PHYSICOCHEMICAL CHARACTERISTICS OF ADVANCED TABLE OLIVE SELECTIONS AT GREEN AND BLACK RIPENESS OBTAINED BY CROSSBREEDING

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Abstract

In this study, table olive characteristics of 17 olive genotypes were evaluated. These genotypes were obtained by advanced olive selections after the cross breeding program. Number of olives per kilogram and flesh to seed ratio of the fruit of these genotypes were determined between 91±10.83-223±27.25 and 3.50±0.17-6.51±0.12. Reduced sugar, hydroxytyrosol and oleuropein contents of olives of genotypes were detected between 1.41±0.12-4.30±0.16 mg/kg, 122.37±35.6-3857.46±171.4 mg/kg and 180.07±9.6-3271.09±387.4 mg/kg respectively. Some olive genotypes showed better table olive characteristics than Gemlik cultivar so that they have potential for registration as a table olive cultivar. Some olives contain high concentration of hydroxytyrosol which are noteworthy for nutritional physiology and some olives contain low concentrations of oleuropein which are remarkable for processing technology. Also some genotypes can be used both table olive and oil production.

Key words: *olive crossing, olive genotype, olive selection, phenolic compound, hydroxytyrosol, oleuropein*

1. INTRODUCTION

Cross breeding has been considered the best method to obtain new olive genotypes with improved physicochemical characteristics. Wide range of olive characteristic variation was obtained by cross breeding programs with different combinations (Parlati et al., 1994).

Olive cross breeding programs have currently been carried out in Tunisia, Greece, Israel, Italy, Turkey, and Spain to develop new olive cultivars for table olive and/or olive oil production (Manai et al., 2006; Alfei et al., 2008; Ersoy et al., 2008). New genotypes obtained from those programs have been evaluated for industrial requirements and compared with standard cultivars by several studies (Fourati et al., 2002; Padula et al., 2008; Ersoy et al., 2008). New cultivars have recently been released by olive cross breeding which showed better table olive characteristic than standard cultivars (Parlati et al., 1994; Ranalli et al., 2000; Pannelli et al., 2008; Bellini et al., 2008).

A breeding program was established for selecting new oil and table olive dual purpose cultivars which began at Italy in 1971. As an output of this breeding program 4 olive genotypes were determined as potential genotype for registration for table olive industry uses. So that agronomic properties, fruit yield, fruit weight, flesh to seed ratio, and oil content of these 4 olive genotypes were determined by Pannelli et al., (2008) which were grown in three locations at central and southern Italy.

A cross breeding program was initiated by Olive Research Institute (Turkey) in 1990 with considering the need to develop new rootstock varieties. In this breeding study, 2700 new olive genotypes were obtained by 13 cross breeding combinations. The aim of this breeding study was to obtain superior quality olive oil and table olive varieties with high and regular yield and early maturity (Telli Karaman et al., 2010). An olive breeding program was also initiated at Ataturk Central Horticultural Research Institute (Turkey) in 1990. From the initial 5000 seedlings, 393 selections had been chosen and were currently cultivated in the observation orchard. This research was aimed to determine the physicochemical characteristics of green and black olives of those genotypes in that observation orchard. They had the potential for registration as a new table olive cultivar according to agronomic characteristics.

2. MATERIALS AND METHODS

2.1. Samples

In this study, 17 olive genotypes were evaluated which were given in Table 1. They come from the crosses of foreign (Ascolana, Belle d'Espagne, Manzanilla and Lucques) and national olive cultivars (Gemlik, Edinciksu, Karamürselsu, Tavşanyüreği and Uslu). These trees were planted at a 1.5 m x 3 m distance in olive genotype observation orchard of Ataturk Central Horticultural Research Institute (Yalova/Turkey)**.** These genotypes were chosen on the basis of their high productivity and resistance to diseases and low periodicity.

Table 1. Evaluated olive genotypes and their origins

No	Olive Genotype code	Parents
1	AT007	Ascolana X Tavşan yüreği
2	AT056	Ascolana X Tavşan yüreği
3	AU019	Ascolana X Uslu
4	BU015	B. D'espagne X Uslu
5	BU016	B. D'espagne X Uslu
6	GE015	Gemlik X Edinciksu
7	GK024	Gemlik X Karamürselsu
8	GK036	Gemlik X Karamürselsu
9	GK131	Gemlik X Karamürselsu
10	GK132	Gemlik X Karamürselsu
11	GK146	Gemlik X Karamürselsu
12	GU118	Gemlik X Uslu
13	GU404	Gemlik X Uslu
14	GU410	Gemlik X Uslu
15	LT011	Lucques X Tavşan yüreği
16	LT019	Lucques X Tavşan yüreği
17	MT038	Manzanilla X Tavşan yüreği
18	Gemlik cultivar	-

Olives were randomly hand-picked at two ripening index. For green olive samples; olives was harvested when the skin color of olives was straw yellow and for black olive samples; olives was harvested when the black color reached the middle of the olive flesh.

