Journal of Applied Horticulture, 24(1): 27-32, 2022
DOI: https://doi.org/10.37855/jah.2022.v24i01.05
Journal of Applied Horticulture
ISSN: 0972-1045

Deficit irrigation effects on five pomegranate cultivars' water use efficiency and biochemical parameters

M.A. Fayek, A.E. Mohamed and A.A. Rashedy*

*Pomology Department, Faculty of Agriculture, Cairo University, Giza, Egypt, *E-mail*: *ahmed.rasheedy@agr.cu.edu.eg*

Abstract

The purpose of this study was to compare the effects of two irrigation levels,100 percent of field capacity (FC) (control) and 30 percent FC (deficit irrigation) on vegetative growth, biochemical status, and water use efficiency of five pomegranate cultivars (Wonderful, Manfalouty, Acco, Assuity and Early 116) over two seasons in 2019 and 2020. The results showed that deficit irrigation reduced the dry weight of the shoots and roots, as well as the chlorophyll content of the leaves, in all the pomegranate cultivars. Under stress, proline content, total soluble sugars, total phenols, and peroxidase activity all increased significantly. The Manfalouty cultivar had the highest shoot and root dry weights, as well as the highest water use efficiency, proline content, and total phenols. Furthermore, when compared to other cultivars, Wonderful cultivar had the highest chlorophyll content during both seasons. In comparison to the other cultivars studied, Wonderful cultivar showed the limited or no significant increase in peroxidase activity. This could be due to a genotype's variable peroxidase activity potential in pomegranate cultivars under water stress. The obtained results indicate that Manfalouty and Wonderful cultivars are more tolerant of deficit irrigation than the other cultivars studied.

Key words: WUE, proline, peroxidase, phenols, soluble sugars, chlorophyll, *Punica granatum*

Introduction

Pomegranate (*Punica granatum* L.) is a commercial fruit crop that requires little water, fertiliser, and pesticides. It is grown and cultivated in a wide range of climates, demonstrating its adaptability and genetic diversity (da Silva *et al.*, 2013). Pomegranates are one of Egypt's most promising export crops, with exports totaling around 122387.2 tonnes (Egyptian ministry of Agriculture and Land Reclamation 2019). Manfalouty (local and late ripening variety) and Wonderful (foreign and late ripening variety) cultivars are considered one of the most common cultivars.

Worldwide, 18 % of arable land is irrigated, contributing up to 40 % of agricultural output (Ritzema, 2016). Irrigated agriculture consumes 70 % of all freshwater (Christ and Burritt, 2017). According to climate change predictions, the Mediterranean basin will be affected by drought now or in the future (Senatore *et al.*, 2011). The lack of fresh water limits plant growth and productivity. The effects of water stress on plants' morphology, physiological, and biochemistry affect yield and quality (Khan *et al.*, 2017). Drought stress adaptation mechanisms and strategies vary between cultivars and species. Martnez-Nicolás *et al.* (2019) found that withholding irrigation during flowering and fruit set reduced total shoot growth of the 7 year old pomegranate cultivar Mollar de Elche compared to daily irrigation at 115 percent ETc. Also, Khattab *et al.* (2011a) found that 7 m^3/tree/year irrigation reduced all vegetative parameters and yield of a pomegranate cultivar (Manfalouty) compared to 13 m^3/tree/year irrigation over two seasons. Plants use compatible solutes like proline, phenols, and sugars to avoid tissue dryness and maintain plant water status under various stresses (Kapoor *et al.*, 2020). The peroxidase enzyme also scavenges H_2O_2 to non-toxic levels (Lin *et al.*, 2015). According to Bahaulddin and Hepaksoy (2020) reducing available water also reduced the chlorophyll content of pomegranate plants. Water use efficiency (WUE) is considered an important characteristic for determining biological and economic yield as well as water consumption (Ullah *et al.*, 2019).

To deal with water scarcity, there are two approaches: (1) use efficient and improved deficit irrigation management practices, and (2) use low water consumption plants that can withstand drought conditions with minimal yield and fruit quality loss (Galindo *et al.*, 2018). The aim of this study was to compare and evaluate five pomegranate cultivars under deficit irrigation in terms of biochemical/morphological parameters and water use efficiency.

Materials and methods

Plant material, growth conditions and treatments: The study was conducted in the nursery and laboratory of the Pomology Department, Faculty of Agriculture, Cairo University, Egypt, during the 2019 and 2020 growing seasons (30°01'04"N, 31°12'30"E; 30m altitude). This study used five pomegranate cultivars namely: Wonderful, Manfalouty, Acco, Assuity and Early 116, which were propagated by hard wood stem cuttings and irrigated with tap water using the drip irrigation system (4 L/h) every two days until complete saturation or leaching (100 percent of field capacity). Table 1 shows the results of soil and water samples analysis at the soil, water, and environmental research institute, Ministry of Agriculture and land reclamation, Giza, Egypt. All pots in the experiment had bricks under them to ensure drainage and prevent root spread.

