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Saline Water Irrigation Effects on Fruit Development, Quality, and Phenolic Composition of Virgin Olive Oils, Cv. Chemlali

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Field-grown olive trees (cv. Chemlali) were used over two growing seasons to determine the effect of different saline water irrigation levels on fruit development characteristics, yield, and virgin olive oil (VOO) quality. The plants were irrigated with fresh water (FW; ECe = 1.2 dS m^{-1}) and saline water (SS; ECe = 7.5 dS m^{-1}). Fruit weight, olive, and oil content decreased under irrigation with saline water. Total oil contents were 27.85 and 25.7% fresh weight (fw) during 2005 in FW and SS irrigated plants, respectively. However, major phenolic compounds (tyrosol, hydroxytyrosol, vanillic,...) and total phenol concentrations in VOO increased under saline water irrigation. In 2005, total phenol contents were 198 and 223 mg/kg of oil in FW and SS treatments, respectively. Furthermore, VOO from SS treated plants showed higher contents of oleic, linoleic, linolenic, and heptadecanoic acids than FW ones, and oil samples of both treatments were classified as "extra virgin".

KEYWORDS: Salinity; oil yield; oil quality parameters; fatty acid composition; oxidative stability

INTRODUCTION

Traditionally, olive trees have been cultivated under rain-fed conditions. In recent decades, the olive plantation has been extended to irrigated lands. However, in arid and semiarid regions such as those in Tunisia, the limited water availability and the increased need for good water quality for urban use restrict the use of fresh water for irrigation. Therefore, large quantities of marginal water, such as saline water, are used for olive tree irrigation [33% of irrigated lands are saline water irrigated (16000 ha)].

Most papers dealing with the assessment of olive water needs have reported that the olive tree is characterized by its limited water requirements and its tolerance to salinity (*1*−*3*). Existing data on the effects of salinity conditions on yield, quality, and phenolic composition of virgin olive oil are few and sometimes contradictory. In 1994, Cresti et al. (*4*) have signaled that salinity alters pollen germination and fruit set and that it causes the increase of both aliphatic and triterpenic alcohol contents in oil and the percentage of linoleic acid. According to Klein et al. (*5*), olive irrigation with saline water (ECe = 6.5 dSm^{-1}) increased the fruit dry weight and oil percentage. The same authors stated that no effects were recorded when irrigation was made with water of <4.5 dSm^{-1}. Similarly, Bouaziz (*6*) did not record any effect of irrigation with brackish water (up to 4 g/L of solid residue) on yield and oil percentage of some olive varieties grown intensively in the central part of Tunisia. However, in Israel, Wiesman et al. (*7*) reported that young Barnea olive trees irrigated with intermediate saline water (4.2 dSm^{-1}) produced 20% higher yield than those irrigated with high-saline water (7.5 dSm^{-1}).

Besides, it has been shown that olive yield response to salinity is planting density dependent (*8*). The effects of salt treatment at ECe = 4.5 dSm^{-1} were a 12% increase in oil yield for the 830 trees/ha planting density and an 18% decrease for the 410 trees/ha one. However, at high salinity (ECe = 7.5 dSm^{-1}), the decreases of oil yield were 89 and 74% of the control in the high and low planting densities, respectively (*8*).

With regard to oil quality, Wiesman et al. (*7*) showed that olive oil produced under high water salinity level has higher amounts of total phenols. In addition, the ratio of unsaturated/saturated fatty acids decreased significantly at moderate and high salinity levels. In contrast, Bouaziz (*6*) did not signal any changes in the fatty acid composition of the oil when olive trees were irrigated, for 12 years, with brackish water.

Overall, olive salt tolerance was assumed to be cultivar dependent (*9*−*11*). According to Benlloch et al. (*9*), 'Lechin de Granada' olive was more tolerant to sodium excess than 'Manzanillo', and the latter was less tolerant to boron excess

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than 'Picual'. Furthermore, Aragüés et al. (*11*) pointed out that salt tolerance of the olive tree, in terms of trunk growth, was high in the first trial year (electrical conductivity of the extract, $ECe_{thr} = 6.7\ dSm^{-1}$), but declined with age and time of exposure to salts by 30% in the second year ($ECe_{thr} = 4.7\ dSm^{-1}$) and by 55% in the third trial year ($ECe_{thr} = 3.0\ dSm^{-1}$). More recently, Weissbein et al. (*12*), comparing vegetative and reproductive response of 12 olive cultivars to moderate saline water irrigation, have shown that the overall yield average of tested olive cultivars varied from 3 to 10 kg/tree. Salt tolerance difference among olive cultivars has been also noted by Al-Absi et al. (*13*), who pointed out that olive tree response to salinity resulted from the interactions of cultivar, ionic composition, and the electrical conductivity of the external solution.

Nowadays, the controlled use of marginal water (saline water, treated wastewater) to improve the qualitative characteristics of horticultural products is becoming more and more important (*14*), particularly under actual conditions of limited water resources and rainfall scarcity in arid regions. For fruit trees, there is some evidence that saline water could improve yield. However, there are few works on the qualitative effects of saline water irrigation on olive tree growing. To the best of our knowledge, there is no study on the effect of saline water on phenolic composition concentrations in virgin olive oil.

The objectives of this study were to determine the effects of saline water used for irrigation on fruit development and the quantitative and qualitative parameters of virgin olive oil (VOO) obtained from trees of the cv. Chemlali grown at a high-density orchard. In particular, we are interested in the fatty acid composition and concentrations of phenols of VOO and the evolution of soil moisture and salinity with soil depth and around the irrigation source. Our experimental approach allows us to improve the understanding of the qualitative response (oil quality) of field-grown Chemlali olive tree to saline water irrigation in arid regions in Tunisia.

MATERIALS AND METHODS

Plant Material, Treatments, and Climatic Conditions. Olive trees (*Olea europaea* L. cv. Chemlali), planted in 1992 in a sandy soil at a density of 625 trees ha^{-1} at Sfax, Tunisia (34° 43 N, 10° 41 E), were used in 2004 and 2005. The sandy soil of the experimental orchard (90.5% sand, 4.5% clay, and 5% silt) was characterized by an organic matter content of 1.1%, 13.4% $CaCO_3$, 1.3% N, pH of 7.6, a field capacity (measured at 33 KPa) of 11.8%, and a wilting point (measured at 1500 kPa) of 5.9%.

In 2004, 10 trees from two adjacent rows (total 20 trees per treatment), with four replications of 5 trees each, were selected to be similar in potential yield and canopy. The Chemlali olive trees were subjected to the following treatments: irrigation with fresh water, 1.2 dS m^{-1} ECe (FW); and saline water, 7.5 dS m^{-1} ECe (salt stress, SS). The water used was either that supplied by the Tunisian National Water Carrier (FW) or saline water (SS) from the local reservoir situated in the area of the Olive Tree Institute in Sfax. The fresh and saline waters used were characterized by 145 and 600 mg/L Na^+, 326 and 1169 mg/L Cl^-, 280 and 520 mg/L K^+, 94 and 261 mg/L Ca^{2+}, 57 and 102 mg/L Mg^{2+}, respectively.

