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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Frost Tolerances of Turkish Olive (*Olea europaea* L.) Cultivars

Türkiye Zeytin Çeşitlerinin Dona Toleransları

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Abstract

The motherland of the olive is considered as east of the Mediterranean Basin. Turkey is partly located within this basin having a rich source of biodiversity. Olive growing in Turkey is carried out in the Aegean, Marmara, Mediterranean, Southeastern Anatolia and Black Sea Regions. Climatic factors determine the cultivation limits of olive which is not very selective in terms of soil demand. In regions where olives are grown, the Mediterranean climate prevails. Winters are warm and rainy in the region, while summers are hot and dry. On the other hand, severe cold damage in some years can cause significant damage especially in the inner parts of Aegean, Marmara and Black Sea Regions. As a matter of fact, it is known that 31.8% of olive plantations in Turkey are exposed to occasional frost damage. This situation has revealed the necessity of determining the varieties with high frost tolerance and determining the suitable varieties for the regions where cold damage may occur. In the study, 40 registered olive cultivars in “Kemalpaşa Olive Germplasm Bank" in Bornova Olive Research Institute, in İzmir were screened in terms of frost tolerance. To evaluate the frost tolerance (LT_{50}) of cultivars, as estimated by ion leakage (electrical conductivity), leaf samples taken in six different periods over two years were exposed to temperatures at +4 °C (control), -2 °C, -5°C, -8 °C, -11°C, -14 °C, -17 °C and -20 °C. In conclusion; it has been determined that frost tolerance of olive varieties revealed significant variability both genetically and seasonally. Butko, Memeli, Otur, Gemlik, Sinop No 5, Yün Çelebi, Kara Yaprak, Satı and Sarı Ulak were determined as cultivars that were more tolerant of frost. Sinop No 1, Marantelli, Ayvalık, Görvele, Çakır, Samsun Tuzlamalık, Erkence, Saurani, Eşek Zeytini (Tekirdağ), Kan Çelebi, İzmir Sofralık, Çilli, Samsun Yağlık, Domat, Eşek Zeytini (Ödemiş), Saçaklı Otur, Sinop No 4, Memecik, Nizip Yağlık, Tekirdağ Çizmelik and Patos were found to be moderately tolerant cultivars while Edincik, Sinop No 6, Çekişte, Mavi, Kiraz, Kilis Yağlık, Çelebi (İznik), Trabzon Yağlık, Uslu and Girit olive cultivars were grouped as cultivars having low tolerance to frost. In addition, data showed that cold acclimation in the olive is quite important, and exposure to low temperatures for a certain period of time has significantly increased the frost tolerance. However, this condition was not stable and could rapidly become reversed when temperature reached to the point at which adapdation did not ensure.

Keywords: Olive, Acclimation, Cultivar, Frost tolerance, LT_{50}, *Olea europaea* L.