In early June, 42 plants of each cultivar were divided into two groups: Control (21 plants of each cultivar) received 100 % of field capacity and drought-stressed plants (21 plants of each cultivar) received 30 % of field capacity. Soil samples were fresh-weighed and oven-dried for 3 days at 85°C to determine field capacity (FC) (Coombs *et al.*, 1987). All plants were irrigated at the previous levels for three months. Every three days, three pots from each treatment were weighed, and the water lost was estimated and added. Plants were fed 0.25 strength Hoagland solution for macronutrients and full strength for micronutrients (Fozouni *et al.*, 2012).

Table 1. Physical and chemical properties of the experimental soil and irrigation water

Characteristics	Soil	Water
Particle size distribution (%)		
Fine sand	44.2	
Coarse sand	41.6	
Silt	11.2	
Clay	3	
Texture	Sandy soil	
EC (dsm^{-1})	1.21	0.48
P^H	7.91	7.53
Soluble cations (meq/L)		
Ca^{+2}	3.8	2.3
Mg^{+2}	1.9	1.4
Na^{+}	60	0.84
K^{+}	0.3	0.18
Soluble anions (meq/L)		
CO_3^{-2}	--	--
HCO_3^{-}	0.37	1.8
Cl^{-}	10	2.5
SO_4^{-2}	1.63	0.24
Soil Moisture constants		
Saturation percentage (SP)	24	
Field capacity (FC)	15 %	
Wilting point (WP)	6.2	
Available water (AW)	8.8	

All the following parameters were recorded at the end of the experimental period (1st September).

Growth parameters: Shoot and root were oven dried at 70° C for 48h before taking dry weight (g) .

Water use efficiency (g/L): It was calculated as described by Kerbiriou (2014) using the following equation:

WUE= Total dry matter/ Water added

Biochemical analysis

Fresh leaf samples taken from the 5th and 6th leaves from shoot tip were used for the following chemical analysis at the end of the experimental period:

Proline content (µmol/g FW): Proline concentration was determined spectrophotometrically by the ninhydrin method according to Bates *et al.* (1973). Leaf samples (0.5 g) were crushed in 10 mL of 3 % sulfosalicylic acid then the homogenates were filtered (2 mL of extract were mixed with 2 mL of ninhydrin reagent (3 % v/v) and 2 mL of glacial acetic acid, and then incubated at 100°C for 1 h. The reaction was terminated by placing it in an ice bath. The reaction mixture was vigorously mixed with 4 mL of toluene. After warming at 25°C, absorbance of the colored solutions was read at 520 nm using, toluene as a blank.

Total soluble sugars (mg/g FW): Soluble sugars were determined according to phenol-sulfuric acid method (Dubois *et al.,* 1956). Leaf samples (0.5 g FW) were extracted for three days in 10 mL of 70 % ethanol. A specific amount of the extract (1 mL) was mixed with 1 mL of phenol (5 %) and 4mL of sulfuric acid (98 %). The mixture was left for 1 hour to stop the reaction before being measured with a spectrophotometer at 490 nm. The total soluble sugars content was calculated using a standard curve with glucose as the standard solution, and the results were expressed as mg/g FW.

Total phenols (mg/g FW): Total phenols content was determined based on the Folin ciocalteu method (Sharma *et al.,* 2019). Leaf samples (0.5 g FW) were extracted for three days in the dark in 20 mL of methanol (80 %). A specific amount of the extract (1 mL), 1 mL Folin 10 %, 5 mL sodium carbonate (20 %), and 3 mL of distilled water were combined to make the final volume of 10 mL. A spectrophotometer was used to measure the absorbance at 765 nm after the mixture had been left for 1 hour. Gallic acid equivalents (GAE) in milligrames per grame of fresh leaf weight were used to express the total phenolic content.

Peroxidase activity (mg/g FW): Leaf samples (0.5 g FW) were maintained at -20°C before being processed according to Ni *et al.* (2001). Cold potassium phosphate buffer (0.1M, pH 7.0) containing 1 % (w/v) polyvinylpyrrolidone and 1 % (v/v) Triton X-100 was used to extract enzymes from frozen plant samples. A specific amount of the extract (1 mL) extraction buffer was used to macerate the samples. A further 1 mL of the extraction buffer was used to grind the samples. Each sample received 2 mL of extraction buffer in total. At 4° C, an aliquot of the extract (1.5 ml) was centrifuged at 10 000 rpm for 10 minutes. The supernatant was frozen right away to be used in future enzyme activity tests. Peroxidase activity was determined according to the procedure given by Hammerschmidt *et al.* (1982). A specific amount of pyrogallol (0.05 M) and 100 µL enzyme extract were added to a spectrophotometer sample cuvette. At 420 nm, the readings were reset to zero. The reaction was started by adding 100 µL of hydrogen peroxide (1 %) to the sample cuvette. Changes in absorbance/min/g sample were used to measure enzyme activity.

