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Yield, fruit quality and physiological responses of melon cv. Khatooni under deficit irrigation

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Key words: antioxidant enzyme, irrigation, melon, proline, water use efficiency.

Abstract: To evaluate the effect of water deficit stress on growth, yield, fruit quality and physiological traits of melon cv. Khatooni, field experiments were conducted in split plot randomized complete block design with three replications. In 2014, irrigation treatments consisted of two deficit irrigation regimes, 33% and 66% of ETc (crop evapotranspiration), and 100% ETc as the control (DI_{33}, DI_{66} and I_{100}). In 2015, irrigation treatments applied were: 40, 70 and 100% ETc (DI_{40}, DI_{70} and I_{100}). The results showed that plant height and leaf area decreased from treatment I_{100} to DI_{40} and DI_{33}. The highest average fruit weigh and yield were obtained from irrigation 100% ETc for both years. The water use efficiency (WUE) significantly increased in response to increase water deficit stress. Deficit irrigation treatments significantly decreased leaf relative water content, vitamin C and fruit firmness, whereas antioxidant enzymes activity, proline and total soluble solid contents increased. These results suggest that the crop is sensitive to water deficits, that moderate water stress (DI_{70} and DI_{66}) reduced yield by about 28.5-38.2% and severe water stress (DI_{40} and DI_{33}) had a much more marked effect, reducing yield by 48.1-61.4%.

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1. Introduction

Melon (*Cucumis melo* L.) is an important horticultural crop in Iran, generally cultivated in arid and semi-arid regions. Iran is the third largest melon-producing country in the world with more than 1476801 tonnes (FAO, 2014) of production. Melon plants are highly productive under adequate irrigation conditions; however water for irrigation is not always available at the time and amount needed by the crop, so water scarcity is a major constraint to horticultural production in arid and semiarid regions (Sharma *et al.,* 2014). Deficit irrigation regime, a practice that supplies water below evapotranspiration (ET) demands, can optimize water productivity when full irrigation is not possible (Fereres and Soriano, 2007). When water supply is limited, plant growth and yield is reduced and plant structure is modified by decreasing in leaf size (Kirnak *et al.,* 2002; Chaves *et al.,* 2003).

The effect of deficit irrigation on fruit yield and quality has been reported by numerous researchers with different results. In melon, deficit irrigation reduced marketable fruit number and yield, average fruit weight, fruit diameter and did not affect rind thickness and seed cavity, but increased total soluble solids content (Sharma *et al.,* 2014). Although deficit irrigation reduce crop yield, may be able to save a significant amount of irrigation water (Sharma *et al.,* 2014). Fabeiro *et al.* (2002) stated that deficit irrigation during blooming stage affected mainly fruit yield, at setting stage both quantity and quality, and the deficit imposed at ripening stage affected sugar content.

Rouphael *et al.* (2008) indicated that water deficit significantly reduced yield, biomass production and leaf water status of mini-watermelon, but increase the water use efficiency.

The soluble solids concentration (SSC) is probably the most important quality parameter that is commonly evaluated by consumers (Cabello *et al.,* 2009). Water deficit studies in melon have been reported to increase (Sharma *et al.,* 2014), decrease (Long *et al.,* 2006), or had no effect (Hartz, 1997) on soluble solid content. Vitamin C content, as a secondary metabolite of plants, did not change with deficit irrigation in watermelons (75% ETc) (Leskovar *et al.,* 2004) and melons (50% ETc) (Sharma *et al.,* 2014).

Oxidative stress is one of the major causes of cellular damage in plants during stress (Miller *et al.,* 2010). However, plants can avoid the drought damage by promoting antioxidant enzymes activity, such as superoxide dismutase (SOD), peroxidases (POD), and catalase (CAT), to scavenge for free radicals and, or accumulate osmotic regulators such as soluble sugar, and proline may play a role in protection of cellular machinery against photo-oxidation by reactive oxygen species (ROS) that increase the drought resistance of plants under water stress (Foyer and Noctor, 2005; Veljovic-Jovanovic *et al.,* 2006).

