

Influence of water stress on the nutritional quality of peach fruits

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Article info

Article history:
Received: 08/02/2022
Accepted: 14/04/2022

Keywords: *Prunus persica* L.,
Cyclic water deficit, stress
indicators, Sugars, Proteins.



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Conflict of Interest : The
authors declare no conflict of
interest.

Abstract

Climate change, especially in arid and semi-arid areas, affects the production of fruit trees. In this region, fruit tree production requires an efficient water supply that maintaining safe and stable yields. The aim of this work is to study the influence of irrigation modes on the nutritional peach fruit quality and the control of water stress indicators rates. Our experiment was carried out at the CRRRA Sidi Bouzid (Central-West of Tunisia). It focused on four varieties of peach (*Prunus persica* L), two early varieties (Flordastar (FS) and Early Maycrest (EMC)), a seasonal variety (Rubirich (RUB)) and a late variety (O'Henry (O'H)). Three different irrigation treatments were applied to the experimental plot: full irrigation (T1; 100% ETc), sustained deficit irrigation (T2; 50% ETc) and cyclical deficit irrigation (T3). The contents of total sugar, protein, and proline as well as some bioactive compounds and stress indicators (MDA, H₂O₂) were quantified in the exocarp and mesocarp of the fruit. The results showed that O'H fruits are the richest in phenolic compounds, as well as they have significant antioxidant activity. While, both FS tissues accumulated more sugar (55.15 and 81.31g/100g in the mesocarp and exocarp, respectively). Protein level was much higher under T2 and T3 treatments compared to the control treatment (T1) in all varieties. Water stress mainly T2 had significantly stimulated the accumulation of proline in the mesocarp of FS (the content increased from 0.61 to 2.1 μmol/100 g MS). In addition, in the four varieties, the cyclic water treatment (T3) has a significant effect on the accumulation of sugar and phenolic compounds. In conclusion, T3 seems to be the most adequate water regime to be applied in semi-arid region, saving water resources and maintaining fruit quality.

1. INTRODUCTION

For several years, meteorologists have observed change in climatic conditions on a global scale leading to global warming. This climate change leads to pronounced summer drought conditions, causing significant water stress (OTEDD, 2007). The Mediterranean climate is temperate, where hot and dry summers alternate with cold and wet periods from autumn to spring. Production capacity during the summer is vulnerable by drought, associated with high temperatures and low rainfall, leading to high evaporative demand and low soil water

availability. Thus, in Tunisia the average annual rainfall varies from 100 mm to more than 1500 mm in the far north of the country. This situation makes Tunisia a country with limited renewable water resources (Stambouli et al, 2016). These climatic conditions with the scarcity of water resources had severely change the production of fruit trees (Fernandes-Silva et al, 2019). The application of deficit irrigation strategies will maintain yields. Generally, a reduction in irrigation, especially during the fruit development stages, is considered a necessary tool to limit vegetative growth (Maatallah et al, 2015) and increase water productivity in

orchard. In addition, deficit irrigation can be used to optimize yield and improve peach fruit quality (Du et al., 2017). Furthermore, Guizani et al, (2019a) showed that a moderate water deficit improves the quality of peach fruits by favoring an increase in sugar content, as well as an increase in the levels of phenolic compound and antioxidant activity (Buendía et al., 2008). Peach is widely consumed for its taste and nutritional quality. Phenolic compounds represent the main sources of antioxidant capacity. Many properties of the peach are found in its skin (exocarp), so it is interesting to consume this fruit without peeling it (Guizani et al, (2019b), Sara et al, (2020)). So, in this context, our work consists in comparing the variation of the biochemical parameters in the mesocarp and the exocarp of the fruit in four varieties of peach (*Prunus persica* L.) under the effect of different water strategies (T1: Full irrigation; T2: which is based on the uniform restriction of irrigation throughout the season; T3: consists of re-irrigating the soil to field capacity each time the latter decreases to 50%). The present work was devoted to study the variation of the contents of anthocyanin, carotenoid, proline and total sugar, in addition to study the influence of irrigation strategies on the indicators of oxidative stress in the peach fruit.