The amount of water supplied to olive trees was estimated according to the Penman−Monteith−FAO equation (*15*) as described by Ben Ahmed et al. (*1*). The irrigation was delivered using a drip system with four drip nozzles (two per side), of 4 L h^{-1} per tree, set in a line along the rows (at 0.5 m from the trunk). Without rainfall taken into account, total water supplied to mature olive trees was 4000 m^3/ha/year. The plants were subjected to the same olive cultivation practices in the area.

The region is characterized by an arid climate of Mediterranean type. Annual rainfall and temperature averages over a 52-year period were 250 mm and 23 °C, respectively. In both crop seasons, precipitation was virtually absent during the summer and it was 218.5 and 285.5 mm, respectively, in the first and second crop seasons. The mean temperatures were 25.6 and 25.1 °C, respectively, and maximum temperatures were 38 and 37 °C, respectively. The evapotranspiration rates were 1413 and 1271 mm in 2004 and 2005, respectively.

Soil Moisture and Salinity Measurements. Analyzed soil samples were taken from the surface until a depth of 1.2 m with a layer of 0.3 m. On these samples, the soil moisture (%) and the electrical conductivity (ECe) of the saturated phase were determined. The ECe was determined also at different distances (0, 0.15, 0.3, and 0.6 m) from the irrigation source.

Fruit Growth Characteristics, Maturation Index, and Yield. Fruit weight (FrW), fruit diameter (FD), fruit volume (FV), and fruit water content (FWC) of harvested olives were taken, during both crop seasons, four times per month from June to December. In every measurement, 60 olives from four plants per treatment (15 olives per plant) were collected for characterization. The whole fruit water content was calculated as:

$$\text{FWC}\ (\%) = \frac{\text{fw} - \text{dw}}{\text{fw}} \times 100$$

where fw is the fresh weight and dw the dry weight of fruit samples.

Immediately before harvest, the olive maturation index was determined according to the procedure described by Motilva et al. (*16*). This method is based on the evaluation of the olive skin and pulp colors of 100 olive fruits. The 0−7 scale of fruit maturity index consists of eight groups: intense green (group $N = 0$), yellowish green (group $N = 1$), green with reddish spots (group $N = 2$), reddish brown (group $N = 3$), black with white flesh (group $N = 4$), black with <50% purple flesh (group $N = 5$), black with ≥50% purple flesh (group $N = 6$), and black with 100% purple flesh (group $N = 7$). The maturation index is expressed as $\Sigma\ (N_i n_i)/100$, where N is the group number and n is the fruit number of its respective group. For the oil analyses, three samples of 4 kg of fruits each were harvested for each treatment at a maturation index of 6. Total oil content (% fw) was determined by extracting material with 50−60 °C petroleum ether using a Soxhlet apparatus (*17*). For olive yield determination, 10 trees per treatment were chosen, and the harvest was made in mid-December of each year manually to guarantee accuracy.

Oil Mechanical Extraction Process. Olive oil used for analysis was extracted using a laboratory olive Bench Hammer Mill (Abencor Analyzer, MC2 Ingenierias y Sistemas, Sevilla, Spain). After fruit crushing and malaxation for 30 min at 25 °C, centrifugation and decantation allow the separation of oil. The amount obtained was measured. Oil samples were filtered, transferred into amber glass bottles, and stored at 4 °C in darkness until analysis. Total oil content was expressed on a fresh weight basis (% fw).

Oil Quality Indices. *Extinction Coefficients.* Extinction coefficients K_{232} and K_{270} were measured at 232 and 270 nm, respectively. Free acidity and peroxide value expressed as milliequivalents of active oxygen per kilogram of oil (mequiv of O_2/kg) were measured following the analytical method described in European Regulation EEC 2568/91 (*18*).

Fatty Acid Composition. Fatty acid composition was determined on the basis of European Regulation EEC 2586/91 method (*18*). The methyl esters were prepared by vigorous shaking of a solution of oil in hexane (0.2 g in 3 mL) with 0.4 mL of 2 N methanolic potassium hydroxide and analyzed by gas chromatography with a FID detector. A fused silica column (15 m length × 0.25 mm) was coated with SGL-1000 phase (0.15 μm thickness). The carrier gas used during the process was nitrogen. The injector and detector temperatures were set at 250 °C, and the oven temperature was set at 180 °C.

Chlorophyll and Carotenoid Concentrations. The chlorophyll fraction at 670 nm and the carotenoid fraction at 470 nm were evaluated from the absorption spectrum of each VOO sample (7.5 g) dissolved in cyclohexane as described by Mínguez-Mosquera et al. (*19*).

Oxidative Stability. Oxidative stability is evaluated using a 679 Rancimat apparatus (Metrohm, Switzerland) at 120 °C and 20 L h^{-1} air flow (*20*). The oil stability is expressed as the induction time (hours) of hydroperoxide decomposition.