2.2. Physical analysis

Number of olives per kilogram and flesh to seed ratio were determined according to official method TS 774 (1992). Fruit weight was calculated by weighting the 100 olive fruits. Flesh to seed ratio was calculated by using the ratio of flesh and seed weight of 100 olive fruits.

2.3. Water and oil analysis

Water content of olive samples was determined in a conventional oven at 105±2°C (Esti et al., 1998). Before the oil analysis, seed of olives were removed and olives were crushed. After that crushed olives were dried. Oil of the dried olive paste was extracted by soxhlet apparatus for at least 8 hours with petroleum ether extraction at 50°C. Oil content of the olives was calculated at fresh weight (Cemeroglu, 2007).

2.4. Reduced sugar analysis

5 g olive paste was weighted and mixed with 5 ml potassium ferrocyanide (%15) ve 5 ml zinc sulfate (%30). This mix was completed to 250 ml with distilled water and filtered through filter paper (40 µm pore diameter). 0.5 ml of the diluted sample, 1.5 ml of distilled water and 1 ml of the dinitrophenol was added into the test tube which was held in 100°C water bath for 6 min and cooled for 3 min with tap water. Absorbance values were determined by spectrophotometer (Shimadzu UV-2900, Japonya) at 600 nm wavelength within 20 minutes (Ross, 1959). The content of reduced sugar in each sample was determined using a standard curve prepared by glucose.

2.5. Phenolic compound analysis

Approximately 1 g of ground olive flesh (from 50 olives) was mixed with 40 ml hexane and agitated for 4 min. The upper phase was recovered and the extraction was repeated twice with the lower phase to remove pigments and lipids. The phenolic compounds were extracted with 80 ml methanol (80%) containing 400 ppm sodium metabisulfite. For the separation of the hydromethanolic phase, the mixture was homogenized for 30 s and this procedure was repeated twice and the hydromethanolic phases were combined. Then hydromethanolic phases were filtered with 0.45 µm nylon syringe filter. Extracted phenolic compounds were dissolved in 1 ml of methanol and analyzed by HPLC. Chromatograms were obtained at 278 nm for hydroxytyrosol and oleuropein and 339 nm for luteolin and rutin (Morello et al., 2004). Chromatographic Conditions: mobile phase A: 0.2 % acetic acid, Mobil phase B: Metanol, flow rate: 1.5 ml/min Total duration: 54 min, column : C18 (5 µm x 250 cm x 4.6 mm) injection volume: 20 µl, detector: Diode Array Detector (279 nm, 339 nm), column temperature: 25^{o}C.

2.6. Statistical analysis

Research plan was performed according to the randomized experimental design. Three replicates were tested for each parameter. Analysis of variance was applied with the Duncan multiple comparison test of the means ($p<0.01$) to determine the presence of significant differences among the samples. Statistical analysis was performed by using the JMP v. 5.0 statistical package program (SAS Institute, Cary, N.C., U.S.A.). The physicochemical characteristics of genotypes were used to perform principal component analysis (PCA) with the PNTSYS statistical package program (Applied Biostatistics Inc., New York, USA).

3. RESULTS AND DISCUSSION

3.1. Fruit weight and flesh to seed ratio

Significant differences among genotypes were observed according to physical characteristics and chemical composition of olives. Genetic diversity of those genotypes was clearly affected physicochemical characteristics of their olives. In general, olive ripening increased the fruit weight and flesh to seed ratio (Table 2). Similar results were also found by Fourati et al. (2002) and Menz and Vriesekoop (2010). Number of olives per kilogram and flesh to seed ratio of olives were given in Table 2. High fruit weight and flesh to seed ratios were found for some genotypes such as 'LT011', 'BU016' and 'AT056'.