Although the effects of water stress have been studied on growth and yield of different crops during the last years, recent information on the response of Iranian melon yield and quality to deficit irrigation remains limited, particularly about the results of restricted water distributions in arid and sub-arid environments. The main goal of this study was to evaluate the effect of controlled deficit irrigation on the physiological parameters and yield of the Khatooni melon cultivar.

2. Materials and Methods

Experimental site

Two field experiments were conducted during the growing season of 2014 and 2015 from June to September at Research farm of Agriculture faculty, University of Zanjan (Iran), to study the effect of water deficit on fruit yield and quality, antioxidant enzymes activities, water use efficiency (WUE), proline and vitamin C content. The soil texture was silty loam with 7.8 pH. Some soil characteristics and irrigation water chemical properties were showed in Table 1 and 2. The daily climate data during the growing seasons (2014 and 2015) was shown in Table 3.

Table 1 - Soil physical and chemical properties at the experiment site

pH	EC (dS m^{-1})	N (%)	Ca (g kg^{-1})	Na (g kg^{-1})	K (g kg^{-1})	OM (%)	Soil texture	Sand (%)	Silt (%)	Clay (%)
7.40	1.49	0.07	0.12	0.13	0.2	0.94	Silt loam	25	38	37

OM= Organic matter.

Table 2 - Irrigation water chemical properties at the experiment site

Bicarbonate (mg L^{-1})	Carbonate (mg L^{-1})	Cl (mg L^{-1})	Mg (mg L^{-1})	Ca (mg L^{-1})	K (mg L^{-1})	Na (mg L^{-1})	EC (dS m^{-1})	pH
195.2	0.0	582.2	103.7	258.45	0.0	50	2.35	6.5

Table 3 - Climatic parameters during the growing seasons

Climatic parameters	June		July		August		Sept	
	2014	2015	2014	2015	2014	2015	2014	2015
Minimum air temperature (°C)	7.60	12.90	10.70	18.53	13.10	16.14	6.80	12.58
Maximum air temperature (°C)	35.80	31.90	39.50	34.46	39.10	35.50	35.40	30.28
Rainfall (mm)	7.30	0.33	17.30	1.13	0.10	0.00	4.00	2.93
Relative humidity (%)	41.50	44.00	43.40	42.00	37.00	39.00	41.40	52.00

Plant materials and irrigation treatments

The experiment was done on a completely randomized block design whit three irrigation levels and three replications. 'Khatooni', yellow-green netted skin color and chimeric stripes, an Iranian melon from the Inodorous group widely cultivated in Iran, was selected for study. The seeds were sown on 1th July 2014 and 23th May 2015 at recommended spacing of 50 cm in row with 200 cm between rows. The irrigation system consisted of one drip line every crop row. Fertilizers was delivered as a pre-plant base comprising 80 kg N/ha, 50 kg p/ha and 80 kg K/ha. At a very early stage, plants were pruned (removing the apex of the main stem), and trained to have two lateral branches.

Three irrigation levels were calculated, based on actual evapotranspiration (ETc). In 2014, irrigation treatments were control or irrigation at 100% ETc (I_{100}), deficit irrigation at 66% ETc (DI_{66}) and at 33% ETc (DI_{33}) of control. According to 2014 results, when water deficit stress treatments strongly reduced fruit yield, in 2015 deficit irrigation treatments were changed, and the irrigation treatments were: 100% ETc (I_{100}), 70% ETc (DI_{70}) and 40% ETc (DI_{40}). Before starting the differential irrigation at five-leaf stage, all treatments were supplied with similar amount of water to maximize stands and uniform crop establishment. All other necessary operations such as pests and weeds control were performed according to recommended package of practices during the crop growth.

Measurements. Plant growth and leaf area

After 30 days of irrigation treatments, the average of leaf area was recorded whit leaf area measurement (DELTA-T Device Ltd, England). After fruit harvest, vine length of each plant was measured. For estimate leaf dry weight, at first fresh weight of leaf was measured; then they were dried in a hot-air oven for 2 days at 72°C, after which the dry weights (%) of leaf was recorded.