Chlorophyll content (mg/g FW): Chlorophyll a (Ch_a) and Chlorophyll b (Ch_b) concentrations were determined according to the Lichtenthaler and Wellburn (1983) method. Leaf samples (0.25 g FW) were immersed in 20 mL of acetone (80 %), and the absorbance was measured using a spectrophotometer at 646 and 663 wave lengths. Ch_a and Ch_b concentrations were calculated according to the following equations:

Chlorophyll a (Ch_a) = $(12.25\times A_{663})-(2.798\times A_{646})$

Chlorophyll b (Ch_b) = $(21.5\times A_{646})-(5.1\times A_{663})$

Total Chlorophyll = (Ch_a) + (Ch_b)

Statistical analysis: The present study used the split plot design with two factors: two irrigation levels as the main plot (factor A) and 5 pomegranate cultivars as a sub-plot (factor B). Each cultivar had 42 plants arranged in a complete randomized block design. Each treatment was represented by 3 replicates. Duncan's multiple range test was used to compare differences between means at a 5 % level of probability, according to (Duncan, 1955).

Results and discussion

Vegetative growth: Tables 2 indicate that, compared to stress treatment (30 % of FC), full irrigated plants (100 % of FC) considerably recorded the highest significant values for both shoot and root dry weight during both seasons. Concerning shoot dry weight, Manfalouty and Wonderful cultivars were the least affected by stress conditions (30 % of FC) among all cultivars and recorded the highest values (5.087, 4.687 and 4.287, 4.517 g) during first and second seasons, respectively, while Assuity and Acco cultivars recorded the lowest values (2.560, 3.127 and 3.547, 3.423 g) during both seasons, respectively. As for root dry weight, Manfalouty cv. recorded the highest values (7.860 and 7.200 g) compared to other pomegranate cultivars during first and second seasons, respectively under drought stress conditions. Many previous researches on pomegranate reported that water stress reduced the whole plant growth (Xie *et al.,* 2015; Bugue˜no *et al.,* 2016; Nasrabadi *et al.,* 2020 and Adiba *et al.,* 2021). Hamdy *et al.* (2016) found that maximum values of vegetative growth parameters of two studied pomegranate cultivars Early 116 and Wonderful were obtained when the plants were irrigated with 100 % of field capacity, while the minimum values were recorded at 40 % of available water. Also in a study under different irrigation levels 100, 75 and 50 % of recommended water levels (11, 8.25 and 5.5m^3/tree/year), it was found that the vegetative growth was enhanced when applying the highest irrigation level (11 m^3/tree/year) in two pomegranate cultivars (Manfalouty and Nab-Elgamal) compared to Wonderful and Wardy cultivars over two seasons (Haleem *et al.,* 2020). Moreover, in Iran, withholding irrigation of pomegranate trees cv. Shahvar starting from the growing season till the end of the fruit set stage during two seasons resulted in delaying initial vegetative growth by ten days and decreased growth rate compared to full irrigated trees (Selahvarzi *et al.,* 2017). The reduced plant growth could be explained that, water stress decreases the transportation of cytokinin from root to shoot and increase the amount of leaf abscisic acid, this hormonal imbalance cause a reduction in cell division, elongation and expansion (Farooq *et al.,* 2012). Also, reduction in growth under water stress conditions could be due to lower photosynthetic rate and stomatal conductance (de Lima *et al.,* 2015).

Table 2. Effect of different irrigation levels on shoot dry weight (g) and root dry weight (g) of five pomegranate cultivars

Cultivars	1st season		Mean	2ed season		Mean
	Irrigation levels			Irrigation levels		
	100 %	30 %		100 %	30 %	
			Shoot dry weight (g)			
Wonderful	8.113b	5.087d	6.600A	8.780c	4.287de	6.533D
Manfalouty	6.773c	4.687d	5.730A	12.39a	4.517d	8.452A
Acco	8.313ab	3.127e	5.720A	10.33b	3.423f	6.875CD
Assuity	9.307a	2.560e	5.933A	10.67b	3.547ef	7.110BC
Early 116	7.987b	4.393d	6.190A	10.79b	4.090def	7.442B
Mean	8.099A	3.971B		10.592A	3.973B	
			Root dry weight (g)			
Wonderful	12.44ab	6.790ef	9.613B	8.717c	7.010d	7.863B
Manfalouty	9.863c	7.860d	8.862C	9.163c	7.200d	8.182B
Acco	13.20a	7.263de	10.23A	11.00b	5.677ef	8.340B
Assuity	11.73b	6.700ef	9.213BC	14.11a	4.620f	9.365A
Early 116	9.360c	6.280f	7.820D	8.700c	6.203de	7.452B
Mean	11.318A	6.979B		10.339A	6.142B	

Means with the same letter for each parameter within each season were significantly equal at L.S.D. 5 % level.

Water use efficiency (WUE): Under water stress conditions all pomegranate cultivars exhibited a significant increase in WUE compared to non-stressed plants (Table 3). Manfalouty and Wonderful cultivars showed the highest significant increase (1.810, 1.693 and 1.687, 1.633 g/L) during first and second seasons, respectively compared to Assuity cv. that showed the lowest significant values. Water use efficiency (WUE) is related to the ability of a plant to absorb higher concentrations of carbon (the maintenance of high photosynthetic rates), and limitation of water loss that is controlled by the stomatal aperture and closure (Flexas *et al.,* 2013; de Santana *et al.,* 2015). WUE is also linked to photosynthetic activity and transpiration efficiency, both of which can be affected by irrigation (de Santana *et al.,* 2015). Many previous studies have observed increase in WUE in different plant species under restricted water conditions (Shi *et al.,* 2014; Shaban *et al.,* 2021). As the plant only assimilates material when the saturation deficit is low, resulting in reduced water loss for each carbon molecule fixed (Bloch *et al.,* 2006).