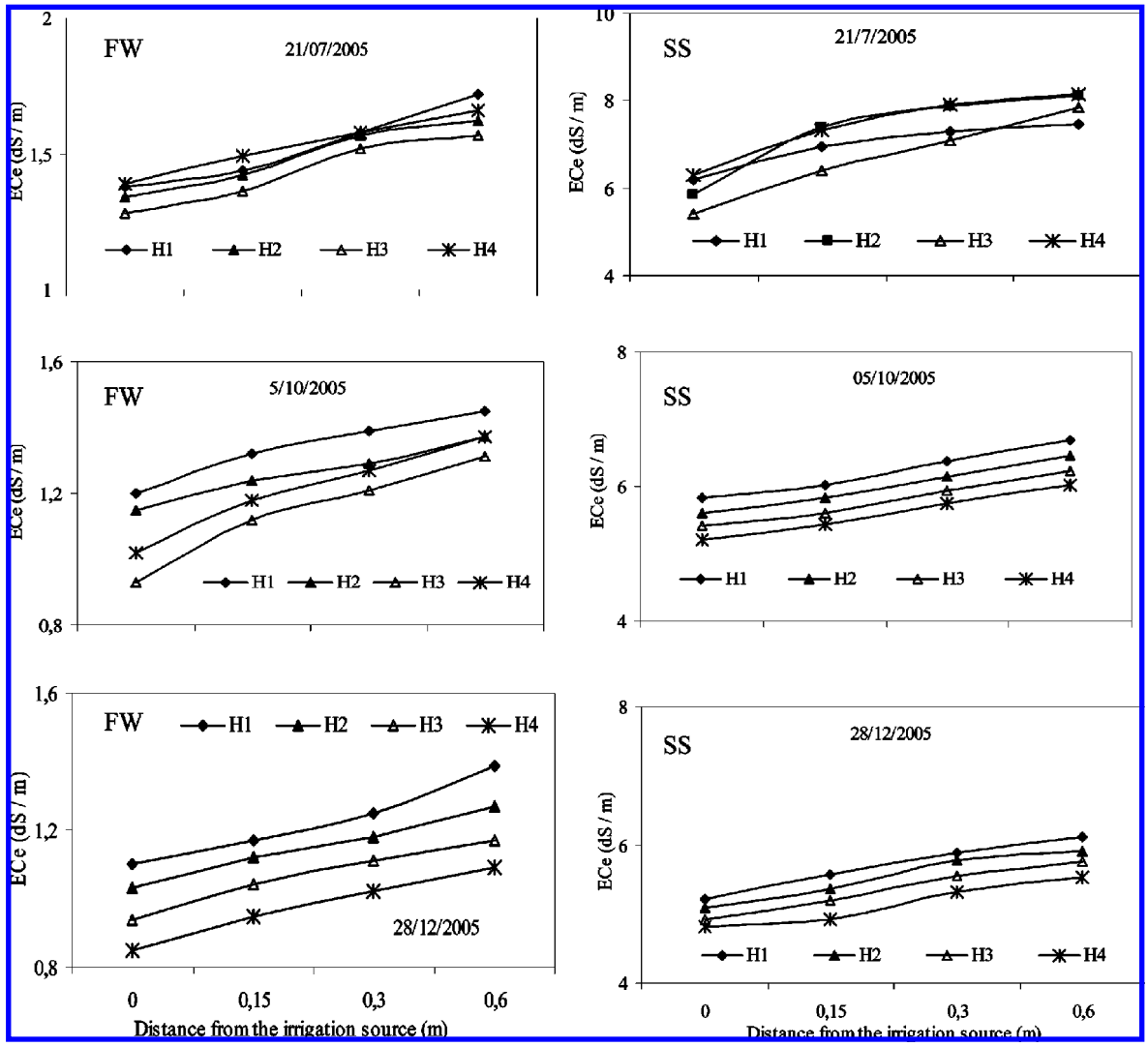

Figure 1. Vertical and horizontal distributions of soil salinity at different distances from the irrigation source in FW (left) and SS (right) during the 2004/2005 crop season. H1, H2, H3, and H4 represent the different soil depths from the surface (30, 60, 90, and 120 cm, respectively). Values are the means of three soil sample measurements ($n = 3$).

Total Phenols and Phenolic Compounds. The concentration of total polyphenols was estimated with Folin−Ciocalteu reagent at 725 nm (*21*). Results were expressed as milligrams of caffeic acid per kilogram of oil.

The different phenolic compounds analyzed were determined from VOO as described by Patumi et al. (*22*). Briefly, a sample of olive oil (14 g) was extracted four times using methanol/water (4:1 v/v). Methanol was removed, and then acetonitrile was added (15 mL) to the residue and washed with hexane. The resulting acetonitrile solution was evaporated under vaccum, and the residue was flushed with nitrogen and dissolved in 1 mL of methanol/water. The final extract was filtered through a membrane filter and transferred into a tube. The extract was injected to the HPLC system as 20 μL. The standards used in the quantification of phenolic substances are tyrosol, hydroxytyrosol, vanillic acid, caffeic acid, syringic acid, *p*-coumaric acid, ferulic acid, *o*-coumaric acid, oleuropein, glycoside oleuropein, and ferulic acid.

The HPLC analysis was performed using an Agilent system 1100 series, which consisted of a Hewlett-Packard Quaternary coupled with HP Chemstation Software, a column, and a diode array UV detector. The analytical separation was achieved on a Lichosphere 100 RP-18.5 μm column (250 mm × 4 mm i.d.). The elutes were detected at 278 nm. The different phenolic compounds were identified according to their order of elution and their retention times compared to those of the standard ones.

Statistical Analysis. Statistical analyses were performed using SPSS 10 for Windows, and treatment means were compared using the least significant difference (LSD) test at $P < 0.05$. At least three replicates were used for each laboratory test.

RESULTS AND DISCUSSION

Soil Moisture and Salinity. The soil salinity variation was greater in soil irrigated with saline water than in that irrigated with fresh water. For both treatments, there is a slight decrease in ECe through the soil depth (**Figures 1** and **2**). Furthermore, higher salt accumulation was registered at a soil layer of 0.3 m. In all soil depths, the salts were more accumulated during the summer season, and salt distribution through the soil depth was affected by autumn−winter rainfall. The seasonal variation of soil salinity in the 1.2 m depth showed that it was lower during the autumn−winter period than during the spring−summer one. The layer with lower ECe showed also higher soil moisture values (**Table 1**). This was displayed more via the relationship determined between the soil moisture and soil salinity (**Figure 3**). In fact, the lower the soil moisture was, the higher the soil salinity. The higher ECe observed in the 30 cm depth, in comparison to other layers (30−120 cm), was due to the higher evaporation occurring in the surface as reported by Aragüés et al. (*2*). Salts are more accumulated in dry layers.