Table 2. Number of olives per kilogram and flesh to seed ratio of olives green and black ripeness

Olives	Fruit weight		Flesh to seed ratio	
	Green	Black	Green	Black
AT007	168±22.01e	123±18.96f	4.58±0.19f	4.59±0.14e
AT056	185±14.52c	124±19.35f	5.95±0.23bc	6.24±0.17a
AU019	179±20.22c-e	182±14.20b	3.79±0.28c	3.50±0.18f
BU015	182±21.47cd	150±16.35d	4.56±0.19f	5.16±0.32c-e
BU016	154±25.69f	143±13.55d	6.47±0.24ab	6.19±0.19a
GE015	190±27.17c	178±11.62c	4.90±0.31d-f	5.92±0.13ab
GK024	172±19.35de	140±18.35de	5.84±0.15ef	6.5±0.19a
GK036	184±19.72cd	150±16.12d	3.76±0.19g	5.44±0.24b-d
GK131	146±28.76f	129±13.91ef	5.41±0.14ce	6.01±0.18ab
GK132	124±30.66h	117±16.43f	5.38±0.21ce	4.84±0.26de
GK146	131±26.94gh	93±17.66g	3.72±0.19g	4.91±0.23c-e
GU118	204±14.20b	175±10.85c	4.62±0.24f	5.52±0.14bc
GU404	208±17.62b	184±16.34c	5.00±0.16d-f	4.55±0.19e
GU410	207±21.94b	201±18.60b	5.54±0.30cd	5.47±0.17b-d
LT011	111±17.25i	91±10.83g	6.51±0.12a	5.92±0.18ab
LT019	223±27.25a	199±16.68a	5.34±0.23ce	6.23±0.13a
MT038	143±23.57fg	146±13.12d	4.84±0.18ef	4.43±0.26b-d
Gemlik	215±24.38b	236±18.52a	5.07±0.20d-f	5.10±0.17c-e

Different letters within a column indicate significant differences at the level P < 0.01

3.2. Moisture, reduced sugar and oil content

Moisture, reduced sugar and oil content of olives were given in Table 3. Moisture and reduced sugar content of some olive genotypes decreased from green to black ripeness whereas oil content increased (Table 3). Similar results have been reported in literature for different olive cultivars (Guillen et al., 1993; Nergiz and Engez, 2000). Reduced sugar content was decreased in all olives of genotypes whereas the oil content increased in all olives of genotypes during color turning from green to black.

Only a small decrease (8.7%) was observed at the oil content of 'BU015'. Similar results were found in literature at green and black ripeness of the olives of different cultivars **(**Romani et al., 1999; Kailis and Harris, 2007; Menz and Vriesekoop, 2010)

Table 3. Moisture, reduced sugar and oil content of olives at green and black ripeness(%)

Olives	Moisture content		Reduced sugar content		Oil content	
	Green	Black	Green	Black	Green	Black
AT007	66.98±2.4d	69.22±2.2a-d	2.83±0.2e	2.03±0.2gh	14.83±0.3h	15.47±0.6f-h
AT056	68.01±2.1d	71.16±2.3a-c	3.22±0.1e	2.62±0.1cd	14.56±0.4h	15.35±0.6f-h
AU019	70.99±2.4ab	69.02±2.0a-d	4.30±0.2a	3.17±0.2a	13.41±0.3i	14.76±0.6h
BU015	70.32±2.8bc	56.56±2.3f	3.50±0.2bc	3.05±0.2ab	23.31±0.3a	21.27±0.6ab
BU016	65.47±1.2ef	68.17±2.4b-d	2.07±0.2f	2.07±0.1ef	12.27±0.3j	20.08±0.6b
GE015	55.06±2.5h	70.65±2.5a-d	3.10±0.1a	2.49±0.1de	18.52±0.3f	20.65±0.7ab
GK024	61.82±2.3g	69.18±2.1a-d	2.78±0.2e	2.14±0.2f	12.46±0.3j	16.83±0.6cd
GK036	68.58±2.6cd	73.92±1.8ab	2.16±0.1fg	1.58±0.1i	14.5±0.3h	21.34±0.6ab
GK131	66.68±2.9de	58.16±2.1f	2.39±0.2f	2.81±0.1h	21.38±0.3bc	17.03±0.6cd
GK132	67.58±2.2d	68.18±1.8b-d	2.94±0.2de	2.42±0.1i	12.43±0.3j	17.28±0.7c
GK146	71.77±2.3ab	75.21±2.2a	1.91±0.5g	1.92±0.2gh	12.30±0.4j	15.49±0.6e-h
GU118	67.49±2.2d	59.46±2.2ef	4.25±0.2a	2.67±0.1c-e	16.71±0.3g	17.08±0.7c-e
GU404	68.23±2.7d	66.43±2.4c-e	2.94±0.2bc	2.36±0.2ef	22.47±0.3ab	14.47±0.6gh
GU410	72.80±3.0a	65.23±2.0c-e	3.37±0.2bc	2.72±0.1bc	20.61±0.3cd	15.92±0.6d-g
LT011	70.84±2.5ab	71.31±3.0a-c	3.61±0.2b	1.41±0.1i	15.1±0.3h	16.45±0.7c-f
LT019	66.80±2.8de	60.92±2.3ef	3.29±0.3cd	1.80±0.2h	19.71±0.4de	11.39±0.6j
MT038	64.57±2.5f	68.23±2.1b-d	3.62±0.2b	2.73±0.1c	16.65±0.2g	17.15±0.7i
Gemlik	62.50±2.1g	64.56±2.1de	2.62±0.2e	2.45±0.2de	19.32±0.3ef	21.85±0.7a