Table 3. Effect of different irrigation levels on WUE (g /L) of five pomegranate cultivars

Cultivars	1st season		Mean	2ed season		Mean
	Irrigation levels			Irrigation levels		
	100 %	30 %		100 %	30 %	
Wonderful	0.857e	1.687b	1.272A	0.730h	1.633a	1.182B
Manfalouty	0.693f	1.810a	1.252AB	0.900f	1.693a	1.297A
Acco	0.897e	1.503c	1.200B	0.890fg	1.317c	1.103C
Assuity	0.877e	1.337d	1.107C	1.027e	1.180d	1.103C
Early 116	0.723f	1.540c	1.132C	0.813g	1.467b	1.140BC
Mean	0.809B	1.575A		0.872B	1.458A	

Means with the same letter within each season were significantly equal at L.S.D. 5 % level.

Biochemical analysis: Decreasing the available water up to 30 % of FC significantly increased leaf proline content in all pomegranate cultivars compared to the plants received 100 % of FC over the two seasons (Table 4). The table showed that the cultivars Acco, Manfalouty and Wonderful recorded a significant higher increase in leaf proline content (1.437, 1.534 and 1.413 µmole /g FW) in the first season and (2.180, 1.567 and 1.607 µmole /g FW) in the second season, respectively under stress conditions compared to Assuity and Early 116 cultivars. Our findings are in harmony with Khattab *et al.* (2011b), Rad *et al.* (2015) and Tatari *et al.* (2020) who found that drought stress increased leaf proline content in various pomegranate cultivars. Moreover, Hamdy *et al.* (2016) noticed a higher proline content in leaves of Wonderful seedlings at all irrigation levels during two seasons than those recorded in Early 116 variety. Also, Pourghayoumi *et al.* (2017) found that sever water stress (two weeks without water supply) significantly increased proline production in five pomegranate cultivars. Furthermore, decreasing irrigation level up to 70 % of irrigation requirements increased proline content of mango trees during two seasons (Shaban *et al.,* 2021). The existence of proline accumulation could have a role in decreasing levels of reactive oxygen species (ROS). Also, it could be employed to maintain cell resistance by hardening of the cell walls (Szabados and Savoure, 2009). Furthermore, proline accumulation is an osmotic adjustment process protecting cell turgor and maintaining the integrity of cell membranes and as well as the stability of enzymes and proteins (Basu *et al.,* 2016). As for total soluble sugars, data revealed that, plants subjected to 30 % of FC significantly increased leaf content of total soluble sugars during both seasons as compared to full irrigated plants (100 % of FC). Wonderful cv. possessed the highest value of total soluble sugars (91.520 mg/g FW) in the first season, while Acco followed by Wonderful cultivar recorded the highest values (121.3 and 120.8 mg/g FW) during second season, respectively. Similar to our findings, it has been reported that, mild water stress (75 and 50 % of FC) increased soluble sugars in two pomegranate cultivars Rabbab and Shishehgap (Ebtedaie and Shekafandeh, 2016). Also, it has been reported that drought stress conditions increased soluble sugars in pomegranate trees (Rad *et al.,* 2015). The osmotic potential of a cell can be altered by raising the concentration of total soluble sugar, which reduces the cell's water potential without impeding enzyme performance and without reducing the cell's turgidity. Under drought stress conditions, accumulated sugars helps in maintaining membrane stability, preventing and protecting membrane fusion and also keeping protein functioning (Lipiec *et al.,* 2013).

Table 4. Effect of different irrigation levels on proline content (μmole/g FW) and total soluble sugars (mg/g FW) of five pomegranate cultivars.

Cultivars	1st season		Mean	2ed season		Mean
	Irrigation levels			Irrigation levels		
	100 %	30 %		100 %	30 %	
Proline content (μmole/g FW)						
Wonderful	0.830cd	1.413a	1.122AB	1.030d	1.607b	1.318BC
Manfalouty	0.873cd	1.537a	1.205A	1.127cd	1.567b	1.347B
Acco	0.703de	1.437a	1.070B	1.210cd	2.180a	1.695A
Assuity	0.606e	0.976c	0.791C	1.187cd	1.233c	1.210C
Early 116	0.866cd	1.220b	1.043B	1.143cd	1.283c	1.213C
Mean	0.776B	1.317A		1.139B	1.574A	
Total soluble sugars (mg/g FW)						
Wonderful	82.600a	91.520a	87.06A	95.61e	120.8a	108.2C
Manfalouty	71.090a	83.193a	77.14C	112.6d	119.0abc	115.8B
Acco	72.953a	84.467a	78.71C	115.6cd	121.3a	118.5A
Assuity	77.673a	86.127a	81.90B	118.6abc	120.1ab	119.3A
Early 116	68.167a	74.297a	71.23D	117.2bc	118.7abc	118.0AB
Mean	74.497B	83.921A		111.955B	119.986A	

Means with the same letter for each parameter within each season were significantly equal at L.S.D. 5 % level.