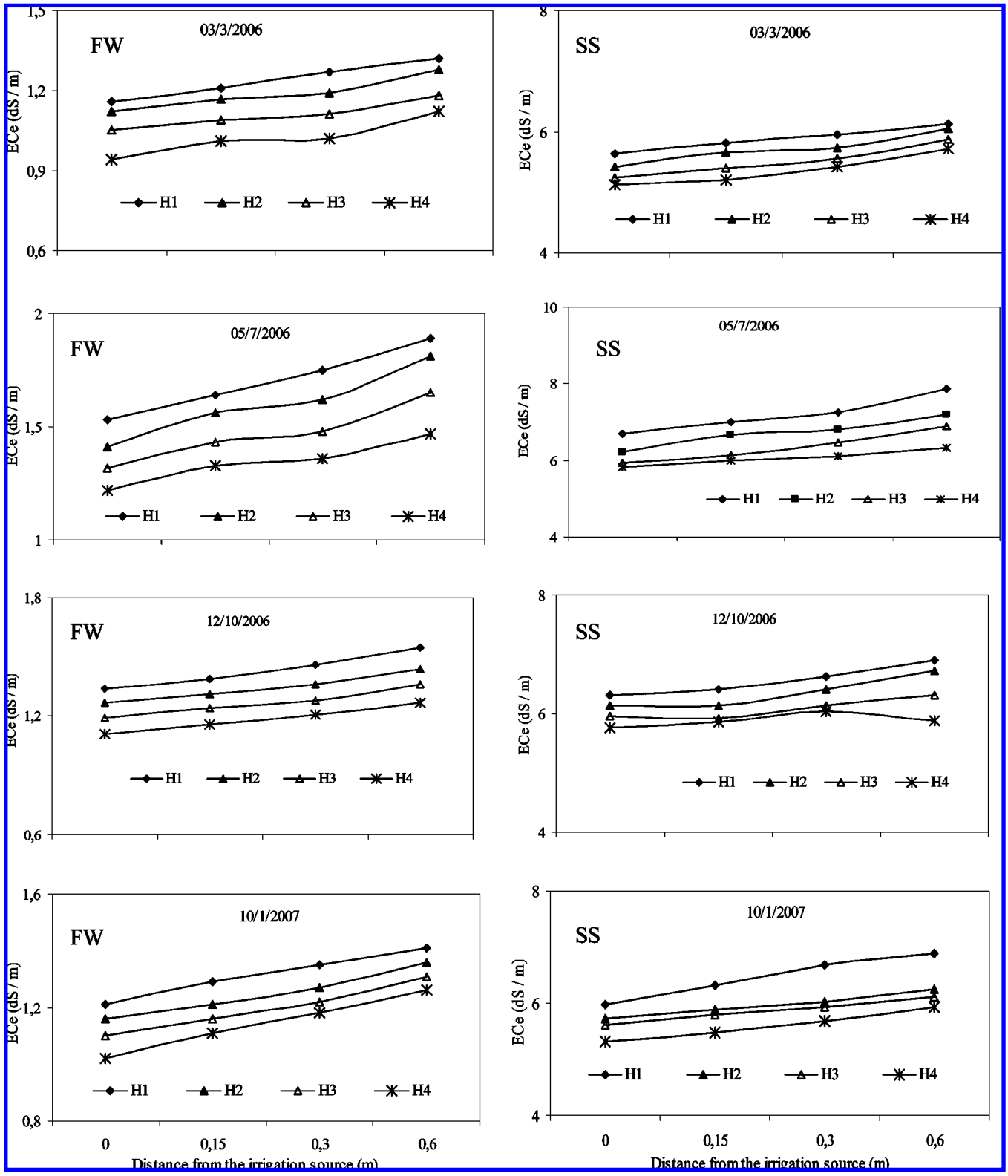

Figure 2. Vertical and horizontal distributions of soil salinity at different distances from the irrigation source in FW (left) and SS (right) during the 2005/2006 crop season. H1, H2, H3, and H4 represent the different soil depths from the surface (30, 60, 90, and 120 cm, respectively). Values are the means of three soil sample measurements ($n = 3$).

The horizontal variation of soil salinity displayed that salts are less accumulated in the drip zone (0−15 cm), in comparison to the more outlying ones (30 and 60 cm). The lower level of soil salinity registered until a depth of 1.2 m, in comparison to the layer of 0.3 m, suggesting that salts are transported and accumulated at higher depth and that the rainfall occurring generally in autumn and winter was sufficient to ensure the leaching of salts, accumulated during summer season, which was facilitated by the sandy soil texture (90.5% sand). Besides, the irrigation source location (at 0.5 m from the trunk) would play a great role in the distribution of soil salinity and consequently in the maintenance of plant water status at

Table 1. Distribution of Soil Moisture (Percent) at Different Layers[a] in FW and SS Treatments during 2004 and 2005 Crop Seasons

	FW				SS			
period	H1	H2	H3	H4	H1	H2	H3	H4
March 2005	9.6	10.9	11.4	12.3	8.9	10.4	11.5	11.9
July 2005	9.5	10.4	11.8	12.5	8	8.9	9.8	10.4
October 2005	8.4	9.3	10.1	10.8	9.4	10.8	11.6	12.2
December 2005	7.2	8.5	9.3	9.9	10.6	12.2	13.2	14.3
March 2006	9.8	11.3	11.9	12.5	9.2	10.5	11.62	12.2
July 2006	8.8	10.5	12.4	12.9	6.8	7.9	8.4	9
October 2006	11.2	12.6	13.5	14.5	8.4	10.2	11.9	12.4
January 2007	10.4	11.6	13.2	14.3	9.9	11.2	12.9	13.9

[a] H1, H2, H3, and H4 represent the different soil depths from the surface (30, 60, 90, and 120 cm, respectively). Values are the means of three soil sample measurements ($n = 3$).

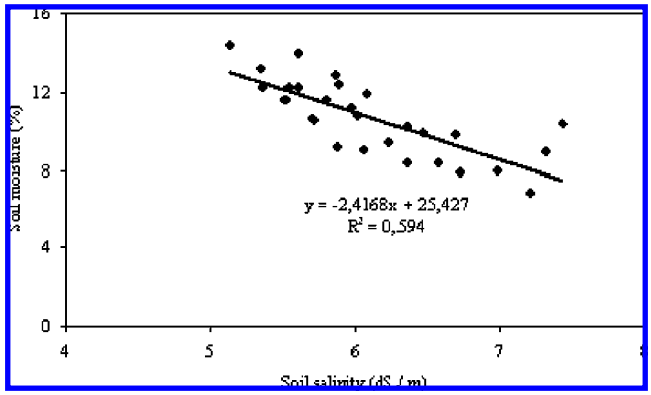

Figure 3. Relationship between soil moisture and soil salinity in SS treatment when soil layers were pooled together ($n = 27$).

acceptable level (*3*). Furthermore, soil salinity distribution is the result of the interaction of water salinity level, irrigation source location, soil texture, and climatic conditions.

In addition, the active root zone in olive could affect soil salinity distribution. Indeed, the high ability of olive trees to accumulate salts in their active roots, generally localized at a depth superior to 30 cm, allows the decrease of soil salinity level. This strategy is commonly developed by the salt-stressed plants to decrease the osmotic potential in the roots, via accumulation of inorganic salts, to activate water retention and transport from the soil to the plant (*3*).

Fruit Characteristics and Yield. During both crop seasons, salinity has altered fruit diameter (FD), fruit volume (FV) (**Figure 4**), and fruit weight (FrW). For both salt treatments, FrW averages increased markedly with time. In June 2004, their values were 0.38 and 0.28 g in FW and SS treated plants, respectively, and they reached 1.33 and 1.05 g, respectively, in December. In 2005, these values were 0.44 and 0.36 g in FW and SS treated plants and reached 1.38 and 1.22 g, respectively, for the respective periods. These results showed that the increase was more important under irrigation with fresh water. Furthermore, the fruit fresh weight in SS plants was statistically lower than that in FW ones ($P = 0.0056$). In 2004, FW plants showed higher fruit water content values than the SS ones (53.39 and 50.88% in FW and SS, respectively). However, in 2005, the two irrigation treatments showed almost similar values (51.43 and 52.27%, respectively) for which differences were not statistically significant. The nonsignificant differences in FWC values between the two crop seasons for both treatments revealed the role of the active root zone of Chemlali olive in upholding a suitable hydration level for its tissues. This was more displayed via the comparable FWC values of SS treated plants, although not significant, in comparison to those of fresh water irrigated ones.

The average olive production of SS plants during the experimental period (15.5 kg tree^{-1}) was much lower (42%) than that of FW ones (27 kg tree^{-1}). For both treatments, the first crop season was marked by higher olive yield (**Table 2**). The yield variation in both treatments could be due to the alternate bearing phenomenon characterizing olive tree production or the effects of climatic conditions characterizing the experimental site. Generally, the fruit development phase (from June to December) coincides with a period of high temperature and radiation which, even under irrigation, can affect fruit growth and, hence, olive yield.

The decrease of fruit weight and olive yield under salinity conditions has been also reported by Klein et al. (*5*). However, Bouaziz (*6*) did not record any effect of irrigation with brackish water on olive production. The increased mean fruit weight recorded in 2005, for both treated plants, could be due to the decrease of fruit set. The same results have been noted in olive cv. Leccino by Gucci et al. (*23*), who have demonstrated that, at harvest, the fruit fresh weight decreased as crop load increased; however, such tendency was not apparent for severe deficit irrigated plants. Similarly, Wiesman et al. (*7*) have indicated that the most productive saline treatment (4.2 dSm^{-1}) yielded smaller olives, whereas high saline treatment (7.5 dSm^{-1}), with lower yield, produced larger olives in terms of both fruit weight and diameter.

During both crop seasons, the irrigation treatments did not affect oil accumulation in the Chemlali olive tree as no statistically significant differences were observed between total oil content (% fw) of the two treatments ($P > 0.05$). It was 27.8 and 30.5% fw in FW and 25.7 and 28.3% fw in SS during the 2004 and 2005 crop seasons, respectively (**Table 3**). If we compare the reduction in olive production with that in oil content, a more drastic effect of salt stress was found on olive yield than on oil yield.