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Öz

Zeytinin anavatanı Akdeniz Havzası'nın doğusu olarak görülmektedir. Türkiye coğrafi konum olarak bu havzayı kısmen içermektedir ve zeytin biyoçeşitliliği bakımından zengin bir kaynağa sahiptir. Türkiye'de zeytin yetiştiriciliği Ege, Marmara, Akdeniz, Güneydoğu Anadolu ve Karadeniz bölgelerinde yapılmaktadır. Toprak isteği bakımından fazla seçici olmayan zeytinin sınırlarını iklimsel faktörler belirlemektedir. Zeytincilik yapılan bölgelerde genellikle Akdeniz iklimi hüküm sürmektedir. Bu bölgelerde kışlar ılık ve yağışlı, yazlar ise sıcak ve kurak geçmektedir. Buna karşın, bazı yıllar oluşan şiddetli soğuklar özellikle İç Ege, Marmara ve Karadeniz bölgelerinde önemli zararlanmalara neden olabilmektedir. Nitekim Ülkemizdeki zeytin işletmelerinin % 31.8'inin dönem dönem don zararına maruz kaldığı bilinmektedir. Bu durum don toleransı yüksek çeşitlerin belirlenmesi ve soğuk zararlarının meydana gelebileceği bölgeler için uygun çeşitlerin saptanması gerekliliğini ortaya çıkarmıştır. Çalışmada Zeytincilik Araştırma Enstitüsü (Bornova/İzmir) bünyesinde bulunan "Kemalpaşa Zeytin Arazi Gen Bankası"ndaki 40 zeytin çeşidinin don toleransı araştırılmıştır. Çeşitlerin dona toleransının araştırmak amacıyla iki yıllık süreçte altı farklı dönemde alınan yaprak örnekleri kontrol (+4°C), -2°C, -5°C, -8°C, -11°C, -14°C, -17°C ve -20°C'lerde don testlerine tabi tutulmuş ve bu testlerde iyon sızıntısı (elektriksel iletkenlik) yönteminden yararlanılmıştır. Sonuç olarak zeytin çeşitlerinin don toleransının gerek genetik olarak gerekse mevsimsel olarak önemli değişkenlikler gösterdiği saptanmıştır. Butko, Memeli, Otur, Gemlik, Sinop No 5, Yün çelebi, Kara yaprak, Satı ve Sarı Ulak çeşitlerinin don toleransının yüksek olduğu belirlenmiştir. Sinop No 1, Marantelli, Ayvalık, Görvele, Çakır, Samsun Tuzlamalık, Erkence, Saurani, Eşek Zeytini (Tekirdağ), Kan Çelebi, İzmir Sofralık, Çilli, Samsun Yağlık, Domat, Eşek Zeytini (Ödemiş), Saçaklı Otur, Sinop No 4, Memecik, Nizip Yağlık, Tekirdağ Çizmelik ve Patos çeşitlerinin dona orta derecede toleranslı olduğu, Edincik, Sinop No 6, Çekişte, Mavi, Kiraz, Kilis Yağlık, Çelebi (İznik), Trabzon Yağlık, Uslu ve Girit Zeytini çeşitlerinin ise don toleransının düşük olduğu tespit edilmiştir. Ayrıca sonuçlar zeytinde soğuk aklimasyonunun oldukça önemli olduğunu ve belirli bir süre düşük sıcaklıklara maruz kalmanın don toleransını önemli derecede arttırdığını göstermiştir. Ancak bu durumun durağan olmadığı ve uyumun gerçekleşmediği sıcaklığa dönüldüğünde hızlıca kaybedilebileceği görülmüştür.

Anahtar Kelimeler: Zeytin, Aklimasyon, Çeşit, Don toleransı, LT_{50}, *Olea europaea* L.

1. Introduction

Between about the 30°-45° latitudes Mediterranean region is considered as economic olive production zone (Bongi and Palliotti, 1994; Mancuso, 2000). Low temperature is one of the most important abiotic stress factors limiting production and quality of olive within this zone (Yang et al., 2005; Sanzani et al., 2012; Aybar et al., 2015). Olive is a plant species with better frost tolerance compared to other subtropical plant species. However, leaf loss and branch dieback may occur in olive trees depending on the period and temperature, specially in the case of lower than -7°C and this leads to severe yield loss (Vitagliano and Sebastiani, 2002). Although it is stated that plants may completely die when temperature drops to -12°C, some cultivars are able to display frost tolerance at temperatures from -12°C to -18°C (Fiorino and Mancuso, 2000; De-Andres Cantero, 2001).

Olive has been cultivated in Aegean, Marmara, Mediterranean, Southeastern Anatolia, and Black Sea Regions of Turkey. Table olive and olive oil are considered as some of the most important agricultural products of this geography (Mete et al., 2016b). Olive trees cultivated in these regions are considerably influenced by climatic factors in some years. It was reported that numerous olive trees were exposed to extremely severe frost damage especially in Marmara region after sudden decreases of weather temperature in the years 1983, 1985, and 1987 (Usanmaz et al., 1988). A similar case occurred in Mudanya district of Bursa province in 2010 and many olive trees got damaged because of low temperature. Likewise, excessively low temperatures in January 2015 caused defoliations and branch dieback in olive trees (Mete et al., 2016a). A study conducted throughout Turkey reported that 31.8% of olive plantations suffered from frost damage periodically (Özaltaş et al., 2016). In addition, probability of low temperature induced injury in olive trees has increased as the result of shift in olive cultivation areas to more marginal areas in recent years.

