Forest Meteorology 107: 167–175. DOI: 10.1016/s0168-1923(00)00242-2.

Boland A.M., Jerie P.H., Mitchell P.D., Goodwin I., Connor D.J. 2000. Long-term effects of restricted root volume and regulated deficit irrigation on peach: II. Productivity and water use. Journal of the American Society for Horticultural Science 125: 143–148. DOI: 10.21273/jashs.125.1.143.

Buesa I., Pérez D., Castel J., Intrigliolo D.S., Castel J.R. 2017. Effect of deficit irrigation on vine performance and grape composition of *Vitis vinifera* L. cv. Muscat of Alexandria. Australian Journal of Grape and Wine Research 23: 251–259. DOI: 10.1111/ajgw.12280.

Çamoğlu G. 2013. The effects of water stress on evapotranspiration and leaf temperatures of two olive (*Olea europaea* L.) cultivars. Zemdirbyste-Agriculture 100: 91–98. DOI: 10.13080/z-a.2013.100.012.

Conesa M.R., de la Rosa J.M., Domingo R., Bañon S., Pérez-Pastor A. 2016. Changes induced by water stress on water relations, stomatal behaviour and morphology of table grapes (cv. Crimson Seedless) grown in pots. Scientia Horticulturae 202: 9–16. DOI: 10.1016/j.scienta.2016.02.002.

De Micco V., Aronne G. 2012. Anatomy and lignin characterisation of twigs in the chaparral shrub *Rhamnus californica*. IAWA Journal 33: 151–162. DOI: 10.1163/22941932-90000086.

Dzung N.A., Khanh V.T.P., Dzung T.T. 2011. Research on impact of chitosan oligomers on biophysical characteristics, growth, development and drought resistance of coffee. Carbohydrate Polymers 84: 751–755. DOI: 10.1016/j.carbpol.2010.07.066.

El-Kenawy M.A. 2017. Effect of chitosan, salicylic acid and fulvic acid on vegetative growth, yield and fruit quality of Thompson Seedless grapevines. Egyptian Journal of Horticulture 44: 45–59. DOI: 10.21608/ejoh.2017.1104.1007.

Ennajeh M., Vadel A.M., Cochard H., Khemira H. 2010. Comparative impacts of water stress on the leaf anatomy of a drought-resistant and a drought-sensitive olive cultivar. Journal of Horticultural Science and Biotechnology 85(4): 289–294. DOI: 10.1080/14620316.2010.11512670.

FAO 2015. Water Use. Food and Agriculture Organization of the United Nations. http://www.fao.org/aquastat/en/overview/methodology/water-use/

Farouk S., Ghoneem K.M., Ali A.A. 2008. Induction and expression of systemic resistance to downy mildew disease in cucumber by elicitors. Egyptian Journal of Phytopathology 36(1–2): 95–111.

Gómez-del-Campo M., Ruiz C., Lissarrague J.R. 2002. Effect of water stress on leaf area development, photosynthesis, and productivity in Chardonnay and Airén grapevines. American Journal of Enology and Viticulture 53: 138–143.

Górnik K., Grzesik M., Romanowska-Duda B. 2008. The effect of chitosan on rooting of grapevine cuttings and on subsequent plant growth under drought and temperature stress. Journal of Fruit and Ornamental Plant Research 16: 333–343.

Guan Y., Hu J., Wang X., Shao C. 2009. Seed priming with chitosan improves maize germination and seedling growth in relation to physiological changes under low temperature stress. Journal of Zhejiang University, Science B 10: 427–433. DOI: 10.1631/jzus.b0820373.

Haider M.S., Zhang C., Kurjogi M.M., Pervaiz T., Zheng T., Zhang C. et al. 2017. Insights into grapevine defense response against drought as revealed by biochemical, physiological and RNA-Seq analysis. Scientific Reports 7; 13134; 15 p. DOI: 10.1038/s41598-017-13464-3.