In contrast with previous studies (*5, 7, 8*), the high saline treatment SS we applied to Chemlali olive tended to decrease oil content relative to FW treated plants (although not significantly so). In the case described by Wiesman et al. (*7*), there were clear increases of total oil content by 25 and 10% under 4.2 and 7.5 dS m^{-1}, respectively, in comparison to control treatment (1.2 dSm^{-1}). In Klein et al. (*5*), the increase of oil content was observed only in the early years of the high-density planted trees treated with moderate saline water. Furthermore, the nonstatistically significant difference in oil content between both saline water treatments, in comparison to olive yield, is certainly an advantage for the use of saline water for Chemlali olive cultivation, being extended to saline irrigated lands in arid regions in Tunisia. Nevertheless, such practice needs a long-term period of saline water irrigation to confirm these results.

Oil Quality Indices. During the 2004 crop season, free acidity ranging from 0.24 to 0.25% and peroxide value ranging from 2.9 to 3.2 mequiv of O_2 kg^{-1} of the different olive oils samples, respectively, in SS and FW were considerably lower than the upper limit of 0.8% as oleic acid and 20 mequiv of O_2 kg^{-1} as the peroxide value established by EU legislation for extra virgin olive oil. Moreover, these two quality indices were not influenced by water quality treatment, because no statistically significant differences were observed between both treatments ($P > 0.05$). In the second crop season, the free acidity and peroxide value increased in both treatments, if compared to those recorded during the first one (**Table 3**). The free fatty acid level

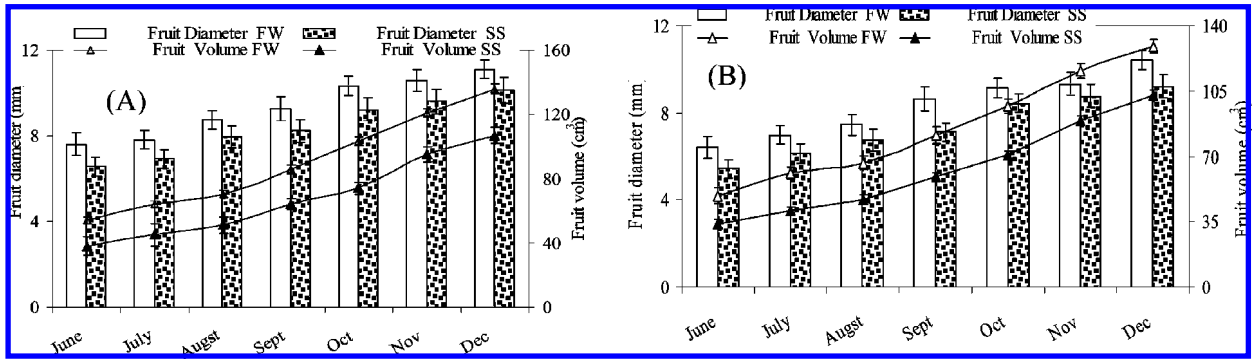

Figure 4. Changes in fruit diameter and fruit volume from olive trees (cv. Chemlali) grown under fresh water irrigation (FW) and saline water irrigation (SS) in 2004 (**A**) and 2005 (**B**). Values are the means of four samples ± standard deviations ($n = 4$).

Table 2. Olive Yield (Kilograms per Tree) of Olive Trees (Cv. Chemlali) Grown under Fresh Water Irrigation (FW) and Saline Water Irrigation (SS) in 2004 and 2005[a]

	FW	SS	relative reduction in SS (%)
2004	38 ± 2.9 aw	22 ± 2.2 bw	42.1
2005	14 ± 2.5 ax	9 ± 2.8 bx	35
mean	27 a	15.5 b	42.6

[a] Values represent the means of 10 samples ± standard deviations. Different letters (a, b) indicate significant differences ($P < 0.05$) between saline water irrigation treatments within each year. Different letters (w, x) indicate significant differences ($P < 0.05$) between crop seasons within each treatment.

of both olive oil samples we analyzed was lower than those recorded in Barnea olive oil subjected to similar salinity treatments (*7*), and differences between FW and SS treated plants were not significant.

Comparison of spectrophotometric absorption characteristics in the UV region at 232 and 270 nm between oils from the two saline irrigated treatments did not show significant differences ($P = 0.433$). Taking into account the values of free acidity, peroxide value, and K_{232} and K_{270}, the oil samples obtained from both treatments met European Union requirements for the extra virgin olive oil category.

The distribution of fatty acid composition of the oil samples of both saline water treatments covers the normal range expected for VOO (**Table 4**). For both treatments, the most abundant acid was the oleic one with values recorded in oil obtained from SS treated plants statistically higher ($P = 0.0024$) than those in oil of FW ones. During both crop seasons, the unsaturated/saturated acid ratios were higher in SS than in FW treatment. However, the monounsaturated/polyunsaturated acid ratios, around 3.5, did not appear to be influenced by saline irrigation treatment. However, the oil obtained from high saline water irrigated plants would be nutritionally better than that obtained in the case of fresh water irrigated ones.

The increase of oleic acid and the decrease of palmitic acid concentrations, under SS treatment, could be due to the triacylglycerol active biosynthesis, involving a decrease of relative percentage of palmitic acid content (*24*). As the palmitic acid is implicated in oleic acid synthesis, the triacylglycerol biosynthesis, which is more important during late fruit ripening stage, as the lipogenesis is more remarkable, induces the increase of oleic acid and the decrease of palmitic acid contents. On the other hand, the higher amounts of linoleic acid recorded in salt stress treated plants and the low level of oleic acid found in the case of Chemlali olive oil, in comparison to other cultivars such as Arbequina (*16*) and Cornicabra (*25*) conducted under different irrigation regimens, may be due, according to Sanchez and Harwood (*26*), to the transformation of oleic acid into linoleic acid by the oleate desaturase activity and/or probably the disturbance of the activities of enzymes involved in the oleic acid synthesis chain by salt stress.

Likewise, El-Agaimy et al. (*27*), focusing on olive oil composition under different saline water levels, have shown that oleic acid percentage (66.6−70.6%) displayed a slight increase under irrigation with saline water (1800, 3600, and 6000 ppm of salts), in comparison to the control treatment (320 ppm). The slight increase of oleic acid content recorded under SS treatment has been also noted by Wiesman et al. (*7*), whereas palmitic acid did not show a similar variation in oil samples of both Chemlali and Barnea olive trees subjected to the same salinity levels.

Although there were differences recorded in fatty acid composition between the two treatments, both olive oil samples showed a variability in the normal range expected for virgin olive oils. Nevertheless, the low values of oleic acid concentrations registered in our study, if compared to data reported by El-Agaimy et al. (*27*) in the case of Picual olive, could result from the interaction of many factors such as the cultivar, stage of fruit maturity, salt exposure duration, and climatic conditions of each environment.