Frost damage occurring in plants depends on genetic structure, phenological period, nutrition and health condition, age of the plant and organs that are exposed to frost stress (Graniti et al., 2011). Air humidity, period of temperature, rate of decrease in temperature, and speed and direction of wind are effective on frost. Depending on this condition, frost tolerance of plants is affected by its genetic structure and environmental factors and thus it shows variation (Beck et al., 2004). Therefore, it is crucial in terms of reliability of the results to take samples homogenously from the trees cultivated under the same conditions to carry out artificial freezing tests.

Different methods have been used in order to determine frost tolerance of olive cultivars. Among these methods, "electrolyte leakage tests" (measured by the electrical conductivity) is considered as the most effective and practical method because it shows the cell membrane injury right after the frost damage (Palliotti and Bongi, 1996; Bartolozzi and Fontanazza, 1999; Mancuso, 2000). Bartolozzi and Fontanazza (1999) noted in their studies caried out to identify tolerance of olive cultivars to low temperatures (indicated by LT_{50}) that olive cultivars 'Bouteillan' (LT_{50}: -18.2°C) and 'Nostrale di Rigali' (LT_{50}: -18°C) were tolerant to frost, whereas the cultivars 'Morcona' (LT_{50}:-11.4 °C) and 'Borsciona' (LT_{50}:-12.2°C) were sensitive. During winter months, mean LT_{50} was determined to be -12 °C for leaves, -13 °C for buds, and -18 °C for offshoots of the olive cultivars 'Nostrale di Rigali', 'Frantoio', 'Leccino', and 'Moralio' (Antognozzi et al., 1990). Mancuso (2000) stated that LT_{50} was between -12.0 °C ('Coratina') and −18.3 °C ('Ascolana') for some olive cultivars. Frost tolerance of olive cultivars 'Roghani', 'Zard', 'Mission', and 'Kroneiki' was determined in the periods of December and October. 'Zard' was found to be the most tolerant one among the studied cultivars (Soleimani et al., 2004). Asl Moshtaghi et al. (2009) investigated frost tolerance of 7 different olive cultivars and determined that 'Delghan' cultivar was the one with the highest frost tolerance. In another study comparing frost damage occurring under natural circumstances and after artificial freezing tests, 'Cornicabra' (-13.3°C) and 'Picual' (-13.2°C) were the most tolerant cultivars, while 'Empeltre' (-9.5°C) cultivar was the most sensitive according to LT_{50} value within 8 different cultivars (Barranco et al., 2005). Cansev et al. (2009) carried out freezing tests on leaves of the cultivars 'Manzanilla', 'Lecques', 'Ascolona', 'Hojoblanca', 'Domat', 'Meski', 'Uslu', 'Gemlik' and 'Samanlı' during dormant and active growth periods. According to the results, 'Manzanilla' was the most sensitive to low temperature stress and 'Domat' was the cultivar with the highest tolerance. Other cultivars were found to be moderately tolerant. A study of frost tolerance of 24 olive cultivars under field conditions in Italy revealed that only 3 cultivars ('Ascolana dura', 'Orbetana', and 'Mignolone') had considerable frost tolerance (Lodolini et al., 2016).

To the best of our knowledge, there are limited studies conducted to determine the tolerance of local olive cultivars to freezing stress. In this manner, determining the freezing tolerance of the cultivars is very important for effective olive growing as well as olive breeding studies in the future. Therefore the study was conducted in order to identify the cultivars with high frost tolerance and determine the cultivars appropriate for the regions where cold damage may occur. In the study, 40 major olive cultivars found in the Olive Germplasm Bank of Turkey were screened by freezing test and clustered based on their cold-hardiness.

2. Materials and Methods

2.1. Plant material

The study was conducted on 47 years old olive trees of 40 olive cultivars planted in Olive Germplasm Bank of Turkey (Kemalpaşa, Izmir / Turkey: 38°27'49.94"K, 27°22'33.64"D). All of the cultivars listed on *Table 1* were planted in the same orchard and were subjected to equal cultural procedures. And to better asses the cold hardiness of cultivars the samples were taken in three periods within a year for two consecutive years. These periods were determined as follows; November when cold occurs, January being one of the coldest months of the year (at which time they are cold-acclimated), and March when the flowering starts.

