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Effects of Growing Season and Ripening Stages on Transcription Level of Geranylgeranyl Reductase (*OeCHL P*) and Some Biochemical Properties in Some Important Olive Cultivars (*Olea europaea* L.)

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ABSTRACT

Transcription level of geranylgeranyl reductase (OeCHL P), and contents of α-tocopherol, oleuropein, hydroxytyrosol and tyrosol of seven olive cultivars obtained from Olive Germplasm Collection, Kemalpasa, Izmir were determined in two ripening stages (green and black fruit) in two consecutive years, 2017 and 2018.

Transcription level of OeCHL P was significantly affected by year, ripening stage and cultivar. The highest values were detected in green fruit of 'Uslu' in both years. In comparison to 2017, a significant increase in gene transcription was observed in 2018 independent of cultivar and ripening stage. The highest α-tocopherol and oleuropein content were obtained from 'Girit Zeytini'. The content of oleuropein decreased with ripening in all cultivars in both years. Tyrosol reached its highest and lowest values in 'Girit Zeytini' at black stage in 2018 and green stage in 2017, respectively. 'Girit Zeytini' stood out for both nutritional value and fruit size. The highest and lowest values of hydroxytyrosol content recorded in 'Girit Zeytini' (2017, black stage) and 'Izmir Sofralık' (2018, green stage), respectively. We also detected positive correlations between OeCHL P relative transcription level and tocopherol, tyrosol contents.

Our overall results indicated that olive CHL P plays an important role in regulation of tocopherol synthesis. A direct relationship was determined between OeCHL P and α-tocopherol, while there was an indirect link between others. These results revealed that more than one factor could affect the evaluated parameters.

Keywords: Gene expression, CHL P, Olive, Oleuropein, α-tocopherol, Tyrosol

1. Introduction

The origin of the olive is Anatolia. There are 97 selected cultivars or types originating from Turkey and 33 cultivars originating from other countries in the Olive Research Institute collection, Turkey (Ozkaya et al. 2004). The 108 olive cultivars grown in Turkey were registered. Thirty of these cultivars are common and others are grown in more limited areas (Efe et al. 2016).

Olive fruit ripening is a long process occurring within 6-8 months. Optimal harvest time varies according to the purpose of the utilization of olives. If olives are used for oil, the best harvesting time is considered between green and black stages. Phenolic compounds, e.g., hydroxytyrosol (HyT) and oleuropein (Ole) are responsible for its peculiar pungent taste and for its high stability in extra virgin olive (Psomiadou & Tsimidou 2002; Visioli et al. 2002).

Recently, some reference genes have been identified during the development and ripening periods of olive fruit. Within these genes, the NADPH-dependent geranylgeranyl reductase (*CHL P*) catalyzes formation of chlorophyll, tocopherols (TcP) and plastoquinone compounds, which are necessary for metabolic pathways associated with plant response to yield and stress, as well as the nutritional value of fruit (Muzzalupo et al. 2011). *CHL P* gene expression modulation follows a complex genetic network under developmental stages and stress conditions. It is reported that *CHL P* message is abundant in chlorophyll-containing tissue and flower organs, rarely in mesocarps and roots in peach genotypes (Giannino et al. 2004). *OeCHL P* transcripts were detected in various organs of olive plants (Threlfall & Whistance 1970; Bollivar et al. 1994; Keller et al. 1998; Tanaka et al. 1999; Munnè-Bosch & Alegre 2002). *CHL P* transcription in 'Coralea' olive cultivar fruits damaged by *Bactrocera oleae* pathogen was locally increased in specific cells. By examination of all the data, it was reported that *OeCHL P* gene

expression responds to biotic and abiotic stress factors in the early period and gene activity may be related to TcP activity under stress (Bruno et al. 2009).

The aim of this work was to study the transcription levels of *OeCHL P* gene during drupe ripening by comparing seven cultivars, to elucidate the possible correlation between *OeCHL P* mRNA level and phenolic compounds (Ole, HyT, and Ty) and α-TcP biomarkers.