Different letters within a column indicate significant differences at the level P <0.01

3.3. Hydrxytrosol, luteolin, rutin and oleuropein content

Hydroxytrosol, luteolin, rutin and oleuropein contents of genotypes showed significant differences (Table 4 and Table 5). Similar results were found in the literature for different genotypes (Tokusoglu et al., 2010). Hydroxytyrosol contents of some olive genotypes could be considered as high, luteolin and rutin contents as similar and oleuropein contents as lower for some genotypes when compared with literature (Esti et al., 1998; Romani et al., 1999). In addition, hydroxytyrosol contents were generally higher than oleuropein concentrations compared with the previous studies of Esti et al. (1998) and Servili et al. (2004). Hydroxytyrosol is one of the degradation products of oleuropein during maturation (Kailis and Harris, 2007; Damak et al., 2008). This observation could be confirmed with this study. In fact, samples had high values for the ratio hydroxytyrosol/oleuropein at black ripeness.

Table 4. Hydroxytyrosol and luteolin content of olives at green and black ripeness (mg/kg)

Olives	Hydroxytyrosol		Luteolin	
	Green	Black	Green	Black
AT007	122,3±35,6q	884,3±45,m	10,3±2,1j	5,5±1,7h
AT056	841,2±103,6	1706,4±92,3d	12,6±1,3i	8,5±2,1e
AU019	2648,1±212,6b	1338,4±80,8h	19,5±1,4g	6,6±2,1e
BU015	1573,6±73,1p	1548,72±83,4f	5,4±2,0kl	4,7±1,0hi
BU016	2396,2±274,9e	1333,62±82,2h	34,3±2,1e	4,1±1,1I
GE015	2566,9±262,5c	2485,60±142,0b	10,5±2,0j	7,0±2,5f
GK024	1317,8±157,4j	683,54±42,3p	40,5±2,3d	7,1±2,2f
GK036	1911,0±122,7g	1868,9±98,9c	21,3±2,4f	16,7±2,8d
GK131	2523,8±208,7d	798,26±48,7n	16,5±3,2h	41,2±3,1a
GK132	1367,1±116,4i	1061,16±52,5k	10,7±2,0j	8,2±1,6e
GK146	2337,4±283,2f	359,46±27,3q	4,4±1,8kl	5,1±1,5hi
GU118	3154,8±214,6a	3857,5±171,4a	17,6±3,4h	18,3±2,1c
GU404	1668,2±138,2h	1290,1±78,6i	53,7±2,7b	20,4±2,0b
GU410	607,0±150,3o	976,1±62,1l	11,0±2,1j	17,2±2,1cd
LT011	676,4±143,2n	788,7±46,7o	60,7±4,4a	7,0±2,1f
LT019	607,1±84,8o	1110,39±65,5j	42,2±3,6c	9,0±2,7e
MT038	1223,6±143,6k	1644,32±88,6e	5,9±1,8k	4,3±1,3i
Gemlik	1051,6±164,5l	1386,2±120,7g	4,1±1,6l	5,6±1,6gh

Different letters within a column indicate significant differences at the level $P < 0.01$

The results of the study indicated that the physicochemical characteristic of olives depends primarily on genetic factors in same agricultural and climatic cultivation. Olive genotypes displayed their own olive weight, flesh to seed ratio, water, reduced sugar, oil and phenolic contents. Similar results were reported in the literature in different olive breeding studies (Parlati et al., 1994; Bellini et al., 2008).