For the freezing test, leaf samples were collected randomly from the middle part of current-year shoots (30-40 cm) located in the northern site of the tree in each cultivar and packed on ice and brought to the laboratory.

Table 1. Olive cultivars used in the study and their region of origin

Cultivars	Region of origin	Cultivars	Region of origin
Ayvalık	Aegean	Sarı Ulak	Mediterranean
Çakır	Aegean	Yün Çelebi	Southeastern
Çekişte	Aegean	Kan Çelebi	Southeastern
Çilli	Aegean	Kilis Yağlık	Southeastern
Girit Zeytini	Aegean	Mavi	Southeastern
Domat	Aegean	Nizip Yağlık	Southeastern
Eşek Zeytini (Ödemiş)	Aegean	Butko	Blacksea
İzmir Sofralık	Aegean	Görvele	Blacksea
Kiraz	Aegean	Marantelli	Blacksea
Memeli	Aegean	Patos	Blacksea
Uslu	Aegean	Otur	Blacksea
Erkence	Aegean	Saçaklı Otur	Blacksea
Memecik	Aegean	Satı	Blacksea
Kara Yaprak	Aegean	Samsun Yağlık	Blacksea
Gemlik	Marmara	Samsun Tuzlamalık	Blacksea
Tekirdağ Çizmelik	Marmara	Trabzon Yağlık	Blacksea
Edincik	Marmara	Sinop No 1	Blacksea
Eşek Zeytini (Tekirdağ)	Marmara	Sinop No 4	Blacksea
Çelebi (İznik)	Marmara	Sinop No 5	Blacksea
Saurani	Mediterranean	Sinop No 6	Blacksea

2.2. Controlled freezing test

In this study, membrane injury of leaf tissues was carried out by ion leakage method developed by Arora et al. (1992) and modified for olive plant by Eriş et al. (2007). Briefly, leaf discs in 10 mm diameter were punched from the leaves, they were then lightly rinsed in distilled water, gently blotted with paper, and placed in test tubes (one disc per tube). Tubes were placed in a glycol freezing bath (JULABO brand F38-ME). Three replications were performed for each temperature and each cultivar in each leaf sampling period. A water bath device with cooler and a 45-lt chamber manufactured from stainless steel was used for applying the freezing tests. The temperature was decreased at 2ºC/h of speed, down to +4ºC to -2, -5, -8, -11, -14, -17, and -20ºC and samples were allowed to gradually thaw inside ice box removing from freezing bath after keeping them for 2 hours at each of temperature.

2.3. Cell membrane injury and cold-hardiness determination

Ten ml of distilled water was added to each tubes from exposed and non-exposed (control) to freezing stress and theywere then shaken on a gyratory shaker (250 rpm) for 4 h at room temperature. Electrical conductivity of each sample was measured using Selecta- CD-2005 conductivity meter. Electrical conductivity of each sample was measured once more after the tubes were autoclaved (0,12 MPa, 120°C, 20 min) and cooled. Proportional injury at each temperature was calculated from ion leakage data using the equation: proportional injury=[(proportional L(t)- proportional L(c)) / (1- proportional L(c))], where proportional L(t) and proportional L(c) are percentage ion leakage data for the treatments and control samples, respectively (Arora et al., 1992). Cold-hardiness (LT_{50}) was defined as the subzero temperature at which 50% injury occurred.

2.4. Statistical analyses

The study was planned with 3 replications according to randomized block experimental design. Resulting data was statistically analyzed using Student's t grouping test in JMP software. Data from cold-hardiness were evaluated according to logistic regresyon analysis (Weisberg, 1985).

3. Results and Discussion

3.1. Cold-hardiness of olive cultivars

Freezing tests were repeated for two years and tests were applied in three different periods for each year. These periods were November 2014, January 2015, March 2015 (1^{st} year), November 2015, January 2016, and March 2016 (2^{nd} year). *Table 2* and *Table 3* show mean LT_{50} values of the cultivars within these periods.