Yield and productivity components

The fruits were harvested when color changed from green to yellow and after the appearance of the netted pattern. Each melon fruit was weighed to determine mean fruit weight (FW). The fruit number per plant and fruit yield per plant was measured to determine of total yield, expressed in t ha^{-1}. Fruit yield was calculated by the mean fruit weight (kg), fruit number per plant and the density (20,000 plants/ha).

Fruit quality

Immediately after harvest, flesh ratio (FR), fruit firmness (ff), total soluble solid (TSS) and vitamin C (VC) were determined. The flesh ratios were calculated using the formulae:

$$FR\ (\%)=[(a+b)^2-(a'+b')^2/(a+b)^2]\times 100$$

where a is the fruit length, a' is the seed cavity length, b is the fruit diameter and b' is the seed cavity diameter.

From the liquid extract obtained by liquefying the mesocarp of each fruit, TSS content was determined by a handheld refractometer and expressed as °Brix. Fruit firmness was measured on the mesocarp tissue at three random locations per fruit using a digital penetrometer (Mc Cormic-FT 327) and recorded as kg cm^{-1}.

Proline content

Proline content in leaf tissue was determined according to the method of Bates *et al*. (1973). Mature leaves of plant were sampled 30 days after the onset of the deficit irrigation treatments. Proline was extracted from a sample of 0.5 g fresh leaves material samples in 3% (w/v) solution sulphosalycylic acid and estimated using the ninhydrin reagent. After reading the absorbance of fraction at a wave length of 520 nm, proline concentration was determined using a calibration curve and expressed as mg g^{-1} FW.

Catalase and peroxidase enzymes activity

Samples were taken from the fully expanded leaf and transferred to the laboratory in the ice. Leaf sample (0.5 g) was frozen in liquid nitrogen and ground using a porcelain mortar and pestle.

Catalase (CAT) activity was measured by following the decomposition of H_2O_2 at 240 nm with a UV spectrophotometer (Havir and McHale, 1987). Samples without H_2O_2 were used as blank. The activity of CAT was calculated by the differences obtained at OD_{240} values at 30 second interval for 2 min after the initial biochemical reaction. Peroxidase (POD) activity was measured using modified method of the Tuna *et al.* (2008) with guaiacol at 470 nm. A change of 0.01 units per minute in absorbance was considered to be equal to one unit POD activity, which was expressed as unit g^{-1} FW min^{-1}.

Leaf relative water content

The relative water content (RWC) in leaves was determined whit sampling fully expanded young leaves at noon according to Yamasaki and Dillenburg, (1999). Leaf relative water content was calculated using the following formula:

$$RWC\ (\%) = [(FW-DW)/(SW-DW)] \times 100$$

where FW stands for fresh weight, DW for dry weight, and SW for saturated weight.

Water use efficiency

Water use efficiency (WUE) was calculated for all treatments based on total crop yield and amount of water applied during growth period. WUE was estimated as the ratio of fruit yield (Y, kg ha^{-1}) and irrigation water applied (W, m^{-3}) (Stanhill, 1986). WUE=Y/W.

Statistical analysis

All data were analyzed statistically using a one-way ANOVA. Because of differences in the treatments, the data for each year were submitted to ANOVA separately. For data analysis, a completely randomized block design was used (3 Irrigation levels × 3 replications × 10 observations per experimental unit). Data were analyzed using the SAS statistical program (SAS Institute Inc., Cary, NC, USA), and means were compared by Duncan's multiple range tests at the 5% and 1% probability levels.