2. Material and Methods

Fruit samples of seven cultivars were obtained from Olive Germplasm Collection, Kemalpasa, Izmir, Turkey (Table 1). The analyzes were carried out at Alata Horticultural Research Estitute, Erdemli, Mersin, Turkey in 2017 and 2018. Olive fruit samples were taken according to the scale of 0-7 based on ripening stages (Cebeci 2007). Green fruit (GF) samples were harvested at 127th day after anthesis (A), scale of 1. Black fruit (BF) samples were collected at 190th day, scale of 6. The container containing dry ice is used to transfer the harvested olive fruits rapidly from orchard to Alata Horticultural Research Enstitute Biotechnology Laboratory. Then fruits were transferred to liquid nitrogen (-196 °C) and were kept at -80 °C until analysis.

Table 1- List of the experimental olive cultivars

Cultivar name	*Origin*	*Use*
Cilli	Kemalpasa- Izmir	Green Table
Esek Zeytini (Odemis)	Odemiş- Izmir	Green Table
Girit Zeytini	Bodrum- Mugla	Oil
Izmir Sofralık	Izmir	Green Table
Nizip Yaglık	Gaziantep, Nizip	Oil, Table
Sinop No 5	Sinop	Table, Oil
Uslu	Akhisar	Black Table

Anthesis dates of cultivars were recorded before olive fruits were collected (Table 2). 'Uslu' and 'Sinop No 5' had the earliest anthesis date in 2017 (May, 13 and May, 15, respectively). Anthesis dates of 'Cilli', 'Esek Zeytini', 'Girit Zeytini', 'Nizip Yaglık' were May, 19. The latest anthesis date was in 'Izmir Sofralık (May, 20)' in 2017. The earliest anthesis date was in 'Uslu (May, 12)' and 'Sinop No 5 (May, 15)' again in 2018. Anthesis date of 'Cilli', 'Girit Zeytini', 'Nizip Yaglık' was May, 18. However, 'Esek Zeytini' was become to anthesis time two days early compared to 2017 (May, 17). Anthesis date of 'Izmir Sofralık' was not changed according to the previous year.

Table 2- The date of anthesis, green fruit (GF) and black fruit (BF) stages of the cultivars (sampling date)

Cultivars	*Anthesis 2017*	*GF Stage 2017*	*BF Stage 2017*	*Anthesis 2018*	*GF Stage 2018*	*BF Stage 2018*
Cilli	May,19	September, 25	November, 25	May, 18	September, 24	November, 24
Esek Zeytini (Odemis)	May,19	September, 25	November, 25	May, 17	September, 23	November, 23
Girit Zeytini	May,19	September, 25	November, 25	May, 18	September, 24	November, 24
Izmir Sofralık	May, 20	September, 26	November, 26	May, 20	September, 26	November, 26
Nizip Yaglık	May,19	September, 25	November, 25	May, 18	September, 24	November, 24
Sinop No 5	May,15	September, 21	November, 21	May, 15	September, 21	November, 21
Uslu	May, 13	September, 19	November, 19	May, 12	September, 14	November, 18

2.1. Transcript analysis in olive pericarp

Total RNA was isolated from pericarp tissues at two different developmental stages and processed separately. It was performed by using trizol (TRI®Reagent T9424 SIGMA) according to Avison (2007)s' protocol. The quality and quantity of total isolated RNA were controlled with a Nanodrop spectrophotometer (Thermo Fisher, Madison WI, USA), according to the manufacturer's instructions (Thermo Fisher). cDNA synthesis was performed from isolated RNA samples. The first sequence of cDNA synthesis was performed using High Reverse Transcriptase cDNA synthesis kit 200 rxn. Quantitative revers-transcription PCR (qRT-PCR) was performed on a Rotor-Gene 6000 Real-Time PCR (Qiagen, USA). The primer used for qRT-PCR analyses of the *OeCHL P* gene (GeneBank DQ424963) are Fw 5'-CCAAGGGAGGCATTTGTAGA-3' and Bw 5'-TGGATTCACAGCCAATTTCA-3'. 18S rRNA was used as a normalization control. The primer sequence of 18S rRNA was Fw 5'-AAACGGCTACCACATCCAAG-3' and Bw 5'-CCTCCAATGGATCCTCGTTA-3'. Amplification reactions were performed according to the procedures of Bruno et al. (2009). The results of qRT-PCR were analyzed via Opticon Monitor. Cycle threshold (CT) values were obtained from Genex Software (Bio-Rad) and data was analyzed through the 2^{-DDCT} method (Livak & Schmittgen 2001). The means of *OeCHL P* transcript levels were calculated from three biological repeats, obtained from three independent experiments.