Chlorophyll and Carotenoid Concentrations. Chlorophyll concentrations in the virgin oils ranged from 9.5 to 10.19 mg/kg and from 9.2 to 10.22 mg/kg in FW and SS, respectively

Table 3. Total Oil Content, Free Acidity, Peroxide Value, and Extinction Coefficients of Virgin Olive Oils from Olive Trees (Cv. Chemlali) Grown under Fresh Water Irrigation (FW) and Saline Water Irrigation (SS) in 2004 and 2005[a]

	treatment	total oil content (% fw)	free acidity (%)	peroxide value (mequiv of O_2/kg)	K_{232}	K_{270}
2004	FW	27.85 ± 2.61 aw	0.25 ± 0.02 aw	3.2 ± 0.31 aw	1.06 ± 0.025 aw	0.05 ± 0.01 aw
	SS	25.7 ± 2.05 aw	0.24 ± 0.02 aw	2.9 ± 0.12 aw	1.05 ± 0.042 aw	0.05 ± 0.023 aw
2005	FW	30.56 ± 3.02 ax	0.34 ± 0.04 ax	4.6 ± 0.34 ax	1.62 ± 0.062 ax	0.11 ± 0.02 ax
	SS	28.32 ± 2.12 ax	0.32 ± 0.05 ax	4.4 ± 0.28 ax	1.76 ± 0.06 ax	0.16 ± 0.018 bx

[a] Values are the means of three different VOO samples ($n = 3$) ± standard deviations. Different letters (a, b) indicate significant differences ($P < 0.05$) between saline water irrigation treatments within each year. Different letters (w, x) indicate significant differences ($P < 0.05$) between crop seasons within each treatment.

Table 4. Fatty Acid Composition (Percent) of Virgin Olive Oils from Olive Trees (Cv. Chemlali) Grown under Fresh Water Irrigation (FW) and Saline Water Irrigation (SS) in 2004 and 2005

	2004		2005	
	FW	SS	FW	SS
palmitic acid	19.82 ± 0.25 aw	16.1 ± 0.35 bw	16.7 ± 0.32 ax	15.51 ± 0.42 bw
palmitoleic acid	2.57 ± 0.15 aw	2.1 ± 0.17 aw	1.76 ± 0.12 ax	1.65 ± 0.11 ax
heptadecanoic acid	0.12 ± 0.01 aw	0.11 ± 0.02 aw	0.14 ± 0.03 aw	0.13 ± 0.03 aw
heptadecenoic acid	0.24 ± 0.02 aw	0.22 ± 0.03 aw	0.27 ± 0.05 aw	0.26 ± 0.08 aw
stearic acid	2.19 ± 0.015 aw	2.01 ± 0.016 aw	2.83 ± 0.016 ax	2.25 ± 0.025 ax
oleic acid	55.58 ± 1.05 aw	59.3 ± 2.03 bw	60.73 ± 2.45 ax	64.59 ± 2.56 bx
linoleic acid	16.14 ± 0.65 aw	17.96 ± 0.52 aw	16.52 ± 1.01 aw	17.26 ± 0.96 aw
linolenic eicosanoic acid	0.56 ± 0.08 aw	0.63 ± 0.078 bw	0.56 ± 0.08 aw	0.55 ± 0.09 ax
eicosanoic acid	0.34 ± 0.03 aw	0.41 ± 0.08 bw	0.4 ± 0.06 ax	0.43 ± 0.07 aw
eicosenoic acid	0.16 ± 0.02 aw	0.14 ± 0.04 aw	0.18 ± 0.05 aw	0.16 ± 0.07 aw
uns/sat ratio	3.34 aw	4.31 bw	3.98 aw	4.61 bw
mono/poly ratio	3.5 aw	3.32 aw	3.68 aw	3.74 aw

[a] Values are the means of three different VOO samples ($n = 3$) ± standard deviations. Different letters (a, b) indicate significant differences ($P < 0.05$) between saline water irrigation treatments within each year. Different letters (w, x) indicate significant differences ($P < 0.05$) between crop seasons within each treatment.

Table 5. Total Chlorophyll and Carotenoids Contents of Olive Oils from Olive Trees (Cv. Chemlali) Grown under Fresh Water Irrigation (FW) and Saline Water Irrigation (SS) in 2004 and 2005[a]

	treatment	total chlorophyll (mg/kg)	carotenoids (mg/kg)
2004	FW	9.5 ± 0.07 aw	0.38 ± 0.07 aw
	SS	9.2 ± 0.09 aw	0.41 ± 0.04 aw
2005	FW	10.19 ± 0.05 aw	0.42 ± 0.06 aw
	SS	10.22 ± 0.04 aw	0.44 ± 0.075 aw

[a] Values are the means of three different VOO samples ($n = 3$) ± standard deviations. Different letters (a, b) indicate significant differences ($P < 0.05$) between saline water irrigation treatments within each year. Different letters (w, x) indicate significant differences ($P < 0.05$) between crop seasons within each treatment.

(**Table 5**). Differences between the treatments were not significant ($P > 0.05$) in either season. As for the chlorophyll contents, during both crop seasons, the carotenoid contents were not influenced by saline irrigation regimens. Indeed, the slight differences in carotenoid compounds between both treatments were not significant.

Total Phenols and Phenolic Composition. Table 6 reports the concentrations of the major phenolic, total phenols, and oxidative stability of VOO samples in both treatments. Total phenol contents of VOO were significantly influenced by the salinity treatments. During the 2004 crop season, the total phenol contents were 181 and 214 mg/kg, respectively, in FW and SS treatments. During the second crop, these values reached 198 and 223 mg/kg, respectively. The use of saline water at 7.5 dS m^{-1} reinforced phenol accumulation and did not alter oil quality. The three phenolic compounds in highest concentrations in both oil samples were hydroxytyrosol, tyrosol, and glycoside oleuropein.

The increment of total phenol and phenolic compound contents under SS treatment could be involved in the antioxidative mechanisms developed by the olive tree in response to oxidative stress induced by salt stress conditions as suggested by Foyer et al. (*28*). In the case of Wiesman et al. (*7*), the higher polyphenol contents recorded in oils of saline irrigated plants has been explained by the acceleration of maturation of the olives, which could account for the higher levels of phenols. Furthermore, as it is known, salt stress could result in both water deficit and salt accumulation. Consequently, the increase of phenol contents in SS treated plants might be due to the effects of water deficit on the activation of phenylalanine ammonia-lyase (PAL), a key enzyme in the biosynthetic pathway of phenolic compounds, which is directly involved in the accumulation of polyphenols in the VOO (*29*, *30*). The increase of phenolic compound concentrations under water deficit conditions has been recently reported in cv. Leccino olive by Servili et al. (*31*). Moreover, periods of severe conditions could influence PAL activity in olive fruit (*32*), and this could be an explanation for the reduced level of phenol concentration (*33*) in FW treated plants.