2. MATERIAL AND METHODS

2.1. Orchard description

The experimental work was conducted in an experimental peach orchard located 3 km south west of Sidi Bouzid (Center-West of Tunisia) (35° 01'21.9" N, 9° 26'31.3" E) and 160 m above sea level during the consecutive seasons of (2016-2017). The region was characterized by typical Mediterranean climate with low rainfall and high temperatures during the summer. The soil is a siltclay-loam. The thirteen year old four peach cultivars Peaches (*P. persica* L. [Batsch]), Flordastar (FS), Early My Creat (EMC), Ribrish (RUB) and O'Henry (OH), were grafted on the Guernem wild rootstock at a spacing of 4m×6m. The four cultivars are divided into two early varieties (FS and EMC), one season (RUB) and one late variety (OH). Irrigation water is pumped from drip irrigation with two pipes per row. During the two experiments seasons all cultivars were similarly fertilized. Soluble fertilizers were applied with the drip irrigation system along the irrigation season. The seasonal fertilizer amounts per year included. Irrigation season

started in late February or early Mars and finished by late September or early October depending on the specific year.

2.2. Irrigation treatments

Three different irrigation treatments were considered in this study. A full irrigation, where trees were irrigated by 100% of crop evapotranspiration (ETc). The irrigations were calculated to replace crop evapotranspiration (ETc), minus any effective rainfall. ETc was estimated by multiplying reference evapotranspiration (ET0) by a crop coefficient (Kc) adapted to peach. Kc was modified according to the stage of fruit development (Ayars et al. 2003): initial Kc was 0.5 during Stage I; mid-season Kc was 0.9 during Stage II and III; and late season Kc was 0.5 after harvest. These crop coefficients corresponded to those usually recommended to fruit growers in the area by agricultural extension services. Continuous water deficit irrigation is below the crop evapotranspiration (50 % ETc) and it is based on the idea of allotting the water deficit uniformly over the whole fruit development cycle and irrigation application reduced to 50% of the Full irrigation (100 % ETc) during the fruit cycle. A cyclic deficit irrigation treatment, it is a moderate stress where soil is re-irrigated to field capacity whenever the soil moisture decreased to 50% (Guizani et al, 2019a).

2.3. Phenolic compounds determination

2.3.1. Dosage of carotenoids

Carotenoids are pigments that are insoluble in water and soluble in a polar solvent such as hexane. The carotenoids were extracted according to the method of Sass-Kiss (2005). A quantity of 0.2g of each variety, was added to 10 ml of solvent containing hexane, acetone, and ethanol (1, 2, 1 V/V/V). After 40 min of stirring, the absorbance is measured at 450 nm. The results are expressed in mg of β -Carotene equivalent per 100g of the dry weight of the fruit.

2.3.2. Dosage of anthocyanins

The anthocyanin concentration was determined according to the procedure described by Melo et al (2006). A mass of crushed peach (1g) is mixed with 10ml of ethanolic solution (ethanol/HCL (1.5M:85:15 V/V) is left in the dark at 4°C overnight. After filtration, the absorbance is

measured at 530 nm. The anthocyanin concentration is obtained using a molar extinction coefficient $\epsilon=38,000 \text{ L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$.