2.2. Biochemical analysis in olive pericarp

The α-TcP analysis was performed by HPLC (High-Performance Liquid Chromatography, Shimadzu Corp, USA) according to Abidi (2000) and Ruperez et al. (2001). Five mg of dry sample was taken and 35 mL of each dioxane [n-hexane (1:1, v:v)] was extracted four times in mortar. Samples were cleaned by evaporation and centrifugation. The residue was dissolved in 10 mL of dioxane [n-hexane (3:97, v:v)]. This solution was analyzed in HPLC using a column filled with C18 Nucleosil 50 (4.6x250 mm) with dioxane (n-hexane (3:97, v: v) at a flow rate of 1.5 mL min^{-1}. α-TcP concentration was determined using a standard curve obtained from the commercial standard (Sigma-Aldrich, USA). α-TcP content was estimated at 295 nm_{ex} and 325 nm_{ex} using a fluorescent detector.

Ole, HyT, and Ty determined by HPLC according to Vinha et al. (2005). Each sample (1.5 g) was mixed with methanol. The methanolic extract was filtered, evaporated to dryness under reduced pressure (40 °C) and redissolved in methanol (4 mL) of which 20 µL were injected for HPLC analysis. 1.5 g of each sample were subjected to extraction. The methanolic extract obtained was taken to dryness under reduced pressure (40 °C) and redissolved in 50 mL of acidified water (pH 2 with HCl). The aqueous solution was passed through a C18 column, previously conditioned with 60 mL of methanol and 140 mL of acidified water (pH 2 with HCl). The loaded cartridge was washed with 60 mL of n-hexane to eliminate the lipid fraction and the retained phenolic compounds were then eluted with methanol (60 mL). The methanolic extract was concentrated to dryness under reduced pressure (40 °C) and redissolved in methanol (4 mL). The injection volume for HPLC analysis was 20 µL. When determining the phenolic content, the peak areas were calculated by multiplying by the dilution factor and the results were expressed as mg g^{-1} (dry weight). Hyt, Ty and Ole (Extrasynthese, France) were used as standard substances. Detection was carried out with a diode array detector, and chromatograms were recorded at 280 nm. The external standard method was used for the quantification of the individual phenolic compounds.

2.3. Statistical Analyses

Data were presented as mean±SD and subjected to 3-way ANOVA with randomized plot design with three replications for each parameter using JPM 5.0.1. software (SAS Institute, 1989) followed by LSD test (P<0.05). Additionally, possible correlations between parameters were determined.

3. Results and Discussion

OeCHL P transcription levels were estimated through RT-PCR analysis in seven cultivars harvested at two stages of drupes ripening. In 2017, 'Sinop No 5 (1.00)' was used as a sample calibrator in GF stages and 'Girit Zeytini (1.17)' was used in BF stages. In 2018, 'Uslu (2.96)' was used as a sample calibrator in GF stages and 'Esek Zeytini (2.95)' was used in BF stages. Transcription level of *OeCHL P* was significantly affected by year, ripening stage and cultivar. The highest values were detected in GFs of 'Uslu' in both years (3.01 in 2017 and 2.96 in 2018). The lowest *OeCHL P* gene expression levels detected in 'Sinop No 5', 'Nizip Yaglık', 'Girit Zeytini', 'Esek Zeytini' cultivars were changed according to ripening stage and years. 'Uslu' showed a stable structure by exhibiting the lowest expression level at the BF stages in both years. When the results of the two years are evaluated in general, almost all cultivars increased their *OeCHL P* expression levels in 2018 compared to the previous year (Figure 1).