3. Results and Discussion

Plant growth

Leaf area, vine length and leaf dry weight (LDW) data of the treatments were presented in Table 4. Leaf area significantly decreased in the water deficit stress treatments in both years, reduction 20.38% (DI_{33}) and 30.4% (DI_{40}) in 2014 and 2015, respectively. In 2014, deficit irrigation stress had no effect on LDW. On the contrary, in 2015, LDW was affected significantly by the irrigation treatments, decreasing 22.05% in I_{100} treatment. Also, water deficit stress significantly reduced vine length in 2014, but no significant effect was observed by water deficit stress in 2015. These findings are similar the results obtained by Pew and Gardner (1983) and Ribas *et al.* (2001) who found that vegetative growth was higher under full irrigation instead of limited irrigation. Growth is an irreversible increase in volume, size, or weight, which includes the phases of cell division, cell elongation, and differentiation. A decrease in plant growth may be due to the limitation of cell division, cell enlargement caused by loss of turgor and inhibition of various growth metabolisms (Farooq *et al.,* 2012), and also decrease in photosynthesis (Huang *et al.,* 2011).

Yield, productivity components and water use efficiency

Fruit yield was affected significantly by the irrigation treatments in both years (Table 4). The highest value of fruit yield (40.37 and 43.43 t h^{-1}) was obtained in the irrigation 100% ETc in 2014 and 2015, respectively. Fruit number and fruit weight significantly reduced under deficit irrigation (Table 4).

The mean fruit number per plant was lower in 2014 (1.8, I_{100}) compared to 2015 (2.7, I_{100}). In contrast, fruit mean weight was higher in 2014 (2.18 kg) against 2015 (1.60 kg) that was obtained under irrigation 100% ETc. The lowest fruit number and fruit weight (1.25 kg) was observed respectively, with irrigation 33% ETc in 2014 and irrigation 40% ETc in 2015. This result agrees with the findings of Ribas *et al.* (2001), Cabello *et al.* (2009) and Sharma *et al.* (2014), who reported that limited irrigation reduced fruit yield of melon.

Table 4 - Effect of deficit irrigation on average leaf area (LA), vine length (VL), fruit weight (FW), number of fruits per vine (FN), yield (Y), and water use efficiency (WUE) in 2014 and 2015 seasons

Year	Irrigation (% ETc)	LA (cm^2)	LDW (%)	VL (cm)	FN	FW (kg)	Y (t ha^{-1})	WUE (kg m^{-3})
2014	100	151.53 a	16.24 a	185.6 a	1.8 a	2.18 a	40.37 a	14.14 ab
	66	130.11 b	16.45 a	133.3 ab	1.3 ab	1.91 ab	24.94 b	15.11 ab
	33	120.64 b	17.23 a	116.33 b	1.1 b	1.36 b	15.55 c	17.65 a
2015	100	183.74 a	16.4 b	148.33 a	2.7 a	1.60 a	43.43 a	14.24 b
	70	151.72 b	19.59 a	138.33 a	2.2 ab	1.42 ab	31.03 b	14.53 b
	40	127.88 b	21.04 a	115.5 a	1.8 b	1.25 b	22.50 c	18.45 a

I_{33}, I_{40}, I_{66}, I_{70} and I_{100} represent the irrigation treatments that received 33, 40, 66, 70 and 100% of ETc, respectively. Values are the average of 10 observation of each replication per irrigation level. Within each column, values followed by the same letters are not significantly different at $p<0.05$.

The reduction in fruit yield under deficit irrigation treatments compare to I_{100} treatment can be explained by the decrease in both mean fruit weight and numbers of fruits per vine (Table 4). Cabello *et al.* (2009) and Sharma *et al.* (2014) also reported the reduction in fruit number and fruit weight under deficit irrigation. Previous studies indicated that fruit weight in melon is more sensitive to water stress than fruit number (Long *et al.,* 2006; Dogan *et al.,* 2008).

Figure 1 presents the correlation between irrigation and fruit yield, fruit weight and fruit number per vine. Correlation between irrigation and fruit yield (R^2= 0.93) was stronger than the correlation with fruit weight (R^2= 0.58) and fruit number per vine (R^2= 0.51) which indicates that the reduction in fruit yield with deficit irrigation was attributed to the significant decrease in average fruit weight and fruit number per vine (Fig. 1 b and c).