Hussain M., Malik M.A., Farooq M., Khan M.B., Akram M., Saleem M.F. 2009. Exogenous glycinebetaine and salicylic acid application improves water relations, allometry and quality of hybrid sunflower under water deficit conditions. Journal of Agronomy and Crop Science 195: 98–109. DOI: 10.1111/j.1439-037x.2008.00354.x.

IPCC 2013. Climate Change 2013. The Physical Science Basis. Intergovernmental Panel on Climate Change. Cambridge University Press, USA.

Israelsen O.W., Hansen V.E. 1962. Irrigation, Principles and Practices, third edition. John Wiley and Sons, USA, 448 p.

Jie Z., Yuncong Y., Streeter J.G., Ferree D.C. 2010. Influence of soil drought stress on photosynthesis, carbohydrates and the nitrogen and phophorus absorb in different section of leaves and stem of Fugi/M.9EML, a young apple seedlings. African Journal of Biotechnology 9: 5320–5325.

Kar M., Mishra D. 1976. Catalase, peroxidase, and polyphenoloxidase activities during rice leaf senescence. Plant Physiology 57: 315–319. DOI: 10.1104/pp.57.2.315.

Karimi S., Abbaspour H., Sinaki J.M., Makarian H. 2012. Effects of water deficit and chitosan spraying on osmotic adjustment and soluble protein of cultivars castor bean (*Ricinus communis* L.). Journal of Stress Physiology and Biochemistry 8: 160–169.

Khalil H.A. 2015. Morphological and physiological performance and drought resistance improvement of pomegranate seedlings by mycorrhizal inoculation. Journal of Plant Production 6: 2145–2162. DOI: 10.21608/jpp.2015.52458.

Khalil H.A., El-Ansary D.O. 2015. Impacts of deficit irrigation and humic acid application on growth, yield and fruit quality of Valencia orange trees. Egyptian Journal of Horticulture 42: 441–452. DOI: 10.21608/ejoh.2015.1309.

Khalil H.A., El-Ansary D.O. 2020. Morphological, physiological and anatomical responses of two olive cultivars to deficit irrigation and mycorrhizal inoculation. European Journal of Horticultural Science 85(1): 51–62. DOI: 10.17660/ejhs.2020/85.1.6.

Khan W.M., Prithiviraj B., Smith D.L. 2002. Effect of foliar application of chitin oligosaccharides on photosynthesis of maize and soybean. Photosynthetica 40: 621–624. DOI: 10.1023/a:1024320606812.

Kumaresapillai N., Basha R.A., Sathish R. 2011. Production and evaluation of chitosan from *Aspergillus niger* MTCC strains. Iranian Journal of Pharmaceutical Research 10: 553–558.

Lai Q., Bao Z., Zhu Z., Qian Q., Mao B. 2007. Effects of osmotic stress on antioxidant enzymes activities in leaf discs of P_{SAG12}-*IPT* modified gerbera. Journal of Zhejiang University, Science B 8: 458–464. DOI: 10.1631/jzus.2007.b0458.

Li Y., Ye W., Wang M., Yan X. 2009. Climate change and drought: a risk assessment of crop-yield impacts. Climate Research 39: 31–46. DOI: 10.3354/cr00797.

Lizárraga-Paulín E.G., Torres-Pacheco I., Moreno-Martínez E., Miranda-Castro S.P. 2011. Chitosan application in maize (*Zea mays*) to counteract the effects of abiotic stress at seedling level. African Journal of Biotechnology 10: 6439–6446. DOI: 10.5897/ajb10.1448.

Malekpoor F., Ghasemi Pirbalouti A., Salimi A. 2016. Effect of foliar application of chitosan on morphological and physiological characteristics of basil under reduced irrigation. Research on Crops 17: 354–359. DOI: 10.5958/2348-7542.2016.00060.7.