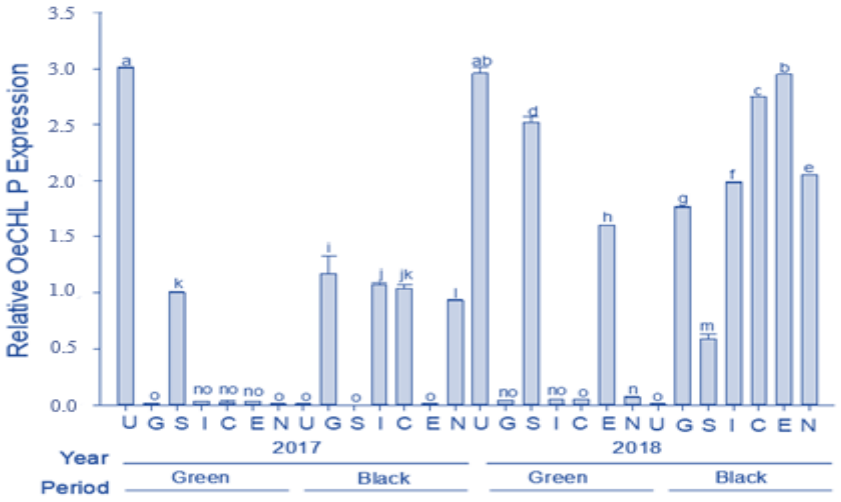

Figure 1- Transcript levels of *OeCHL P* gene in pericaps of cultivars (U: Uslu, G: Girit Zeytini, S: Sinop No 5, I: Izmir Sofralik, C: Cilli, E: Esek Zeytini, N: Nizip Yaglık) harvested at GF and BF ripening in the same cultivation areas (Kemalpasa, Izmir). Values represent the means of three independent biological replicates. Vertical bars show the relative *OeCHL P* level of cultivars. Significant differences between means are shown by different letters (P≤0.05)

OeCHL P gene expression responds to biotic and abiotic stress factors early and independently from the olive genotypes. The expression of *CHL P* was investigated in the different tissues. *CHL P* mRNAs in the cotyledon were induced by light but repressed by dark (Park et al. 2010; Zhou et al. 2013). Similar *CHL P* expression levels in cold stress condition have been also reported in peach (Giannino et al. 2004). Plants often encounter wounding and pathogen attacks because of the temperature changes (Hernandez et al. 2011). One of the main stress factors for the olive plants is the *Bactrocera oleae* (Burrack and Zalom, 2008). The average temperature and precipitation value of Kemalpasa location was recorded during experimental years from the 1st to the 12th month (Table 3). In 2018, olive fruit fly unexpectedly and intensely attacked and significantly increased the *OeCHL P* gene expression rate of cultivars compared to the previous year. Besides, olive fruit fly was variable and uncontrolled conditions such as early increased weather temperature and low precipitation compared to the previous year significantly affected the *OeCHL P* expression level of the cultivars. The same result was shown by Bruno et al. (2009). It was reported that *OeCHL P* gene expression level was 2.5 fold-higher in fruits attacked by *Bactrocera oleae* than healthy ones.

Table 3- The average temperature and precipitation values for the months of 2017 and 2018

Month	*Year*	*Average Temperature (°C)*	*Average Precipitation (mm)*	*Month*	*Year*	*Average Temperature (°C)*	*Average Precipitation (mm)*
1	2017	3.3	246.4	1	2018	6.1	81.8
2	2017	7.9	50.2	2	2018	9.8	119.6
3	2017	11.0	88.0	3	2018	13.0	58.4
4	2017	14.2	15.4	4	2018	17.2	10.0
5	2017	19.2	43.4	5	2018	21.5	26.2
6	2017	24.4	18.4	6	2018	24.1	46.8
7	2017	27.7	13.0	7	2018	27.3	24.0
8	2017	27.2	0.0	8	2018	27.4	6.6
9	2017	22.9	0.0	9	2018	23.1	6.6
10	2017	16.2	0.0	10	2018	16.6	61.6
11	2017	10.0	72.2	11	2018	12.4	83.0
12	2017	9.1	116.0	12	2018	8.7	108.4