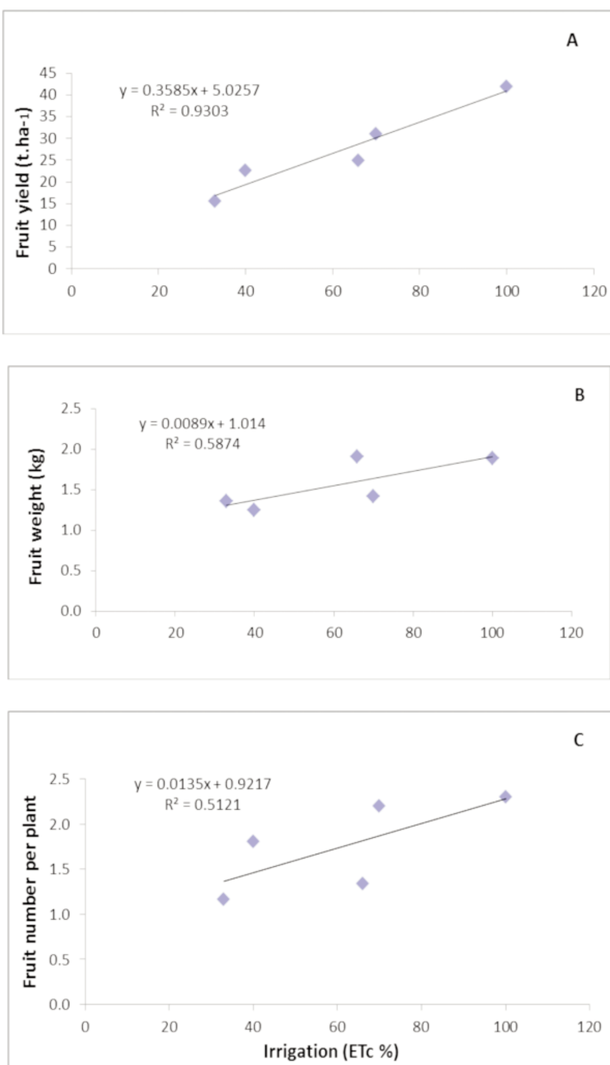

Fig. 1 - Relationship between irrigation by fruit yield (a), fruit weight (b) and fruit number per plant (c) in 2014 and 2015. Values are the mean of 3 replications/10 observations each irrigation level, in two years.

WUE is the relation between yield and the quantity of irrigation water (Zeng *et al.,* 2009). In both years, WUE was lowest for irrigation 100% ETc. Overall; deficit irrigation resulted in 19.88% and 22.81% WUE increased in DI_{33} and DI_{40}, respectively (Table 4). WUE had negative correlation (R^2 = 0.64) with irrigation water amount (Fig. 2). Higher WUE has also been achieved in watermelon (Leskovar *et al.,* 2004), muskmelon (Kirnak *et al.,* 2005; Zeng *et al.,* 2009), Mission and Da Vinci melon cultivars (Sharma *et al.,* 2014) in response to deficit irrigation.

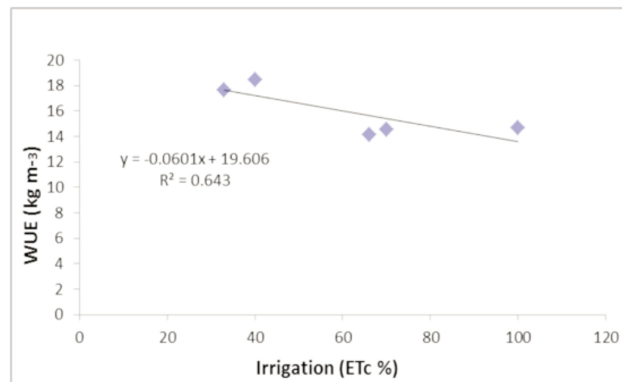

Fig. 2 - Relationship between irrigation by water use efficiency (WUE) in 2014 and 2015. Values are the mean of 3 replications/10 observations each irrigation level, in two years.