2.3.3. Dosage of Proanthocyanidins

The concentration of proanthocyanidins was determined according to the method described by (Maksimovic et al, (2005). Peach dried fruit extract (0.5 ml) was mixed with 3 ml of butanol-HCl (95: 5; v / v) and 0.1 ml iron sulphate (2g/100 ml) The mixture was incubated at 90°C for 1 h The absorbance was determined at 530 nm The results were expressed in mg equivalent cyanide (EC)/100 g using a molar extinction coefficient of cyanidin:

$$\epsilon = 34.700 \times Mm \text{ cyanidin} \times \text{dilution}$$

2.4. Oxidative stress indicators

2.4.1. Malondialdehyde (MDA) dosage

Lipid peroxidation is estimated by determining the amounts of malondialdehyde according to the method described by Draper. (1990). For the assay, 0.5 g of the plant material was homogenized at 4°C with 5 mL of 5% trichloroacetic acid to precipitate the proteins. The precipitate was pelleted by centrifugation for 30 min at 4°C 5000 rpm and an aliquot of supernatant was reacted with an equal volume of buffer (0.5% TBA solution + 20% TCA + H₂O). Then the solution is boiled in a water bath for 30 min at 95°C. After thermal shock for 2 min and cooling, a second centrifugation was carried out for 5 min. Absorbance was read at 532 nm.

2.4.2. Hydrogen peroxide (H₂O₂)

For the H₂O₂ assay was determined according to the method of Junglee et al. (2014). 0.5g of the dry fruit powder was dissolved in 5mL of a TCA solution (0.1%). Then, centrifugation was performed for 15 min at 1200 rpm. Then, 0.5 mL of supernatant was homogenized with 0.5 mL of phosphate buffer (NaH₂ PO₄ + Na₂HPO at pH=7, and 0.5 mL KI (1M). The solution is then incubated in a water bath at 25° C, for 15 min. Finally, the absorbance was read at 390 nm. The samples were analyzed in triplicate.

2.4.3. Proline dosage

The proline content was measured according to the method of Sánchez et al. (2001). For the assay, 200 mg of fresh material ground in liquid nitrogen was dissolved in 3 mL of sulfosalicylic

acid (filtering if necessary). Then, centrifugation was performed for 15 min at 9000 rpm and at room temperature. Then, 1 ml of ninhydrin solution mixed with 1 ml of acetic acid was added. Then, the samples were put in a water bath at 90° C. for 1 hour. Subsequently, the samples were placed in ice to stop the reaction, then 2 ml of toluene was added with vigorous stirring. Finally, 1 ml of the colored phase was read at 520 nm against a blank (toluene).

2.5. Dosage of total sugars

The extraction of sugars was done according to the method described by Kader et al, (1993) with some modifications. A quantity of 0.1 g of crushed fruit was mixed with 15 ml of 80% ethanol then incubated in a water bath for 15 min at 95°C. The mixture was filtered and then centrifuged at 5000 rpm/10min.

The carbohydrate content is determined according to the method of Dubois et al, (1995). A volume of 0.3 mL of supernatant is mixed with 0.3 mL of phenol (5% m/v) and 1.5 mL of concentrated sulfuric acid. After incubation at 105°C for 5 min, the absorbance is measured at 490 nm. The total sugar content is calculated by referring to a calibration curve obtained with a glucose solution. The results are expressed in mg equivalent of glucose per 100 g of dried fruit.

2.6. Protein assay

Protein extraction was done according to the Bradford method. 1 g of the ground freeze-dried dry matter is mixed with 25 mL of physiological water on the ice cube then filtered. Subsequently, 0.1 mL of the extract (from the filtrate) is taken directly, and 3 mL of Bradford's reagent is added. Finally, the absorbance is measured at 595 nm. Results were expressed in g protein (BSA) per 100 g of dried fruit.

2.7. Statistical analyses

Statistical analyses were performed using SPSS software (version 26 for Windows, SPSS, Chicago, IL, USA). Analysis of variance (ANOVA) was used. Duncan's test was used to compare the means between varieties for each treatment and to compare the treatments of each variety. Values were represented by mean \pm standard deviation. Statistically significant differences between groups were considered when $p < 0.05$.