Table 6. Phenolic Composition Concentrations (Milligrams per Kilogram), Total Phenol Contents, and Oxidative Stability of Olive Oils from Olive Trees (Cv. Chemlali) Grown under Fresh Water Irrigation (FW) and Saline Water Irrigation (SS) in 2004 and 2005[a]

	2004		2005	
	FW	SS	FW	SS
tyrosol	42.5 ± 2.36 aw	66.4 ± 3.21 bw	45 ± 2.56 aw	72.5 ± 2.36 bx
hydroxytyrosol	84.5 ± 3.56 aw	96.7 ± 2.06 bw	88.5 ± 2.47 aw	105.4 ± 3.56 bx
oleuropein	10.5 ± 2.84 aw	12.7 ± 2.56 aw	12.5 ± 3.3 aw	16.4 ± 2.84 bx
glycoside oleuropein	23.4 ± 2.56 aw	34.6 ± 2.47 bw	25.5 ± 2.5 aw	37.1 ± 2.56 bw
vanillic	13.2 ± 1.56 aw	15.6 ± 1.04 aw	16.4 ± 2.3 ax	17.6 ± 2.45 aw
caffeic	10.5 ± 1.24 aw	13.6 ± 1.03 bw	13.2 ± 2.45 ax	16.9 ± 2.12 bx
syringic	8.6 ± 1.2 aw	10.7 ± 1.24 bw	10.7 ± 1.24 ax	12.4 ± 1.24 bx
p-coumaric	4.2 ± 1.32 aw	5.39 ± 1.42 aw	6.6 ± 1.2 aw	8.2 ± 1.2 ax
o-coumaric	3.2 ± 1.02 aw	4.6 ± 1.25 aw	5.3 ± 1.32 aw	7.1 ± 1.32 bx
ferulic	2.96 ± 1.43 aw	4.5 ± 1.65 bw	4.25 ± 1.02 ax	6.8 ± 1.22 bx
total phenols (mg/kg of oil)	181.46 ± 2.35 aw	214.17 ± 2.45 bw	198.08 ± 4.56 ax	223.67 ± 3.47 bx
oxidative stability (h)	16.02 ± 1.02 aw	18.84 ± 1.45 bw	16.43 ± 2.03 aw	21.73 ± 2.14 bx

[a] Values are the means of three different VOO samples ($n = 3$) ± standard deviations. Different letters (a, b) indicate significant differences ($P < 0.05$) between saline water irrigation treatments within each year. Different letters (w, x) indicate significant differences ($P < 0.05$) between crop seasons within each treatment.

The higher values of phenolic compound concentrations recorded in SS plants during the second crop season, in comparison to the first one, testified to the tolerance of the Chemlali variety to saline water irrigation. In addition, olive oil obtained from salt-stressed plants was classified as extra virgin with higher levels of phenolic compounds than that of plants irrigated with fresh water. Besides, the significant differences between severe parameters of VOO between the two crop seasons, regardless of treatment, confirm the effects of environmental conditions on these characteristics. Indeed, the VOO quality is dependent not only on saline water level but also on cultivar, salt exposure duration, and climatic conditions of each environment. Preliminary results of a study on the effects of environmental conditions on oil quality confirm this statement (Ben Ahmed et al., unpublished data).

Regardless of the treatment, oxidative stability of Chemlali olive oil under different saline water level conditions ranged from 16 to 22 h. During both crop seasons, the oxidative stability values of olive oil of SS were higher than those observed in the case of VOO from fresh water irrigated plants. The oxidative stability of VOO of SS treated plants significantly increased during the second crop season, in comparison to that characterizing the first crop one. However, in fresh water irrigated plants, the oxidative stability of VOO samples did not show a significant variation between crop seasons. These results were comparable to those obtained by Motilva et al. (*16*) in the case of Arbequina olive cultivar conducted under different irrigation treatments but lower than those characterizing the Cornicabra virgin olive oil (*24*). The same authors have stated that the oxidative stability of VOO depends on a multitude of factors such as the extraction system, climate, latitude, and maturity stage of collected olive fruits. Moreover, the higher oxidative stability of VOO obtained from SS treated plants could be due to the higher total phenols and phenolic compound contents as has been suggested by several papers (*16*, *24*, *34*).

In conclusion, saline water (EC = 7.5 dSm^{-1}) used for olive tree (cv. Chemlali) irrigation appears not only to be beneficial for water resource management but also to have direct effects on both quantitative and qualitative characteristics of virgin olive oil in the case of the Chemlali olive cultivar tested in this experiment. Changes in VOO fatty acid and phenolic compositions were induced perhaps by water deficit, resulting from saline water irrigation, as has been suggested by different researchers (*16*, *22*, *24*, *27*). Recent research has confirmed the protective role of phenols, as natural antioxidants, against cardiovascular diseases and colon, breast, and skin cancers (*35*). The higher levels of phenolic compounds found in Tunisian olive oil would provide more benefits for people. Furthermore, studies have reported different pharmacological activities of olive oil phenols, other than antioxidant potential (*36*).

On the other hand, faced with the large genetic diversity in olive tree and the cultivar dependence of olive salt tolerance, it would be important that saline water resource management be developed for different cultivars grown under certain climatic and soil conditions. Besides, the determination of soil moisture and soil salinity variation along the depth is very important. Results in this study showed that these two parameters not only are dependent on water salinity level but are also controlled by a multitude of factors, particularly the soil texture, distance from the irrigation source, and climatic conditions (rainfall pattern, temperature average,...). Our results are further evidence of direct effects of saline water irrigation on qualitative parameters of olive oil, particularly the fatty acid and phenolic composition in virgin olive oil.

ABBREVIATIONS USED

VOO, virgin olive oil; FW, fresh water treatment; SS, saline water treatment; fw, fresh weight; ECe, electrical conductivity; FrW, fruit weight; FD, fruit diameter; FV, fruit volume; FWC, fruit water content; MI, maturation index; LSD, least significant differences; PAL, phenylalanine ammonia-lyase.

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LITERATURE CITED

(1) Ben Ahmed, C.; Ben Rouina, B.; Boukhriss, M. Effects of water deficit on olive trees cv. Chemlali under field conditions in arid region in Tunisia. *Sci. Hortic.* **2007**, *133*, 267–277.

(2) Aragüés, R.; Puy, J.; Isidoro, D. Vegetative growth response of young olive trees (*Olea europaea* L., cv. Arbequina) to soil salinity and waterlogging. *Plant Soil* **2004**, *258*, 69–80.

(3) Ben Ahmed, C.; Ben Rouina, B.; Athar, H. R.; Boukhriss, M. Olive tree (*Olea europaea* L. cv. "Chemlali") under salt stress: water relations and ions content. *Pak. J. Bot.* **2006**, *38*, 1477–1484.

(4) Cresti, M.; Ciampolini, F.; Tattini, M.; Cimato, A. Effect of salinity on productivity and oil quality of olive (*Olea europaea* L.) plants. *Adv. Hortic. Sci.* **1994**, *8*, 211–214.

(5) Klein, I.; Ben-Tal, Y.; Lavee, S.; De Malach, Y.; David, I. Saline irrigation of cv. Manzanillo and Uovo De Piccione trees. *Acta Hortic.* **1994**, *356*, 176–180.

(6) Bouaziz, A. Behaviour of some olive varieties irrigated with brackish water and grown intensively in the central part of Tunisia. *Acta Hortic.* **1990**, *286*, 247–250.

(7) Wiesman, Z.; Itzhak, D.; Ben Dom, N. Optimisation of saline water level for sustainable Barnea olive and oil production in desert conditions. *Sci. Hortic.* **2004**, *100*, 257–266.

(8) Gucci, R.; Tattini, M. Salinity tolerance in olive. *Hortic. Reviews* **1997**, *21*, 177–214.