Fruit quality

Fruit quality as indicated with fruit firmness, flesh ratio, total soluble solid (TSS) and vitamin C was presented in Table 5. In both years, fruit firmness decreased as the irrigation was restricted. The lowest fruit firmness was 1.49 kg cm^{-1} under irrigation 33% ETc, although there was no significant difference

Table 5 - Effect of deficit irrigation on fruit firmness (FF), flesh ratio (FR), total soluble solid (TSS) and vitamin C (VC) in 2014 and 2015 seasons

Year	Irrigation (% ETc)	FF (kg cm^{-1})	FR (%)	TSS (°Brix)	VC (mg 100 ml^{-1})
2014	100	2.38 a	49.55 a	10.06 b	10.002 a
	66	1.79 ab	49.53 a	11 ab	8.082 b
	33	1.49 b	48.29 a	12.06 a	6.98 c
2015	100	3.15 a	54.07 a	9.03 b	10.68 a
	70	3.00 a	49.04 ab	10.7 ab	9.21 b
	40	2.1 b	45.77 b	11.76 a	7.88 c

I_{33}, $I4_{40}$, I_{66}, I_{70} and I_{100} represent the irrigation treatments that received 33, 40, 66, 70 and 100% of ETc, respectively. Values are the average of 10 observation of each replication per irrigation level. Within each column, values followed by the same letters are not significantly different at p<0.05.

between I_{100} whit DI_{66} and DI_{70} in 2014 and 2015, respectively. These results was agreement with Cabello *et al.* (2009) in melon, who reported that increasing irrigation water improved flesh firmness, but obtained a reduction in flesh firmness when irrigation water increased in following year. Also, Sharma *et al.* (2014) did not obtain a significant difference of irrigation treatments on fruit firmness with approximately positive effect of optimal irrigation.

The flesh ratio was unaffected by the irrigation rates in 2014 season. However, flesh ratio varied significantly in 2015. The largest flesh ratio (54.07%) was obtained under irrigation 100% ETc in 2015. These results are in agreement with the results of Dogan *et al.* (2008) in melon. The results indicated that optimal irrigation water could increase flesh thickness while water stress has a negative effect on it. It is not in accordance with Ribas *et al.* (2003) who reported that the flesh and skin ratios are not usually affected by the irrigation levels.

TSS is a very important index of quality in melon fruits (Zeng *et al.,* 2009). In both years, larger amounts of irrigation water resulted in lower TSS. The highest TSS was recorded with 12.06 and 11.76 °Brix in irrigation 33 and 40% ETc, respectively. The similar results were also observed by some other researchers (Lester *et al.,* 1994; Fabeiro *et al.,* 2002). Dogan *et al.* (2008) showed that fruit sugar content affected positively by water stress. Furthermore, other studies have shown that in muskmelon, TSS decreased with the decrease in irrigation water levels (Long *et al.,* 2006; Zeng *et al.,* 2009; Li *et al.,* 2012). Gonzalez *et al.* (2009) found no significant differences for watermelon fruit soluble solids between well-watered and regulated deficit irrigation treatments, although it was 9.5% higher, for the regulated deficit irrigation treatment.

Deficit irrigation markedly ($P<0.05$) reduced vitamin C content. The highest value of vitamin C was found in treatments I_{100} in both years (Table 5), which high decrease value (30.21%) was recorded in irrigation 33% ETc. The results indicated that vitamin C content was highly sensitive to deficit irrigation. Our results are agreement with Li *et al.* (2012) and Wang *et al.* (2017) who showed that severe water deficit stress reduced significantly the fruit vitamin C content, but these results differ from the findings of Cui *et al.* (2008) who stated that water deficit during the fruit growth and maturation stages increased significantly vitamin C content.