The results presented in Table 5 revealed that compared to control treatment (100 % of FC), exposing pomegranate cultivars to water stress conditions led to a significant increase in total phenols in all studied cultivars over the two seasons. Moreover, it can be noticed that Manfalouty cv. exhibited the highest significant value (200.4 mg/g FW) compared to other cultivars during first season, meanwhile, Manfalouty followed by Wonderful cultivar recorded the higher values (142.9 and 137.6 mg/g FW) during the second season, respectively. In contrast, Assuity cv. had the lowest concentrations of total phenols (169.4 and 127.9 mg/g FW) in first and second season, respectively. Our results match with previous study of Petridis *et al.* (2012) who reported an increase in total phenol content when four olive cultivars were subjected to 33 % of FC compared to control treatment. Similar results were observed by Rezayian *et al.* (2018) who found that water stress (15 % of polyethylene glycol) increased total phenol content in canola plants. Accumulation of phenolic compounds is very important to counteract the adverse effects of drought stress in plants (Naikoo *et al.,* 2019). Previous studies reported that phenols could act as antioxidants to scavenge the reactive oxygen species to maintain normal biochemical and physiological process in tolerant cultivars (Jogaiah *et al.,* 2014; Sharma *et al.,* 2019).

Table 5. Effect of different irrigation levels on total phenols (mg/g FW) of five pomegranate cultivars

Cultivars	1st season		Mean	2ed season		Mean
	Irrigation levels			Irrigation levels		
	100 %	30 %		100 %	30 %	
Wonderful	134.3f	155.3de	144.8C	98.42f	137.6ab	118.0B
Manfalouty	151.4e	200.4a	175.9A	105.3de	142.9a	124.1A
Acco	155.2e	170.3c	162.9B	108.5d	136.3b	122.4A
Assuity	153.2e	169.4c	161.3B	108.1d	127.9c	118.0B
Early 116	159.3d	194.0b	176.6A	101.4ef	130.6c	116.0B
Mean	150.702B	177.882A		104.340B	135.077A	

Means with the same letter within each season were significantly equal at L.S.D. 5 % level.

Peroxidase (POD) activity due to deficit water irrigation varied among pomegranate cultivars during both seasons (Table 6). Compared to full irrigated plants (100 % of FC), all pomegranate cultivars under water stress conditions had significantly increased peroxidase activity in leaves during both seasons. Meanwhile, Wonderful cv. stressed plants had only slight increase during the first season (29.07 mg/g FW) while in the second season showed a small reduction in POD activity (56.67 mg/g FW), but this decrease was not significant. This concurs well with Tatari *et al.* (2020) who reported that two pomegranate cultivars GT-Yazd and M-Pishva showed a low POD activity under water stress compared to control treatment. HongBo *et al.* (2005) considered that in stressed plants, the high enzyme activity may reflect a genotype's potential to degrade harmful compounds such as free radicals generated under stress conditions. Increased scavenging antioxidant enzymes activities in stressed pomegranate plants revealed that these enzymes play an important role in controlling cellular levels of ROS under water restriction conditions (Pourghayoumi *et al.,* 2017). Previous reports on antioxidant enzyme activities under water stress conditions have shown that they are heterogeneous and levels of these enzymes differ, and they may increase, remain unchanged or even decrease (Sun *et al.,* 2013; Slabbert and Kru¨ger, 2014; Tatari *et al.,* 2020).

Table 6. Effect of different irrigation levels on peroxidase activity (mg/g FW) and chlorophyll content (mg/g FW) of five pomegranate cultivars

Cultivars	1st season		Mean	2ed season		Mean
	Irrigation levels			Irrigation levels		
	100 %	30 %		100 %	30 %	
Peroxidase activity (mg/g FW)						
Wonderful	27.17 f	29.07 e	28.12 D	57.57 e	56.67 e	57.12 C
Manfalouty	27.03 f	38.57 c	32.80 C	41.63 g	63.70 d	52.67 D
Acco	26.07 f	43.07 b	34.57 B	47.13 f	67.53 c	57.33 C
Assuity	33.47 d	48.10 a	40.78 A	43.80 fg	79.20 b	61.50 B
Early 116	26.93 f	39.33 c	33.13 C	42.27 g	94.83 a	68.55 A
Mean	28.133 B	39.627 A		46.480 B	72.387 A	
Chlorophyll content (mg/g FW)						
Wonderful	1.660a	1.477bcd	1.568A	1.890c	1.460f	1.675B
Manfalouty	1.620ab	1.340de	1.480A	1.640e	1.297g	1.468C
Acco	1.380cd	1.193ef	1.287B	2.243a	1.410f	1.827A
Assuity	1.530abc	0.797g	1.163C	2.020b	1.240g	1.630B
Early 116	1.587ab	1.050f	1.318B	1.737d	1.207g	1.472C
Mean	1.555A	1.171B		1.906A	1.323B	

Means with the same letter for each parameter within each season were significantly equal at L.S.D. 5 % level.