The α-TcP levels of cultivars were significantly influenced by all factors and their interactions (Table 4). The highest α-TcP value was detected in 'Girit Zeytini' (2017, BF stage) as 362.14 mg kg^{-1} of total oil and this was followed by 'Izmir Sofralık' (2017, BF stage) as 349.74 mg kg^{-1} of total oil. The lowest α-TcP value was detected in 'Izmir Sofralık' (2017 and 2018, GFs stage) as 146.55 and 143.41 mg kg^{-1} of total oil, respectively. The content of α-TcP is decreased in 2018 in comparison to 2017 in all cultivars except for 'Esek Zeytini'.

CHL P is a minor component (Collakova and DellaPenna 2003) and required for synthesis of TcPs (Tanaka et al. 1999). α-Tocopherol comprises 90% of the total tocopherol content in olive oil (Paiva-Martins & Kritsakis 2017). Aguilera et al. (2005) reported that the amount of α-TcP sharply decreases in the earlier stages of ripening, but then maintains its concentration more or less constantly during ripening, with a further decrease at the latest ripening stage. But, Muzzalupo et al. (2011) reported that the biosynthesis of TcPs increased during the process of pericarp ripening. In our study, the amount of α-TcP decreased with the ripening in early ripening cultivars ('Uslu' and 'Sinop No 5') but increased in another medium late and latest ripening cultivars ('Cilli', 'Esek Zeytini (Odemis)', 'Girit Zeytini', 'Izmir Sofralık', and 'Nizip Yaglık'). Andrikopoulos & Hassapidou (1989) specified the α-TcP content in different types of Greek olive oil and found that virgin olive oil contained an average of 113.00 mg kg^{-1} of α-TcP. Bruno et al. (2009) determined that α-TcP content of 'Carolea' cultivar as 109.98 ug g^{-1} DW in GF stage and 151.44 ug g^{-1} DW in dark fruit. In our study, α-TcP content was found between 257.16-146.55 mg kg^{-1} of total oil in GF stage and 362.14-204.22 mg kg^{-1} of total oil in BF stage. Our α-TcP results are higher than those reported by Andrikopoulos & Hassapidou (1989) and Bruno et al. (2009).

The Ole content of cultivars were significantly influenced by all factors and their interactions except for year × ripening stage (Table 4). Fruit size (small or large drupe) affects the content of Ole (Amiot et al. 1989). The content of Ole in small drupe cultivars was greater than that of large drupes (Amiot et al. 1986). 'Girit Zeytini', which was quite small drupes cultivar, is determined as the highest cultivar of Ole content (65.67 mg g^{-1} DW at GFs in 2017, and 49.71 mg g^{-1} DW at BFs in 2017). The lowest Ole contents were detected in 'Cilli' as 3.73 and 2.99 mg g^{-1} DW at BFs in 2017 and 2018, respectively. When the results were evaulated, the content of Ole decreased with ripening in all cultivars in both years. The highest α-TcP and Ole content were obtained from 'Girit Zeytini'.

Table 4- α-Tocopherols (α-TcP), oleuropein (Ole), hydroxytyrosol (HyT) and tyrosol (Ty) content in pericarp of cultivars at different ripening stages