In November of the year 1, though the cultivar 'Yün Çelebi' had the highest LT_{50} value with -14.25°C. The cultivars 'Memeli' (-14.25°C, LT_{50}) and 'Otur' (-14.01°C, LT_{50}) were statistically found within the same group as 'Yün Çelebi' cultivar. These were followed by the cultivars 'Sinop no 5' (-13.97°C, LT_{50}) and 'Butko' (-13.83°C, LT_{50}) . The cultivar with the lowest frost tolerance was 'Çelebi (İznik)' which had -9.30°C of LT_{50} result. Other cultivars with relatively low frost tolerance within this period were 'Uslu' (-10.18°C, LT_{50}), 'Girit Zeytini' (-10.59°C, LT_{50}), 'Eşek Zeytini' (Ödemiş) (-10.84°C, LT_{50}), 'Tekirdağ Çizmelik' (-10.85°C, LT_{50}), and 'Trabzon Yağlık' (-10.88°C, LT_{50}). LT_{50} values increased based on cold acclimation in January 2015 when air temperature is lowest among all the periods. LT_{50} values of the cultivars varied between -22.53°C ('Satı') and -13.51°C ('Çelebi, İznik'). The cultivar 'Gemlik' which is one of the most important table olive cultivars in Turkey had -22.42 °C of LT_{50} value and statistically involved in the same group with cultivar 'Satı'. These cultivars were followed by the cultivars 'Otur' (-22.06°C, LT_{50}), 'Memeli' (-21.98°C, LT_{50}), and 'Kan Çelebi' (-21.47°C, LT_{50}), respectively. The cultivars 'Uslu' (-14.78°C, LT_{50}), 'Girit Zeytini' (-14.83°C, LT_{50}), 'Çekişte' (-14.89°C, LT_{50}), and 'Trabzon Yağlık' (-15.23°C, LT_{50}) as well as 'Çelebi (İznik)' shared the last five ranks. In March, which was the last period of the tests for the year 1, the cultivar having the highest frost tolerance with -21.63°C of LT_{50} was 'Butko'. This cultivar was followed by the cultivars 'Sinop no 5' (-20.50°C, LT_{50}), 'Kara Yaprak' (-20.30°C, LT_{50}), 'Yün Çelebi' (-19.63°C, LT_{50}), and 'Memeli' (-18.42°C, LT_{50}). The last 5 ranks within this period were listed as follows; 'Girit Zeytini' ($LT_{50,}$ -11.60°C), 'Sinop no 6' (-11.80°C, LT_{50}), 'Trabzon Yağlık' (-11.87°C, LT_{50}), 'Uslu' (-12.63°C, LT_{50}), and 'Eşek Zeytini (Ödemiş)' (-12.82°C, LT_{50}).

The cultivar with the highest LT_{50} value obtained from 2^{nd} year freezing tests conducted in November was 'Otur'. This cultivar with -14.40°C of LT_{50} value was followed by the cultivars 'Çakır '(-14.35°C, LT_{50}), 'Butko' (-14.30°C, $LT_{50,}$), 'Memeli' ($LT_{50,}$ -13.57°C) and 'Kara Yaprak' (-12.94°C, LT_{50}). The cultivars 'Çekişte' ($LT_{50,}$ -10.21°C), 'Kilis Yağlık' ($LT_{50,}$ -10.14°C), 'Kiraz' (-9.83°C, LT_{50}), 'Mavi' ($LT_{50,}$ -9.78°C) and 'Sinop no 5' (-9.68°C, LT_{50}) were the cultivars with the lowest frost tolerance. LT_{50} values obtained from 2^{nd} year freezing tests in January were found to be lower compared to same period in previous year. LT_{50} values of the cultivars varied between -12.06°C ('Girit Zeytini') and -17.96°C ('Saçaklı Otur'). The first 5 cultivars with high frost tolerance within this period were determined as Saçaklı Otur' (-17.96°C, LT_{50}), 'Butko' (-17.71°C, LT_{50}), 'Memeli' (-17.59°C, LT_{50}), 'Yün Çelebi' (-17.52°C, LT_{50}), and 'Gemlik' (-17.24°C, LT_{50}). Within the last 5 ranks were the cultivars 'Girit Zeytini' (-12.06°C, LT_{50}), 'Kilis Yağlık' (-12.57°C, LT_{50}), 'Uslu' (-12.60°C, LT_{50}), 'Çekişte' (-13.04°C, LT_{50}), and 'Domat' (-13.08°C, LT_{50}). The first 5 cultivars with the lowest frost tolerance in March were 'Edincik' (-9.43°C, LT_{50}), 'Girit Zeytini' (-9.85°C, LT_{50}), 'Trabzon Yağlık' (-10.34°C, LT_{50}), 'Uslu' (-10.60°C, LT_{50}), and 'Kiraz' (-10.74°C, $LT_{50,}$). Within this period, the cultivar 'Memeli' had the highest frost tolerance with -14.59 °C of LT_{50} value. This cultivar was followed by 'Erkence' (-13.97°C, $LT_{50,}$), 'Butko' (-13.74°C, LT_{50}), 'Ayvalık' (-13.74°C, LT_{50}), and 'Kara Yaprak' (-13.65°C, $LT_{50,}$), respectively. The other cultivars were aligned between these groups.