In terms of chlorophyll content, the data showed that water stress at 30% FC reduced total chlorophyll concentration significantly when compared to fully irrigated plants during the two seasons. Also, it is observed that Wonderful cv. recorded the highest values (1.477 and 1.460 mg/g FW) during both seasons respectively, meanwhile, Assuity and Early 116 cultivars exhibited the lowest chlorophyll concentration (0.797 and 1.050 mg/g FW) in the first season, and (1.240 and 1.207 mg/g FW) in the second season with no significant difference. Our results were supported by those of Khattab *et al.* (2011b) who observed that decreasing the available water was concurrent with reducing total chlorophyll in pomegranate cultivar Manfalouty over two seasons. Moreover, Pourghayoumi *et al.* (2017) mentioned that severe water stress declined total chlorophyll content in five pomegranate cultivars. Also, Bahaulddin and Hepaksoy, (2020) reported an increase in chlorophyll content with irrigated pomegranate trees compared to non-irrigated ones. Furthermore, it has been reported that deficit irrigation resulted in a significant reduction in total chlorophyll content in eleven pomegranate cultivars (Adiba *et al.,* 2021). During photosynthesis process, chlorophyll is considered the primary unit of plant energy system (Guidi *et al.*, 2017). The reduction in chlorophyll concentration could be attributable to decreasing uptake of macronutrients especially N and Mg which are the major elements in chlorophyll (Hepaksoy *et al.,* 2016).

The results demonstrated that deficit irrigation reduced the dry weight of the shoots and roots, as well as the chlorophyll content of the leaves, in all pomegranate cultivars. Under stress, proline concentration, total soluble sugars, total phenols, and peroxidase activity, all rose dramatically. The Manfalouty cultivar had the largest shoot and root dry weights, as well as the maximum water use efficiency, proline content, and total phenols. Furthermore, when compared to other cultivars, the Wonderful cultivar had the maximum chlorophyll concentration during both seasons. Ascompared to the other cultivars studied, the Wonderful cultivar had the weakest or no significant increase in peroxidase activity. This could be related to a genotype's varied peroxidase activity potential in pomegranate cultivars under water stress. The results indicate that the Manfalouty and Wonderful cultivars are more tolerant to deficit irrigation than the other cultivars investigated.

References

Adiba, A., R. Razouk, J. Charafi, A. Haddioui and A. Hamdani, 2021. Assessment of water stress tolerance in eleven pomegranate cultivars based on agronomic traits. *Agric. Water Manag.,* 243: 106419.

Bahaulddin, A. and S. Hepaksoy, 2020. The effects of irrigation on chlorophyll content of pomegranate (*Punica granatum* L.) trees. *Int J Agric Biol.,* 13: 174-180.

Basu, S., V. Ramegowda, A. Kumar and A. Pereira, 2016. Plant Adaptation to Drought Stress. F1000Research 2016, 5(F1000 Faculty Rev):1554.

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

Bloch, D., C.M. Hoffmann and B. Märländer, 2006. Impact of water supply on photosynthesis, water use and carbon isotope discrimination of sugar beet genotypes. *Eur. J. Agron.*, 24: 218-225.

Bugue˜no, F., N. Livellara, F. Varas, P. Undurraga, M. Castro and E. Salgado, 2016. Responses of young *Punica granatum* plants under four different water regimes. *Ciencia e Investigación Agraria*., 43: 49-56.

Christ, K.L. and R.L. Burritt, 2017. Water management accounting: A framework for corporate practice. *J. Clean. Prod*., 152: 379-386.

Coombs, J., D.O. Hall, S.P. Long and J.M. Scurlock, 1987. Techniques in Bioproductivity and Photosynthesis. Pergamon press, Oxford.

da Silva, J.A.T., T.S. Rana, D. Narzary, N. Verma, D.T. Meshram and S.A. Ranade, 2013. Pomegranate biology and biotechnology: A review. *Sci. Hort.,* 160: 85-107.

de Santana, T.A., P.S. Oliveira, L.D. Silva, B.G. Laviola, A.A.F. de Almeida and F.P. Gomes, 2015. Water use efficiency and consumption in different Brazilian genotypes of *Jatropha curcas* L. subjected to soil water deficit. *Biomass Bioenergy,* 75: 119-125.

de Lima, R.S.N., F.A.M.M. de Assis, A.O. Martins, B.C.D.S. de Deus, T.M. Ferraz, M.D.M. de Assis Gomes, E.F. de Sousa, D.M. Glenn, and E. Campostrini, 2015. Partial rootzone drying (PRD) and regulated deficit irrigation (RDI) effects on stomatal conductance, growth, photosynthetic capacity, and water-use efficiency of papaya. *Sci. Hort.,* 183: 13-22.

Duncan, D.B. 1955. Multiple range and multiple F tests. *Biometrics.* 11: 1-42.

Dubois, M., K.A. Gilles, J.K. Hamilton, P.A. Rebers and F. Smith, 1956. Colorimetric method for determination of sugars and related substances. *J. Anal. Chem*., 28: 350-356.

Ebtedaie, M. and A. Shekafandeh, 2016. Antioxidant and carbohydrate changes of two pomegranate cultivars under deficit irrigation stress. *Span. J. Agric. Res.,* 14: 1-9.

Egyptian Ministry of Agriculture, 2019. Pomegranate Production. Annual Report. Egyptian Ministry of Agriculture, Cairo, Egypt.