Year	*Ripening Stage*	*Cultivar*	*Parameters*			
			α-TcP (mg kg^{-1} oil)	*Ole (mg g^{-1} DW)*	*HyT (mg g^{-1} DW)*	*Ty (mg g^{-1} DW)*
2017	Green	Uslu	234.34±4.49^{j}	22.54±1.02^{m}	2.34±0.29^{cd}	1.75±0.61^{b}
		Girit Zeytini	257.16±0.58^{e}	65.67±0.34^{a}	2.24±0.24^{cd}	0.31±0.12^{efgh}
		Sinop No 5	247.66±0.15^{g}	26.55±1.34^{j}	1.53±0.38^{ghi}	0.56±0.18^{de}
		Izmir Sofralık	146.55±0.01^{t}	30.94±0.19^{h}	2.32±0.23^{cd}	0.15±0.09^{gh}
		Cilli	207.11±0.02^{op}	20.91±1.00^{n}	2.31±0.03^{cd}	1.49±0.63^{b}
		Esek Zeytini	204.27±0.03^{q}	29.45±0.39^{i}	2.08±0.03^{def}	0.01±0.00^{h}
		Nizip Yaglık	205.00±0.02^{pq}	46.48±0.67^{c}	2.14±0.01^{cde}	0.23±0.01^{fgh}
	Black	Uslu	213.23±0.19^{n}	5.58±0.58^{rs}	2.87±0.76^{ab}	0.50±0.08^{def}
		Girit Zeytini	362.14±0.65^{a}	49.71±0.45^{b}	3.00±0.94^{a}	1.66±0.39^{b}
		Sinop No 5	215.99±0.01^{m}	6.68±0.12^{q}	2.10±0.08^{cdef}	1.06±0.06^{c}
		Izmir Sofralık	349.75±0.45^{b}	18.39±0.20^{p}	2.15±0.10^{cde}	1.15±0.09^{c}
		Cilli	238.71±0.51^{i}	3.73±0.32^{t}	2.26±0.12^{cd}	1.55±0.16^{b}
		Esek Zeytini	208.15±1.75^{o}	19.32±0.06^{o}	2.15±0.02^{cde}	1.12±0.01^{c}
		Nizip YaGlık	236.09±0.03^{j}	35.74±0.66^{f}	3.02±0.02^{a}	1.14±0.01^{c}
2018	Green	Uslu	228.76±1.01^{k}	19.46±0.71^{o}	2.24±0.03^{abc}	1.06±0.05^{c}
		Girit Zeytini	255.66±0.10^{e}	31.19±0.07^{h}	2.02±0.04^{defg}	0.16±0.04^{gh}
		Sinop No 5	245.25±0.04^{h}	24.09±0.29^{l}	2.25±0.14^{cd}	0.30±0.05^{efgh}
		Izmir Sofralık	143.41±0.14^{u}	41.40±0.22^{e}	1.12±0.02^{i}	0.43±0.02^{defg}
		Cilli	198.17±0.01^{r}	22.77±0.51^{m}	1.72±0.29^{efgh}	0.22±0.06^{fgh}
		Esek Zeytini	222.41±1.47^{l}	43.11±0.39^{d}	1.32±0.12^{hi}	0.57±0.07^{de}
		Nizip Yağlık	175.13±4.33^{s}	33.96±0.63^{g}	1.61±0.36^{fghi}	0.09±0.08^{h}
	Black	Uslu	213.26±0.02^{n}	4.79±0.98^{s}	2.15±0.39^{cd}	0.27±0.17^{efgh}
		Girit Zeytini	297.86±0.16^{c}	25.13±0.12^{k}	2.30±0.04^{cd}	2.15±0.01^{a}
		Sinop No 5	204.22±0.49^{q}	5.90±0.11^{qr}	2.14±0.08^{cde}	1.04±0.02^{c}
		Izmir Sofralık	286.32±0.04^{d}	23.40±0.15^{lm}	2.17±0.07^{cde}	1.09±0.02^{c}
		Cilli	226.81±2.06^{k}	2.99±0.39^{t}	2.40±0.31^{bcd}	0.70±0.14^{d}
		Esek Zeytini	250.44±1.15^{f}	27.34±0.47^{j}	2.60± 0.62^{abc}	1.04±0.04^{c}
		Nizip Yaglık	234.72±1.63^{j}	23.86±0.19^{l}	2.46±0.22^{bcd}	0.48±0.02^{def}
3-way ANOVA						
Year			<0.0001	<0.0001	0.0001	<0.0001
Stage			<0.0001	<0.0001	<0.0001	<0.0001
Cultivar			<0.0001	<0.0001	0.0012	<0.0001
Year × Stage			<0.0001	0.6383	0.1667	0.6879
Year × Cultivar			<0.0001	<0.0001	0.0068	<0.0001
Stage × Cultivar			<0.0001	<0.0001	0.1227	<0.0001
Year × Stage × Cultivar			<0.0001	<0.0001	0.0002	0.0003