Table 5. Rutin and oleuropein content of olives at green and black ripeness (mg/kg)

Olives	Rutin		Oleuropein	
	Green	Black	Green	Black
AT007	225,9±29,2d	199,1±7,5d	1273,3±113,6m	514,5±26,3i
AT056	189,3±31,8e	277,0±11,9b	3271,1±387,4a	840,6±41,2e
AU019	586,9±52,1a	63,9±4,4ik	2393,6±319,5c	189,7±17,5n
BU015	80,4±3,5i	111,3±5,2g	1038,8±95,3n	219,1±14,6m
BU016	44,2±2,3l	166,0±6,6e	1268,4±98,6m	377,1±14,8l
GE015	369,8±32,1b	97,3±3,5h	1987,1±120,6e	410,9±16,0j
GK024	57,1±3,9k	31,0±4,2l	929,4±61,3o	555,0±26,3h
GK036	69,5±3,7j	92,5±4,9h	855,5±47,4p	869,8±37,4d
GK131	71,6±4,2j	229,1±12,4c	1371,2±106,4j	889,4±39,8c
GK132	54,3±3,6k	61,5±3,5jk	1352,7±78,3k	590,8±27,1g
GK146	172,1±12,5f	132,7±6,6f	2485,2±387,4b	681,2±27,3f
GU118	132,7±11,7g	98,6±4,8h	1759,3±144,2h	2572,4±97,8a
GU404	133,0±16,4g	136,8±5,5f	1035,8±73,5n	180,1±9,6n
GU410	174,1±24,7f	135,9±6,4f	1800,7±126,5g	550,4±17,9h
LT011	239,6±30,4c	59,1±4,1k	2165,2±164,9d	370,6±21,3l
LT019	185,3±33,5e	66,2±3,8i	1920,6±153,6f	896,0±36,0c
MT038	124,6±15,0h	466,3±10,7a	1289,5±83,1 l	397,8±17,4k
Gemlik	81,2±8,2h	68,3±4,2i	1521,6±115,8i	1028,9±46,9b

Different letters within a column indicate significant differences at the level P < 0.01

3.4. Principal component analysis (PCA)

PCA was also performed on the results of the olive analysis. PCA results accounted for green and black olives which had 74 and 67% of the total variance respectively (Fig. 1 and 2). The biplot score of PCA of green olives showed four groups according to the analyzed physicochemical characteristics (Fig. 1).

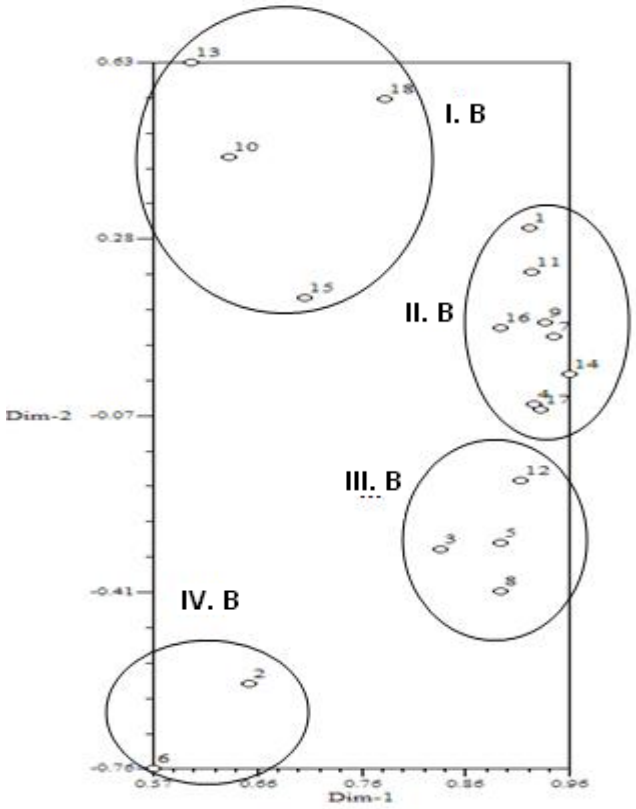

Fig. 1: PCA scores for black olives on plot. Indicate the four principal groups described in the text.