3. RESULTS AND DISCUSSION

The impact of different water treatments in the content of phenolic compounds of peach fruit in the four varieties studied is presented in Table 1. The results showed a significant difference between the varieties and between the compartments of the fruit (mesocarp and exocarp). Indeed, β -carotene contents varied significantly depending on the varieties and water treatment (Table 1). In control treatment, this compound varied from 5.79 mg/100gDW in to 9.01 mg/100g DW in the mesocarp of RUB and O'H fruits, respectively. While, the highest contents were registered in the exocarp of EMC fruits (12.06 mg/100g DW). Under sustained deficit irrigation (T2), O'H and EMC generated the highest contents especially in the mesocarp (8.4 and 11.32mg/100g DM, respectively). In various cultivars, the concentration of β -carotene recorded in the exocarp was higher than those of the mesocarp. As well, deficit irrigation (sustained and cyclic) has a negative effect on the content of β -carotene in the fruit. Table 1 also illustrated the variation of anthocyanin and proanthocyanin contents among the different water treatments. Indeed,

the ANOVA study showed an inter- and intra-specific effect (varietal effect and irrigation effect), as well as a significant difference between mesocarp and exocarp. Under T1, the exocarp of RUB fruit was the richest in anthocyanins and proanthocyanins (185.5mg/100g DW and 0.82mg /100g DW, respectively), while the lowest concentration was found in FS and EMC. Generally, in the most varieties, these two compounds were concentrated in the exocarp of fruit.

Deficit irrigation significantly increased anthocyanin and proanthocyanin contents in various fruit tissues except in RUB.

T2 and T3 induced a significant increase in the contents of phenolic compounds that varied among variety and treatment applied. Pliakoni and al (2010) and Falagán and al (2015) found similar results in nectarines. In addition, according to Boudiaf (2006), the content of polyphenols reflects the antioxidant power through the direct scavenging of reactive oxygen species (ROS).

Fig. 1 showed MDA variation in mesocarp and exocarp tissues under different water treatments. Under control conditions, the mesocarp of O'H and the exocarp of RUB fruits

Table 1. Phenolic compounds' concentrations (mg /100g DW) evaluated in the mesocarps and exocarp of four *Prunus persica* cultivars subjected to three irrigation treatments during the 2016 and 2017 seasons.

Varieties	Treatments	B-carotene (mg/100gDW)		Anthocyanin (mg/100gDW)		Pro-anthocyanin (mg/100gMS)	
		Mesocarp	Exocarp	Mesocarp	Exocarp	Mesocarp	Exocarp
FS	T1	6.47Bb	10.88Wx	22.46Ba	173.01Wx	0.14Aa	0.25Xx
	T2	3.47Aa	4.93Xy	21.11Ca	110.62Xy	0.12Ba	0.11Yy
	T3	4.15Bb	5.09 Wy	7.97Cb	105.69Xy	0.09Ca	0.12Xy
RUB	T1	5.79 Ba	11.48Wx	76.5Aa	185.5Wx	0.17Aa	0.82Wx
	T2	5.5Ba	6.04 W Xy	44.98Ab	133.5Wy	0.13Ba	0.82Wx
	T3	5.86 ABa	3.5 Xy	25.01Bc	117.6Wz	0.13Ca	0.28Wy
OH	T1	9.01Aa	10.53Wx	27.27Bc	27.77Xx	0.21 Ab	0.25Xy
	T2	8.4Ba	11.2Wx	34.4Bb	36.05Yx	0.30Aa	0.35 Xx
	T3	8.07 Aa	5.0Wx	63.67 Aa	11.8Zy	0.33Bab	0.11Xz
EMC	T1	5.96Ba	12.06Wx	22.01Bb	31.5Xy	0.19Ab	0.33Wx
	T2	5.06 Ba	11.32Wx	45.49Aa	30.01Zz	0.23Bb	0.26Xx
	T3	5.8ABa	4.58W Xy	25.53Ba	38.34Yx	0.49Aa	0.10Xy