Table 2. The cold-hardiness (LT_{50}) of the olive cultivars in three different periods in the first year

November 2014		January 2015		March 2015	
Cultivar	**LT_{50} (- °C)***	**Cultivar**	**LT_{50} (- °C)***	**Cultivar**	**LT_{50} (- °C)***
Yün çelebi	14.25 a	Satı	22.53 a	Butko	21.63 a
Memeli	14.25 a	Gemlik	22.42 a	Sinop No 5	20.50 ab
Otur	14.01 a	Otur	22.06 ab	Kara Yaprak	20.30 b
Sinop No 5	13.97 ab	Memeli	21.98 a-c	Yün Çelebi	19.63 bc
Butko	13.83 a-c	Kan Çelebi	21.47 a-d	Memeli	18.42 cd
Çekişte	13.38 b-d	Eşek Zeytini (Ö)	21.17 b-e	Sinop No 1	18.39 cd
Domat	13.25 c-e	Görvele	21.06 b-e	Gemlik	18.30 d
Satı	13.24 c-e	Sarı Ulak	20.77 c-e	Nizip Yağlık	18.00 de
Sinop No 6	13.08 d-f	Eşek Zeytini (T)	20.60 de	Otur	17.97 de
Samsun Yağlık	12.99 d-g	Butko	20.43 de	Ayvalık	17.88 de
Marantelli	12.98 d-g	Sinop No 1	20.36 de	Marantelli	17.88 de
Çakır	12.89 d-h	Samsun Tuzlamalık	20.09 e	Çakır	17.80 d-f
Eşek Zeytini (T)	12.81 d-ı	Sinop No 5	20.03 e	Satı	17.71 d-f
Saurani	12.73 e-j	Yün Çelebi	18.68 f	Saurani	17.58 d-f
Sinop No 4	12.72 e-j	Erkence	18.67 f	Sarı Ulak	17.52 d-f
Samsun Tuzlamalık	12.68 e-k	İzmir Sofralık	18.50 fg	Domat	17.44 d-f
Saçaklı Otur	12.64 e-l	Marantelli	18.50 fg	Tekirdağ Çizmelik	16.89 e-g
Sarı Ulak	12.56 fg-m	Çilli	18.36 f-h	Samsun Tuzlamalık	16.85 e-g
Kara Yaprak	12.43 g-n	Ayvalık	18.36 f-h	Samsun Yağlık	16.79 e-h
Erkence	12.31 h-no	Domat	18.31 f-h	Kan Çelebi	16.56 f-ı
Nizip Yağlık	12.27 ı-o	Mavi	18.12 f-ı	Görvele	16.18 g-j
Patos	12.22 ı-o	Edincik	18.09 f-ı	Eşek Zeytini (T)	15.64 h-k
Memecik	12.19 j-o	Kilis Yağlık	17.40 g-j	Sinop No 4	15.40 ı-l
Kan Çelebi	12.15 j-o	Kiraz	17.30 g-j	İzmir Sofralık	15.35 ı-l
Sinop No 1	12.10 k-p	Memecik	17.23 g-j	Çilli	15.34 ı-l
Edincik	12.06 l-p	Saçaklı Otur	17.18 h-j	Saçaklı Otur	15.25 j-l
Ayvalık	12.03 l-p	Saurani	17.03 ı-k	Erkence	15.19 j-l
Görvele	11.98 m-p	Çakır	16.89 ı-k	Patos	15.14 j-l
İzmir Sofralık	11.96 m-p	Sinop No 6	16.89 ı-k	Edincik	15.08 j-l
Kilis Yağlık	11.86 n-q	Kara Yaprak	16.72 jk	Çekişte	14.74 k-m
Kiraz	11.75 o-q	Tekirdağ Çizmelik	16.45 j-l	Kiraz	14.68 k-m
Gemlik	11.74 o-q	Sinop No 4	16.39 j-l	Memecik	14.39 k-m
Çilli	11.51 pq	Nizip Yağlık	16.28 j-l	Kilis Yağlık	14.16 l-n
Mavi	11.25 qr	Samsun Yağlık	15.97 k-m	Çelebi (İznik)	13.55 m-o
Trabzon Yağlık	10.88 rs	Patos	15.25 lm	Mavi	13.07 n-p
Tekirdağ Çizmelik	10.85 rs	Trabzon Yağlık	15.23 lm	Eşek Zeytini (Ö)	12.82 o-q
Eşek Zeytini (Ö)	10.84 rs	Çekişte	14.89 m	Uslu	12.63 o-q
Girit Zeytini	10.59 st	Girit Zeytini	14.83 m	Trabzon Yağlık	11.87 pq
Uslu	10.18 t	Uslu	14.78 m	Sinop No 6	11.80 q
Çelebi (İznik)	9.30 u	Çelebi (İznik)	13.51 n	Girit Zeytini	11.60 q