Group I.B contained three genotypes (‘GU404’, ‘LT011’ and ‘GK132’) and ‘Gemlik’ cultivar characterized by high flesh to seed ratio, oil and rutin content and medium oleuropein content. Group II.B (‘AT007’, ‘GK146’, ‘LT019’, ‘GK131’, ‘GK024’, ‘GU410’, ‘BU015’ and ‘MT038’) was characterized by a medium number of olives per kg and flesh to seed ratio and low luteolin content. Group III.B (‘AU019’, ‘BU016’, ‘GK036’ and ‘GU118’) had low oil and high hydroxytyrosol content. Group IV.B (‘AT056’ and ‘GE015’) showed a low number of olives per kg and flesh to seed ratio, high rutin and medium-high oleuropein content.

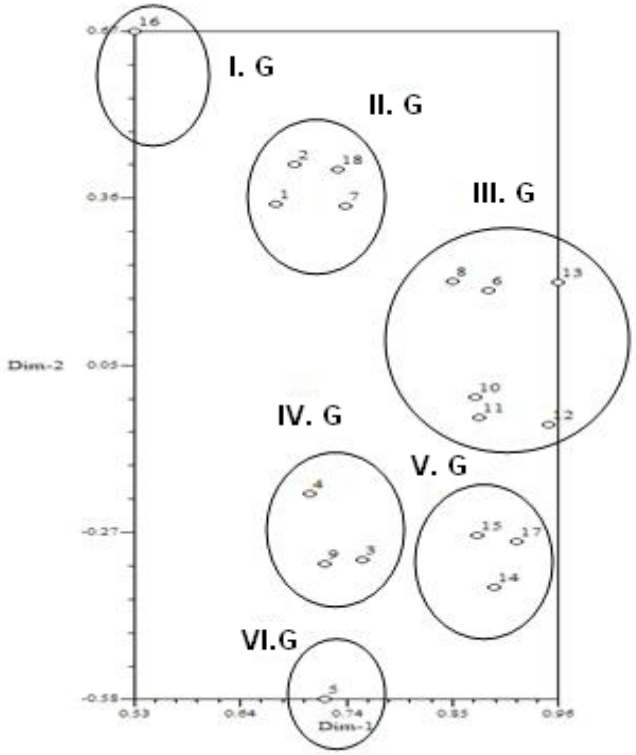

Fig. 2: PCA scores for green olives on plot. Indicate the six principal groups described in the text.

The biplot score of PCA of green olives showed six groups according to the analyzed physicochemical characteristics (Fig. 2). Group I.G had only one genotype (LT019) which contained high oil and oleuropein and low rutin content. Group II.G (AT056, Gemlik, AT007 and GK024) was characterized by low-medium water, high-medium hydroxytyrosol and oleuropein content. Group III.G ('GE015', 'GK036', 'GK132', 'GK146', 'GU118', and 'GU404') had a high flesh to seed ratio, low-medium reduced sugar and medium–high rutin content. Group IV.G ('AU019', 'BU015' and 'GK131') showed high hydroxytyrosol and medium-high oil content. Group V.G ('GU410', 'LT011' and 'MT038') was characterized by medium oil and low hydroxytyrosol and oleuropein content. Group VI.G had only one genotype ('BU016') which had highest flesh to seed ratio and high water and oil content.

4. CONCLUSION

These results of this research indicated that 17 advanced olive genotypes had different physicochemical characteristics. Also PCA scores of physicochemical characteristics of genotype's fruit allowed to classification into four groups for black genotypes and six groups for green genotypes. Olives of AT056, GK024, GK131 and LT011 genotypes showed better table olive characteristics than olive of Gemlik cultivar. They have potential for registration as new table olive cultivars. Olives of GU118, AU019, GE015, GK131 and BU016 genotypes contain high concentration of hydroxytyrosol which are noteworthy for nutritional physiology and olives of GU404, LT011, BU016, BU015 and AU019 genotypes contain low concentrations of oleuropein which are remarkable for table olive processing technology. Low oleuropein concentration simplifies and shortens debittering procedures of olives which is the fundamental and most important step of table olive processing. According to result of oil content and table olive characteristics, olives of some genotypes such as BU015, GK036 and GE015 can be registered for table olive cultivar or double purpose cultvar to produce both table olive and oil production because of their favorable table olive characteristics and high oil content.

ACKNOWLEDGMENT

This work was supported by project (TAGEM/GY/09/03/06/154) Ataturk Central Horticultural Research Institute and funded by the Ministry of Food, Agriculture and Livestock of Turkey.

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