Values are the means of three different peach fruit samples ($n = 3$) \pm standard deviation. The lowercase letters [a], [b], [c], [d] indicate significant differences ($p \leq 0.05$) among the four cultivars for each treatment separately. The uppercase letters [A], [B], [C], indicate significant differences ($p < 0.05$) among the three irrigation treatments, respectively for each season separately. T1, full irrigation; T2, sustained deficit irrigation; T3, cyclic deficit irrigation; FS, Flordastar; EMC, Early May crest; RUB, Rubirich; O'H, O'Henry

were the highest producers of MDA (0.75 and 3,33 $\mu\text{mol}/100\text{g DW}$, respectively). However, deficit irrigation did not affect significantly the MDA content (except EMC). As the product of plant oxidative damage, MDA reflects the degree of membrane lipid peroxidation and oxidative stress (Lyons, 1973). The results of the variation of H_2O_2 contents under different irrigation strategies is illustrated in Fig. 2. H_2O_2 concentrations fluctuated between

0.05 and 0.23 $\mu\text{mol}/100\text{g DW}$ in the mesocarp of control treatment. In the exocarp, the highest levels were measured in RUB and the lowest were those quantified in EMC (0.23 $\mu\text{mol}/100\text{g DW}$). Water treatments (T2 and T3) induced a high production of H_2O_2 in all varieties. Under cyclic deficit irrigation, the O'H variety recorded a significant increase in H_2O_2 (0.53 $\mu\text{mol}/100\text{g DW}$ in the mesocarp) followed by EMC (0.35 $\mu\text{mol}/100\text{g DW}$).

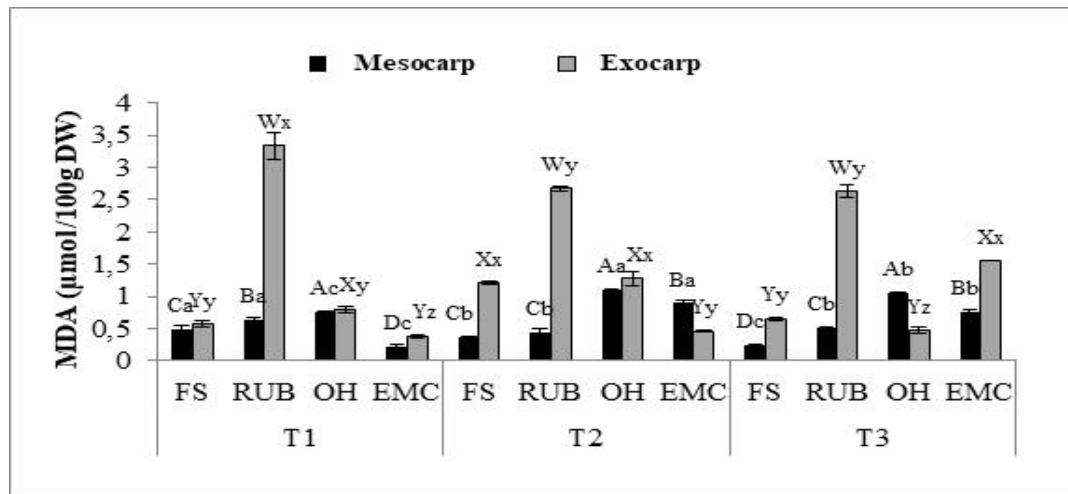


Fig. 1. Variation in the MDA concentration in the exocarp and mesocarp of the fruits of four *Prunus persica* L. Cultivars exposed to three different irrigation strategies.