* Means grouped by Student's t test ($p<0.05$)

The statistical grouping of all the cultivars based on their periodic mean LT_{50} values was shown in *Table 4*. Periodic mean LT_{50} value of all cultivars was determined as -12.32°C (November), -18.27°C (January), and -16.20°C (March) for the 1st year; -11.72°C (November), -15.19°C (January), and -12.17°C (March) for the 2nd year. The average daily temperatures for the last 30 days before application of freezing tests in this period (*Table 4*) were determined to be 4.39°C (January 2015), 8.10°C (March 2015), 6.25°C (January 2016), 12.52°C (November 2014), -12.85°C (March 2016), and -13.97°C (November 2015). Upon examination of the data, a negative correlation between frost tolerance and temperature was found to be notable. In the northern hemisphere, cold acclimation starts in fall when day-time begins to get shorter and non-optimal temperature starts for vegetation.

In addition, subzero degrees that are not lower than critical damage threshold elevates frost resistance (Vitagliano and Sebastiani, 2002). Similar results were also obtained from this study. The highest frost tolerance was obtained in January of 1st year (2015) when average daily temperature was the lowest and temperature was measured to be the highest in November of 2nd year (2015) when frost tolerance was lower. There was a difference only between mean LT_{50} values in March of 1st year (2015) and mean LT_{50} values in January of 2nd year (2016) in terms of temperature correlation. This situation may be associated with substantially increased cold acclimation of cultivars depending on low temperatures in the period of January in 1st year (2015). Low temperatures occurring within February of the same year ensured continuity of cold adaptation process and were considered to cause high frost tolerance in March of 1st year (2015). Moreover, there was a sharp fluctuation between daily average temperatures in January of 2nd year (2016). This might have hadnegative effects on cold acclimation of the cultivars.

Table 3. The cold-hardiness (LT_{50}) of the olive cultivars in three different periods in the second year