Farooq, M., M. Hussain, A. Wahid and K.H.M. Siddique, 2012. Drought stress in plants: an overview. In: Plant Responses to Drought Stress. Aroca, R. ed. (Springer, Berlin, Heidelberg) 1-33.

Flexas, J., U. Niinemets, A. Gallé, M.M. Barbour, M. Centritto, A. Diaz-Espejo, C. Douthe, J. Galmés, M. Ribas-Carbo, P.L. Rodriguez and F. Rosselló, 2013. Diffusional conductances to CO_2 as a target for increasing photosynthesis and photosynthetic water-use efficiency. *Photosynth. Res*., 117: 45-59.

Fozouni, M., N. Abbaspour and H.D. Banch, 2012. Short term response of grapevine grown hydroponically to salinity: Mineral composition and growth parameters. *Vitis,* 51: 95- 101.

Galindo, A., J. Collado-González, I. Griñán, M. Corell, A. Centeno, Martín- M.J. Palomo, I.F. Girón, P. Rodríguez, Z.N. Cruz, H. Memmi, and A.A. Carbonell-Barrachina, 2018. Deficit irrigation and emerging fruit crops as a strategy to save water in Mediterranean semiarid agrosystems. *Agric. Water Manag*., 202: 311-324.

Guidi, L., M. Tattini and M. Landi, 2017. How does chloroplast protect chlorophyll against excessive light? In: Chlorophyll, Jacob-Lopes E, Zepka LQ, Queiroz MI (eds).

Haleem, A.Y., W.M. Ghieth and A.A.H. Hegazy, 2020. Performance of Some Pomegranate Cultivars under Different Irrigation levels in North Sinai. *Mid. East. J. Agric. Res.*, 9: 1021-1031.

Hamdy, A.E., S.M. Khalifa, S.S. Shawer and A.A.G. Mancy, 2016. Effect of water stress on the growth, nutritional and biochemical status of two varieties of pomegranate seedlings*. J. Plant Prod*., 7: 1321-1329.

Hammerschmidt, R., F. Nuckles and J. Kuc, 1982. Association of enhanced peroxidase activity with induced systemic resistance of cucumber to *Colletotrchum lagenarium*. *Physiol. Plant Pathol.,* 20: 73-82.

Hepaksoy, S., A. Bahaulddin and Y.S. Kukul Kurttas, 2016. The effects of irrigation on leaf nutrient content in pomegranate 'İzmir 1513', *Acta Hort.,* 1139: 581-586.

HongBo, S. Z. Liang and M. Shao, 2005. Changes of some anti-oxidative enzymes under soil water deficits among 10 wheat genotypes at maturation stage. *Colloids Surf. B*: *Biointerfaces.,* 45:7-13.

Jogaiah, S., S.D. Ramteke, J. Sharma and A.K. Upadhyay, 2014. Moisture and salinity stress induced changes in biochemical constituents and water relations of different grape rootstock cultivars*. Int. J. Agron.,* 1-8. https://doi.org/10.1155/2014/789087

Kapoor, D., S. Bhardwaj, M. Landi, A. Sharma, M. Ramakrishnan and A. Sharma, 2020. The Impact of drought in plant metabolism: how to exploit tolerance mechanisms to increase crop production*. Appl. Sci*., 10: 1-19.

Kerbiriou, P.J. 2014. Physiology and genetics of root growth, resource capture and resource use efficiency in lettuce (*Lactuca sativa* L.). Ph.D. Diss.,Wageningen University.

Khan, A., Y. Anwar, M. Hasan, A. Iqbal, M. Ali, H.F. Alharby, K.R. Hakeem and M. Hasanuzzaman, 2017. Attenuation of drought stress in *Brassica* seedlings with exogenous application of Ca^{2+} and H_2O_2. *Plants,* 6: 20. doi.org/10.3390/plants6020020

Khattab, M.M., A.E. Shaban, A.H. El-Shrief, and A.E.D. Mohamed, 2011a. Growth and productivity of pomegranate trees under different irrigation levels I: vegetative growth and fruiting. *J. Hort. Sci. & Orna. Plants*. 3:194-198.

Khattab, M.M., A.E. Shaban, A.H. El-Shrief and A.S.E. Mohamed, 2011b. Growth and productivity of pomegranate trees under different irrigation levels. III: leaf pigments, proline and mineral content. *J. Hort. Sci. & Orna. Plants*. 3: 265-269.

Lichtenthaler, H.K. and A.R. Wellburn, 1983. Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. Biochemical Society Transactions, 11: 591-592.

Lin, H., Y.L. Peng, J. Chen, and L. Liang, 2015. Effect of heavy metal stress on antioxidase enzymes. In: Proceedings of international conference on manufacturing science and engineering (ICMSE 2015) 871-876.

Lipiec, J., C. Doussan, A. Nosalewicz, and K. Kondracka, 2013. Effect of drought and heat stresses on plant growth and yield: a review. *Int Agrophys*., 27: 463-477.

Martínez-Nicolás, J.J., A. Galindo, I. Griñán, P. Rodríguez, Z.N. Cruz, R. Martínez-Font, A.A. Carbonell-Barrachina, H. Nouri and P. Melgarejo, 2019. Irrigation water saving during pomegranate flowering and fruit set period do not affect Wonderful and Mollar de Elche cultivars yield and fruit composition*. Agric. Water Manag.,* 226: 105781.