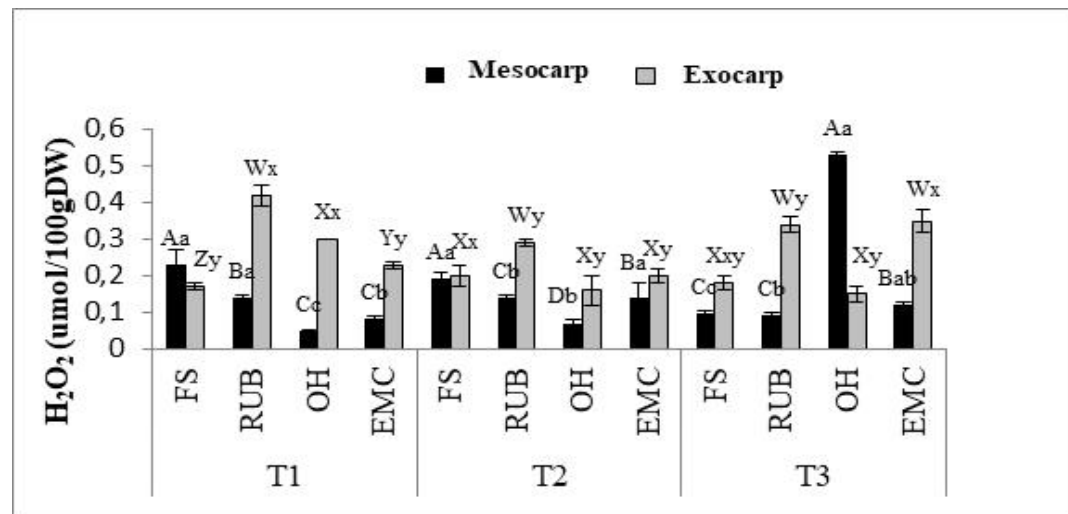


Fig. 2. Variation in the H_2O_2 concentration in the exocarp and mesocarp of the fruits of four *Prunus persica* L. Cultivars exposed to three different irrigation strategies.

Values are the means of three different peach fruit samples ($n = 3$) \pm standard deviation. The lowercase letters [a], [b], [c], [d] indicate significant differences ($p \leq 0.05$) among the four cultivars for each treatment separately.

The uppercase letters [A], [B], [C], indicate significant differences ($p < 0.05$) among the three irrigation treatments, respectively for each season separately. T1, full irrigation; T2, sustained deficit irrigation; T3, cyclic deficit irrigation; FS, Flordastar; EMC, Early May crest; RUB, Rubirich; O'H, O'Henry

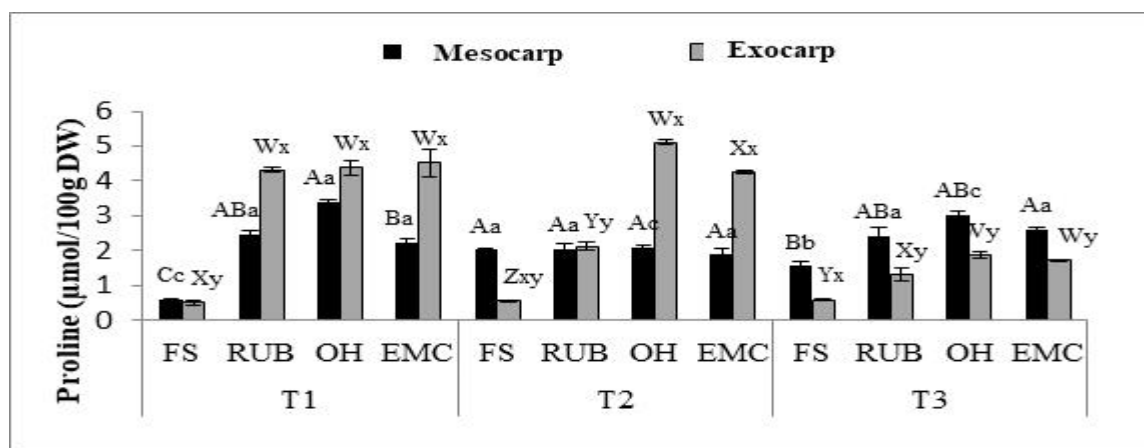


Fig. 3. Variation in the Proline concentrations in the exocarp and mesocarp of the fruits of four *Prunus persica* L. cultivars exposed to three different irrigation strategies.