Data represent mean ± SD of three independent biological replicates. Significant differences between means are shown by different letters within each parameter (P≤0.05)

The Hyt content of cultivars were significantly influenced by all factors and their interactions except for year × stage and stage × cultivar interactions (Table 4). The highest Hyt content was found in 'Nizip Yaglık' and 'Girit Zeytini' (2017, BF stages) as 3.02 and 3.00 mg g^{-1} DW, respectively. The lowest contents were detected in 'Izmir Sofralık' as 1.12 mg g^{-1} DW and 'Esek Zeytini' as 1.32 mg g^{-1} DW at BF stages in 2018. The Ty contents of cultivars were significantly influenced by all factors and their interactions except for year × stage (Table 4). The highest Ty content was found in BFs of 'Girit Zeytini' as 2.15 and 1.66 mg g^{-1} DW in 2017 and 2018, respectively. The lowest content was found in Esek Zeytini as 0.01 mg/g DW at GF stage of 2017. When the Ty content of all cultivars were evaluated, Ty contents increased with ripening except for 'Uslu' in both years. Ty and HyT are phenolic products of hydrolyzed oleuropein (Montedoro et al. 1992; Tsimidou et al. 1992) and Ty content may be a harvest criterion. The HyT contents of cultivars ranged from 3.00 to 1.12 mg kg^{-1}. These values were quite higher than the findings (ranged from 0.006 to 0.057 mmol kg^{-1}) of Yousfi et al. (2006). The highest Ty content was determined in BFs of 'Girit Zeytini'. As a result of the evaluation of both years, the fastest conversion to tyrosol was in BFs of the 'Girit Zeytini'. The Ty contents of cultivars were increased with the ripening except 'Uslu'. 'Uslu' is earlier blooming cultivar and ripening period is short. Similar to results Bengana et al. (2013) stated that the Ty content of 'Chemlal' cultivar decreased as maturity progressed. Yıldırım et al. (2016) also reported that Ty content increased with the progress of maturity.

In the present study, a positive correlation was found between *OeCHL P* gene expression and α-TcP, and Ty. Muzzalupo et al. (2011) reported that a positive linear trend between *OeCHL P* relative transcription levels and α-TcP content during pericarp ripening. Furthermore, Boskou et al. (2004) reported that as the content of Ole content decreased with ripening, the content of HyT and Ty decreased in parallel. However, there was no connection between Ole and other parameters. But, a positive correlation was also found between α-TcP and HyT, Ty and between Hyt and Ty (Table 5).

Table 5- Correlation coefficients of the parameters obtained from two years averages

Variables	*OeCHL P*	*α-TcP*	*Ole*	*HyT*
α-TcP	0.3926**			
Ole	-0.1447	0.1080		
HyT	0.2005	0.3589*	-0.1216	
Ty	0.3926*	0.3926*	-0.2066	0.3497*

*P≤0.05; **P≤0.01

5. Conclusions

OeCHL P gene expression level varied between two consecutive years among the cultivars. Although these differences might be due to biotic and abiotic factors such as ripening stage, temperature, precipitation and *Bactrocera oleae*, some other factors, which have not been established yet, might be responsible as well. These results indicated that olive CHL P played an important role in response to the regulation of TcP synthesis. CHL P could be a good candidate gene for genetic improvement of plant growth and changing conditions. There was not a marked connection between oleuropein and other parameters. But, we suggest that oleuropein might be an important parameter for in determination of cultivars that can be utilized as functional products. On the other hand, Hyt can give an idea about the hydrolysis rate of oleuropein and Ty can be important criteria in determining the harvest date. Further studies need to be carried out, considering more factors and different ripening stages.

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Abbreviations	
BF(s)	Black Fruits
GF(s)	Green Fruits
HyT	Hydroxytyrosol
NADPH	Nicotinamide adenine dinucleotide phosphate
OeCHL P	*Olea europaea* geranylgeranyl reductase
Ole	Oleuropein
Phe(s)	Phenols
ROS	Reactive oxygen species
Ty	Tyrosol
α-TcP(s)	Tocopherol

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