(9) Benlloch, M.; Arboleda, F.; Barranco, D.; Fernandez-Escobar, R. Response of young olive trees to sodium and boron excess in irrigation water. *HortScience* **1991**, *26*, 867–870.

(10) Gucci, R.; Lombardini, L.; Tattini, M. Analysis of leaf water relations in leaves of two olive (*Olea europaea*) cultivars differing in tolerance to salinity. *Tree Physiol.* **1997**, *17*, 13–21.

(11) Aragüés, R.; Puy, J.; Royo, A.; Espada, J. L. Three-year field response of young olive trees (*Olea europaea* L., cv. Arbequina) to soil salinity: trunk growth and leaf ion accumulation. *Plant Soil* **2005**, *271*, 265–273.

(12) Weissbein, S.; Wiesman, Z.; Ephrath, Y.; Silberbush, M. Vegetative and reproductive response of olive cultivars to moderate saline water irrigation. *HortScience* **2008**, *43*, 286–293.

(13) Al-Absi, K.; Qrunfleh, M.; Abu-Sharar, T. Mechanism of salt tolerance of two olive *Olea europaea* L. cultivars as related to electrolyte concentration and toxicity. *Acta Hortic* **2003**, *618*, 281–290.

(14) Al-Absi, K. M.; Al-Nasir, F. M.; Mahadeen, A. Y. Mineral content of three olive cultivars irrigated with treated industrial wastewater. *Agric. Water Manag.* **2008** (in press).

(15) Doorenbos, J.; Pruitt, W. O. *Guidelines for Predicting Crop Water Requirements*, 2nd ed.; FAO Irrigation and Drainage 24; FAO: Rome, Italy, 1977.

(16) Motilva, M. J.; Tovar, M. J.; Romero, M. P.; Alegre, S.; Girona, J. Influence of regulated deficit irrigation strategies applied to olive trees (Arbequina cultivar) on oil yield and oil composition during the fruit ripening period. *J. Sci. Food Agric* **2000**, *80*, 2037–2043.
(17) Donaire, J. P.; Sanchez-Roya, A. J.; Lopez-George, J. L.; Recal de, L. Etudes physiologiques et biochimiques de l'olive: I. Variations de la concentration de divers métabolites pendant son cycle évolutif. *Agrochimica* **1977**, *21*, 311–321.
(18) European Union Commission. Regulation EEC 2568/91 on the characteristics of olive oils and their analytical methods. *Off. J. Eur. Communities* **1991**, 295/57–17/12/1991.
(19) Minguez-Mosquera, M. I.; Gandul, B.; Garrido, J. Pigments presents in virgin olive oil. *J. Am. Oil Chem. Soc*. **1990**, *67*, 192–196.
(20) Laübli, M. W.; Bruttel, P. A. Determination of the oxidative stability of fats and oils by the Rancimat method. *J. Am. Oil Chem. Soc*. **1986**, *63*, 792–795.
(21) Singleton, V. L.; Rossi, J. A. Colorimetry of total phenolics with phosphomolybdic−phosphotungstic acid reagents. *Am. J. Enol. Vitic*. **1965**, *16*, 144–158.
(22) Patumi, M.; d'Andria, R.; Fontanazza, G.; Morelli, G.; Giorio, P.; Sorrentino, G. Yield and oil quality of intensively trained trees of three cultivars of olive (*Olea europaea* L.) under different irrigation regimes. *J. Hortic. Sci. Biotechnol*. **1999**, *74*, 729–737.
(23) Gucci, R.; Lodolini, E.; Rapoport, H. F. Productivity of olive trees with different water status and crop load. *J. Hortic. Sci. Biotechnol.* **2007**, *82*, 648–656.
(24) Salvador, M. D.; Aranda, F.; Fregapane, G. Influence of fruit ripening on Cornicabra virgin olive oil. A study for four crop seasons. *Food Chem*. **2001**, *73*, 45–53.
(25) Gomez-Rico, A.; Salvador, M. D.; Moriana, M. D.; Pérez, D.; Olmedilla, N.; Ribas, F.; Fregapane, G. Influence of different strategies in a traditional Cornicabra cv. olive orchard on virgin olive oil composition and quality. *Food Chem*. **2007**, *100*, 568–578.
(26) Sanchez, J.; Harwood, J. L. Biosynthesis of triacylglycerols and volatiles in olives. *Eur. J. Lipid Sci. Technol*. **2002**, *104*, 564–573.
(27) El-Agaimy, M. A.; Neff, W. E.; El-Sayed, M.; Awatif, I. I. Effect of saline irrigation water on olive oil composition. *J. Am. Oil Chem. Soc*. **1994**, *71*, 1287–1289.
(28) Foyer, C. H.; Lopez-Delgado, H.; Dat, J. F.; Scott, I. M. Hydrogen-peroxide and glutathione- associated mechanisms of acclamatory stress tolerance and signalling. *Physiol. Plant*. **1997**, *100*, 241–254.
(29) Romero, M. P.; Tovar, M. J.; Girona, J.; Motilva, M. J. Changes in the HPLC phenolic profile of virgin olive oil from young trees (*Olea europaea* L. cv. Arbequina) grown under different deficit irrigation strategies. *J. Agric. Food Chem*. **2002**, *50*, 5349–5354.
(30) Tovar, M. J.; Romero, M. P. J.; Motilva, M. J. Changes in the phenolic composition of olive oil from young trees (*Olea europaea* L. cv. Arbequina) grown under linear irrigation strategies. *J. Agric. Food Chem*. **2001**, *49*, 5502–5508.
(31) Servili, M.; Esposto, S.; Lodolini, E.; Selvaggini, R.; Taticchi, A.; Urbani, S.; Montedoro, G.; Serravalle, M.; Gucci, R. Irrigation effects on quality, phenolic composition and selected volatiles of virgin olive oils cv. Leccino. *J. Agric. Food Chem*. **2007**, *55*, 6609–6618.
(32) Tovar, M. J.; Romero, M. P.; Girona, J.; Motilva, M. J. Phenylalanine ammonia-lyase activity and concentration of phenolics in developing olive (*Olea europaea* L. cv. Arbequina) fruit grown under different irrigation regimes. *J. Sci. Food Agric*. **2002**, *82*, 892–898.
(33) Gomez-Rico, A.; Salvador, M. D.; La Greca, M.; Fregapane, G. Phenolic and volatile compounds of extra virgin olive oil (*Olea europaea* L. cv. Cornicabra) with regard to fruit ripening and irrigation management. *J. Agric. Food Chem*. **2006**, *54*, 7130–7136.
(34) Tripoli, E.; Giammanco, H.; Tabacchi, G.; Di Majo, D.; Giammanco, S.; LaGuardio, M. The phenolic compounds of olive oil: structure, biological activity and beneficial effects on human health. *Nutr. Res. Rev*. **2005**, *18*, 98–112.
(35) Tuck, K. L.; Hayball, P. J. Major phenolic compounds in olive oil: metabolism and health benefits. *J. Nutr. Biochem*. **2002**, *13*, 636–644.
(36) Yang, D. P.; Kong, D. X.; Zhang, H. Y. Multiple pharmacological effects of olive oil phenols. *Food Chem*. **2007**, *104*, 1269–1271.

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