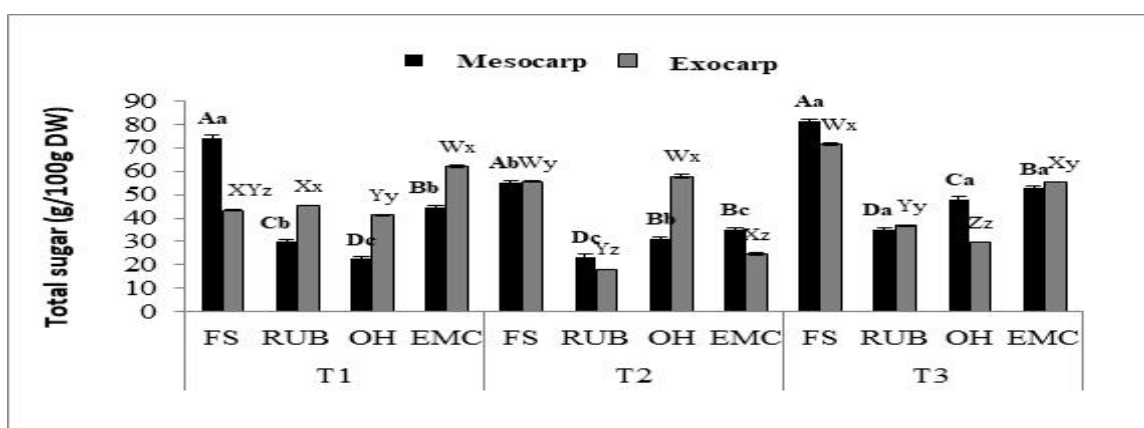


Fig. 4. Variation in the Total sugar concentration in the exocarp and mesocarp of the fruits of four *Prunus persica* L. cultivars exposed to three different irrigation strategies.

For Figs. 3 & 4, values are the means of three different peach fruit samples ($n = 3$) \pm standard deviation. The lowercase letters [a], [b], [c], [d] indicate significant differences ($p \leq 0.05$) among the four cultivars for each treatment separately. The uppercase letters [A], [B], [C], indicate significant differences ($p < 0.05$) among the three irrigation treatments, respectively for each season separately. T1, full irrigation; T2, sustained deficit irrigation; T3, cyclic deficit irrigation; FS, Flordastar; EMC, Early May crest; RUB, Rubirich; O'H, O'Henry.

Under control treatment, proline concentrations varied in the mesocarp from 0.38 to 0.61 $\mu\text{mol}/100\text{g DW}$ (Fig. 3). Water deficit, mainly T2 treatment, significantly stimulated the accumulation of proline in the mesocarp of FS (2.1 $\mu\text{mol}/100\text{gMS}$). Fig. 3 also showed a significant increase in the content of this amino acid in the mesocarp of EMC and RUB varieties (2.61 and 2.4 $\mu\text{mol}/100\text{g DW}$, respectively). Proline plays an important role in stress tolerance. Its accumulation is the first response of plants exposed to water deficit and it reduced the alterations inside the cells (Anjum et al., 2011). In the mesocarp of the FS, EMC, O'H and EMC varieties, T2 treatment caused a significant rise in proline content compared to T3. Proline plays an important role not only as an osmolyte

conferring osmotic adjustment, but also in ROS detoxification and cell stability (Anwar Hossain et al., 2014).

The analysis of the results presented in Fig. 4 showed that soluble sugar contents changed with variety and water treatment. Under T1 treatment, FS fruits were the sweetest. While those of O'H had the lowest content (41.13 g/100g DW). The application of water deficit significantly increased the sugar contents in all varieties. Indeed, T2 and T3 caused the accumulation of sugar contents specially in FS tissues (55.15 and 81.31 g/100g DW in the mesocarp and the exocarp, respectively). Our study confirmed that fruits from trees exposed to water stress have the richest fruits in sugars, making it possible to maintain high cellular