Martorell S., Diaz-Espejo A., Tomàs M., Pou A., El Aou-ouad H., Escalona J.M. et al. 2015. Differences in water-use-efficiency between two *Vitis vinifera* cultivars (Grenache and Tempranillo) explained by the combined response of stomata to hydraulic and chemical signals during water stress. Agricultural Water Management 156: 1–9. DOI: 10.1016/j.agwat.2015.03.011.

Massacci A., Nabiev S.M., Pietrosanti L., Nematov S.K., Chernikova T.N., Thor K., Leipner J. 2008. Response of the photosynthetic apparatus of cotton (*Gossypium hirsutum* L.) to the onset of drought stress under field conditions studied by gas-exchange analysis and chlorophyll fluorescence imaging. Plant Physiology and Biochemistry 46: 189–195. DOI: 10.1016/j.plaphy.2007.10.006.

Medrano H., Escalona J.M., Bota J., Gulías J., Flexas J. 2002. Regulation of photosynthesis of C_3 plants in response to progressive drought: stomatal conductance as a reference parameter. Annals of Botany 89: 895–905. DOI: 10.1093/aob/mcf079.

Murkute A.A., Sharma S., Singh S.K. 2006. Studies on salt stress tolerance of citrus rootstock genotypes with arbuscular mycorrhizal fungi. Horticultural Science 33: 70–76. DOI: 10.17221/3742-hortsci.

Nazarli H., Faraji F., Zardashti M.R. 2011. Effect of drought stress and polymer on osmotic adjustment and photosynthetic pigments of sunflower. Cercetări Agronomice în Moldova 44: 35–41. DOI: 10.2478/v10298-012-0022-9.

Pérez-Pastor A., Ruiz-Sánchez M.C., Domingo R. 2014. Effects of timing and intensity of deficit irrigation on vegetative and fruit growth of apricot trees. Agricultural Water Management 134: 110–118. DOI: 10.1016/j.agwat.2013.12.007.

Pongprayoon W., Roytrakul S., Pichayangkura R., Chadchawan S. 2013. The role of hydrogen peroxide in chitosan-induced resistance to osmotic stress in rice (*Oryza sativa* L.). Plant Growth Regulation 70: 159–173. DOI: 10.1007/s10725-013-9789-4.

Shahnazari A., Liu F., Andersen M.N., Jacobsen S.E., Jensen C.R. 2007. Effects of partial root-zone drying on yield, tuber size and water use efficiency in potato under field conditions. Field Crops Research 100: 117–124. DOI: 10.1016/j.fcr.2006.05.010.

Shibuya N., Minami E. 2001. Oligosaccharide signalling for defense responses in plant. Physiological and Molecular Plant Pathology 59: 223–233. DOI: 10.1006/pmpp.2001.0364.

Smart R.E., Bingham G.E. 1974. Rapid estimates of relative water content. Plant Physiology 53: 258–260. DOI: 10.1104/pp.53.2.258.

Tehrani M.M., Kamgar-Haghighi A.A., Razzaghi F., Sepaskhah A.R., Zand-Parsa Sh., Eshghi S. 2016. Physiological and yield responses of rainfed grapevine under different supplemental irrigation regimes in Fars province, Iran. Scientia Horticulturae 202: 133–141. DOI: 10.1016/j.scienta.2016.02.036.

Thimmaiah S.K. 2004. Standard Methods of Biochemical Analysis. Kalyani Publishers, Ludhiana, India, 545 p.

Toscano S., Scuderi D., Giuffrida F., Romano D. 2014. Responses of Mediterranean ornamental shrubs to drought stress and recovery. Scientia Horticulturae 178: 145–153. DOI: 10.1016/j.scienta.2014.08.014.

Yang F., Hu J., Li J., Wu X., Qian Y. 2009. Chitosan enhances leaf membrane stability and antioxidant enzyme activities in apple seedlings under drought stress. Plant Growth Regulation 58: 131–136. DOI: 10.1007/s10725-009-9361-4.

Yadava U.L. 1986. A rapid and nondestructive method to determine chlorophyll in intact leaves. HortScience 21: 1449–1450.