Naikoo, M.I., M.I. Dar, F. Raghib, H. Jaleel, B. Ahmad, A. Raina, F.A. Khan and F. Naushin, 2019. Role and regulation of plants phenolics in abiotic stress tolerance: an overview. *Plant Signal. Molec.,* 157-168.

Nasrabadi, M., A. Ramezanian, S. Eshghi and A. Sarkhosh, 2020. Chilling and heat requirement of pomegranate (*Punica granatum* L.) trees grown under sustained deficit irrigation. *Sci. Hort.*, 263: 109117.

Ni, X., S.S. Quisenberry, T. Heng-Moss, J. Markwell, G. Sarath, R. Klucasand F. Baxendale, 2001. Oxidative responses of resistant and susceptible cereal leaves to symptomatic and non symptomatic cereal aphid (Hemiptera:Aphididae) feeding. *J. Econ. Entomol*., 94: 743-751.

Petridis, A., I. Therios, G. Samouris, S. Koundouras and A. Giannakoula, 2012. Effect of water deficit on leaf phenolic composition, gas exchange, oxidative damage and antioxidant activity of four Greek olive (*Olea europaea* L.) cultivars. *Plant Physiol. Biochem*., 60: 1-11.

Pourghayoumi, M., M. Rahemi, D. Bakhshi, A. Aalami and A.A. Kamgar-Haghighi, 2017. Responses of pomegranate cultivars to severe water stress and recovery: changes on antioxidant enzyme activities, gene expression patterns and water stress responsive metabolites. *Physiol Mol. Biol. Plants,* 23: 321-330.

Rad, M.H., M.R. Asghari and M.H. Asareh, 2015. The effects of drought stress on growth, yield and fruit quality of pomegranate (*Punica granatum* L.) cv. Rababe Niriz under dry climate condition. *Seed Plant Prod. J.,* 31: 75-90.

Rezayian, M., V. Niknam and H. Ebrahimzadeh, 2018. Differential responses of phenolic compounds of *Brassica napus* under drought stress. *Iran. J. Plant Physiol.*, 8: 2417-2425.

Ritzema, H.P. 2016. Drain for Gain: Managing salinity in irrigated lands—A review. *Agric. Water Manag.,* 176: 18-28.

Selahvarzi, Y., Z. Zamani, R. Fatahi and A.R. Talaei, 2017. Effect of deficit irrigation on flowering and fruit properties of pomegranate (*Punica granatum* cv. Shahvar*). Agric. Water Manag*., 192: 189-197.

Senatore, A., G., Mendicino, G. Smiatek and H. Kunstmann, 2011. Regional climate change projections and hydrological impact analysis for a Mediterranean basin in Southern Italy. *J. Hydrol.,* 399: 70-92.

Shaban, A.E., M.I. El-Banna and A.A. Rashedy, 2021. Mitigation of excessive solar radiation and water stress on 'Keitt'mango *Mangifera indica* trees through shading. *Acta Sci. Pol. Hortorum Cultus*., 20: 77-88

Sharma, A., B. Shahzad, A. Rehman, R. Bhardwaj, M. Landi and B. Zheng, 2019. Response of phenylpropanoid pathway and the role of polyphenols in plants under abiotic stress. *Molecules,* 24: 1-22.

Shi, J., H. Yasuor, U. Yermiyahu, Q. Zuo and A. Ben-Gal, 2014. Dynamic responses of wheat to drought and nitrogen stresses during re-watering cycles. *Agric. Water Manag.,* 146: 163-172.

Slabbert, M.M. and G.H.J. Krüger, 2014. Antioxidant enzyme activity, proline accumulation, leaf area and cell membrane stability in water stressed Amaranthus leaves. *S. Afr. J. Bot.,* 95: 123-128.

Sun, J., J. Gu, J. Zeng, S. Han, A. Song, F. Chen, W. Fang, J. Jiang and S. Chen, 2013. Changes in leaf morphology, antioxidant activity and photosynthesis capacity in two different drought-tolerant cultivars of chrysanthemum during and after water stress. *Sci. Hort.,* 161: 249-258.

Szabados L. and A. Savoure, 2009. Proline: A multifunctional amino acid. *Trends Plant Sci.,* 15: 89-97.

Tatari, M., E. Jadidi and E. Shahmansouri, 2020. Study of some physiological responses of different pomegranate (*Punica granatum* L.) cultivars under drought stress to screen for drought tolerance. *Int. J. Fruit Sci*., 20: 1798-1813.

Ullah, H., R. Santiago-Arenas, Z. Ferdous, A. Attia and A. Datta, 2019. Improving water use efficiency, nitrogen use efficiency, and radiation use efficiency in field crops under drought stress: A review. *Adv. Agron,* 156: 109-157.

Xie, S., X. Lu, X. Zhao and Q. Nie, 2015. Effect of water stress on vegetative growth and nitrogen metabolism of pomegranate seedling. *Acta Hort.*, 1089: 63-70.

Received: October, 2021; Revised: December, 2021; Accepted: December, 2021