integrity in plant tissues (Kameli and Losel,

Acknowledgements

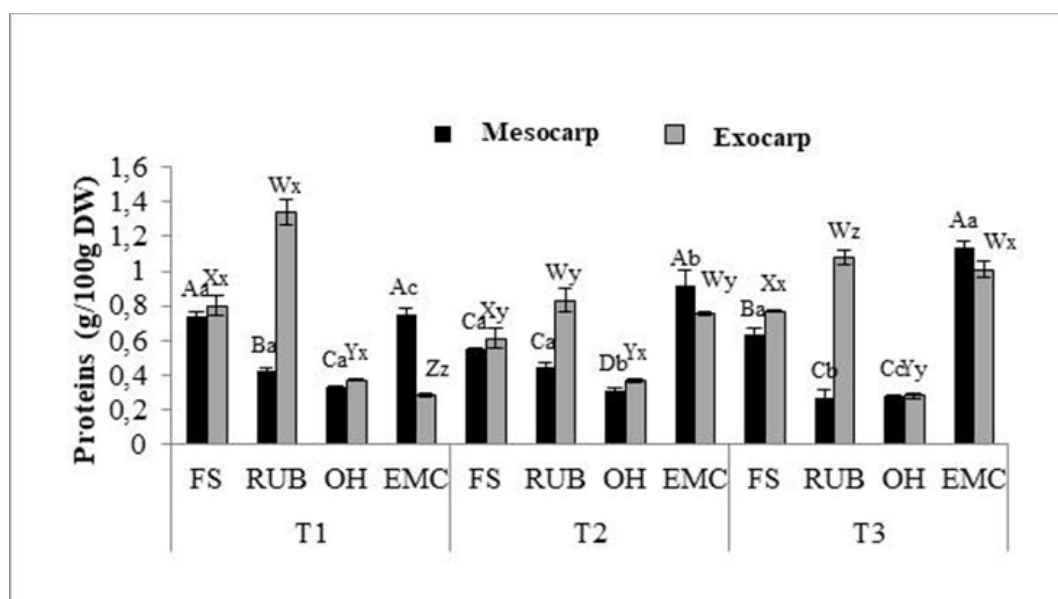


Fig. 5. Variation in the Protein concentrations in the exocarp and mesocarp of the fruits of four *Prunus persica* L. cultivars exposed to three different irrigation strategies.

Values are the means of three different peach fruit samples ($n = 3$) \pm standard deviation. The lowercase letters [a], [b], [c], [d] indicate significant differences ($p \leq 0.05$) among the four cultivars for each treatment separately. The uppercase letters [A], [B], [C], indicate significant differences ($p < 0.05$) among the three irrigation treatments, respectively for each season separately. T1, full irrigation; T2, sustained deficit irrigation; T3, cyclic deficit irrigation; FS, Flordastar; EMC, Early May crest; RUB, Rubirich; O'H, O'Henry.

1995).

The results of the variation of proteins in the mesocarp and the exocarp under various water treatments are illustrated in Fig. 5. FS and EMC was the most concentrated in proteins (0.74 and 0.75 g/100g DW, respectively). However, in the exocarp, RUB fruits showed the highest protein contents (1.34 g/100g DW). The application of sustained or cyclic deficit irrigation did not affect the protein content in the various cultivars except in EMC (1.14 g/100g DW under T3 treatment).

4. CONCLUSION

Peach fruits (*Prunus persica* L.) are considered an inexhaustible source of nutritious substances and bioactive compounds, such as phenolic compounds (anthocyanins, pro-anthocyanins, ...) which have significant antioxidant activity that can be exploited in the food industry and even in medicine. This study confirmed that dark colored fruits (RUB and O'H) were the most concentrated in total phenolic compounds and had the highest anthocyanin contents.

According to the various parameters studied, sustained and cyclic deficit irrigation, in the study area, could improve fruit quality while conserving water resources.

This research was financially supported by the Tunisian Ministry of Higher Education and Scientific Research for Monia Guizani's scholarship. We thank the technical staff of the Regional Center of Agricultural Research, Sidi Bouzid, Tunisia for their assistance in the experimental work and orchard.

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