Proline accumulation

The exposure to water deficit stress significantly ($P<0.05$) increased proline content (Table 6). The maximum value of proline content was 1.97 and 1.8 mg g^{-1} FW under irrigation 33 and 40% ETc, respectively. Accumulation of proline plays an important role in plants to adaptive on environmental stresses, particularly low water stress (Kavas *et al.*, 2013). The proline that accumulated in the leaves under water-limited environment is a cellular regulator that helping to sustain the activity of the cell and tissue in water deficit condition by preventing injuries in the internal apparatus of cell (Ahmed *et al.,* 2009).

Catalase and peroxidase enzymes activity

Significant differences among treatments were observed for CAT enzyme activity (Table 6). CAT activity was the highest (7.47 and 6.97 µmol H_2O_2 g^{-1} FW min^{-1}) with DI_{33} and DI_{40} treatments. Similar to CAT, the POD activity in both seasons increased in response to an increase in water deficit stress (Table 6), which high POD activity was found by irrigation 33% ETc in 2014.

In present study, the antioxidant enzyme activates increased with the decrease of irrigation water

Table 6 - Effect of deficit irrigation on proline, catalase enzyme activity (CAT), peroxidase enzyme activity (POD) and relative water content (RWC)

Year	Irrigation (% ETc)	Proline (mg g^{-1}FW)	CAT (µmol H_2O_2 g^{-1} FW min^{-1})	POD (unit g^{-1} FW min^{-1})	RWC (%)
2014	100	0.77 b	4.52 b	0.422 b	78.63 a
	66	1.5 a	5.62 b	0.5 b	67.45 ab
	33	1.97 a	7.47 a	0.789 a	55.19 b
2015	100	0.97 c	4.4 b	0.356 b	73.13 a
	70	1.302 b	5.19 b	0.486 a	64.26 ab
	40	1.808 a	6.97 a	0.511 a	58.74 b

I_{33}, $I4_{40}$, I_{66}, I_{70} and I_{100} represent the irrigation treatments that received 33, 40, 66, 70 and 100% of ETc, respectively. Values are the average of 10 observation of each replication per irrigation level. Within each column, values followed by the same letters are not significantly different at $p<0.05$.

applied. As found by Kavas *et al.* (2013) in melon and Huseynova (2012) in wheat, the antioxidant activity of CAT significantly increased by drought stress. Antioxidative enzymes like POD and CAT play a major role in conferring drought tolerance and CTA and POD activity of drought tolerance genotypes were higher than sensitive genotypes under drought stress (Hameed *et al.,* 2013).

Relative water content

As applied irrigation water decreased, the relative water content of leaf decreased (Table 4). The results showed that different irrigation treatments had similar effects on RWC in both seasons. The highest value of RWC was recorded in irrigation 100% ETc. The decrease in RWC being respectively, 29.8 and 19.67% for DI_{33} and DI_{40} compared to I_{100}. RWC decreased linearly in response to an increase in water deficit stress in melon (Kavas *et al.,* 2013), watermelon (Kirnak and Dogan, 2009) and mini-watermelon (Rouphael *et al.,* 2008). The results indicated that the RWC was improved by the increasing irrigation water. Kirnak and Dogan (2009) stated the higher leaf relative water content values are generally indication of enough soil water in root zone.

4. Conclusions

Water deficit has been shown to adversely affect leaf area, yield, and leaf water status of melon, but led to increase the WUE and TSS. Since the water scarcity is a key factor for plant production under arid and semi-arid regions, thus achieving great values of WUE is more reasonable than maximum yield. WUE in DI40 and DI33 was greater than full irrigation treatment. Irrigation water increased yields not only by increasing the mean weight of the fruits, but also by increasing fruit number per vine. In both years, the physiological parameters showed significant differences. Results indicated that the change of CAT and POD activity and proline accumulation cooperated with water deficit; indeed CAT and POD activities and proline content (60.1% and 46% in DI33 and DI40, respectively) increased with enhancement of drought intensity, and in stressed plants were significantly higher than full irrigated plants. The results suggested that antioxidant enzyme activities (CAT and POD) as well as proline accumulation may play an important role in protecting 'Khatooni' melon plants against drought stress.

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