November 2015		January 2016		March 2016	
Cultivar	**LT_{50} (- °C)***	**Cultivar**	**LT_{50} (- °C)***	**Cultivar**	**LT_{50} (- °C)***
Otur	14.40 a	Saçaklı Otur	17.96 a	Memeli	14.59 a
Çakır	14.35 a	Butko	17.71 ab	Erkence	13.97 ab
Butko	14.30 ab	Memeli	17.59 ab	Butko	13.74 bc
Memeli	13.57 bc	Yün Çelebi	17.52 ab	Ayvalık	13.74 bc
Kara Yaprak	12.94 cd	Gemlik	17.24 a-c	Kara Yaprak	13.65 b-d
Samsun Tuzlamalık	12.82 de	Otur	16.96 a-d	Çilli	13.60 b-d
Sinop No 1	12.78 de	Sinop No 5	16.79 b-e	Eşek Zeytini (Ö)	13.51 b-d
Sarı Ulak	12.55 d-f	Görvele	16.79 b-e	İzmir Sofralık	13.33 c-e
Ayvalık	12.52 d-g	Marantelli	16.78 b-e	Saurani	13.12 c-f
Patos	12.47 d-g	İzmir Sofralık	16.70 b-e	Sinop No 1	13.07 d-f
Erkence	12.39 d-h	Çilli	16.36 c-f	Yün Çelebi	13.07 d-f
Samsun Yağlık	12.39 d-h	Saurani	16.24 c-f	Gemlik	12.85 e-g
Eşek Zeytini (T)	12.35 d-h	Satı	16.14 c-f	Çakır	12.68 f-h
Çilli	12.27 d-ı	Kara Yaprak	16.09 d-g	Sarı Ulak	12.56 f-ı
Marantelli	12.14 e-j	Kan Çelebi	16.07 d-g	Samsun Yağlık	12.51 f-ı
Çelebi (İznik)	11.90 f-k	Erkence	15.91 d-h	Görvele	12.40 g-j
Domat	11.78 g-l	Sinop No 4	15.81 e-ı	Sinop No 5	12.39 g-j
Sinop No 4	11.72 h-l	Memecik	15.75 e-j	Satı	12.35 g-j
Gemlik	11.71 h-l	Eşek Zeytini (Ö)	15.55 f-k	Samsun Tuzlamalık	12.35 g-j
Nizip Yağlık	11.68 h-l	Ayvalık	15.03 g-l	Çekişte	12.26 g-j
Satı	11.60 ı-m	Sarı Ulak	14.94 h-l	Sinop No 4	12.14 h-k
Saurani	11.59 ı-m	Eşek Zeytini (T)	14.93 h-m	Otur	12.07 h-k
İzmir Sofralık	11.48 j-n	Sinop No 6	14.80 ı-n	Memecik	12.02 ı-l
Görvele	11.44 k-n	Samsun Yağlık	14.79 ı-n	Patos	11.98 ı-l
Girit Zeytini	11.32 k-o	Samsun Tuzlamalık	14.73 ı-o	Nizip Yağlık	11.94 ı-l
Sinop No 6	11.32 k-o	Mavi	14.69 j-o	Çelebi (İznik)	11.83 j-m
Tekirdağ Çizmelik	11.21 k-p	Edincik	14.64 k-o	Marantelli	11.79 j-m
Memecik	11.13 l-p	Tekirdağ Çizmelik	14.64 k-o	Sinop No 6	11.78 j-m
Trabzon Yağlık	11.10 l-p	Çakır	14.26 l-p	Mavi	11.59 k-n
Uslu	10.91 m-q	Trabzon Yağlık	13.83 m-q	Eşek Zeytini (T)	11.59 k-n
Yün Çelebi	10.88 m-r	Patos	13.69 n-r	Tekirdağ Çizmelik	11.40 l-o
Kan Çelebi	10.75 n-r	Sinop No 1	13.65 o-s	Domat	11.25 m-p
Edincik	10.59 o-r	Nizip Yağlık	13.34 p-s	Kan Çelebi	11.10 n-q
Saçaklı Otur	10.50 p-s	Çelebi (İznik)	13.16 p-t	Saçaklı Otur	10.88 o-r
Eşek Zeytini (Ö)	10.35 q-t	Kiraz	13.11 q-t	Kilis Yağlık	10.85 o-r
Çekişte	10.21 q-t	Domat	13.08 q-t	Kiraz	10.74 p-r
Kilis Yağlık	10.14 r-t	Çekişte	13.04 q-t	Uslu	10.60 qr
Kiraz	9.83 st	Uslu	12.60 r-t	Trabzon Yağlık	10.34 rs
Mavi	9.78 st	Kilis Yağlık	12.57 st	Girit Zeytini	9.85 st
Sinop No 5	9.68 t	Girit Zeytini	12.06 t	Edincik	9.43 t

* Means grouped by Student's t test ($p<0.05$)

Table 4. Daily average temperatures (°C) 30 days before the frost tests and periodic average LT_{50} (-°C) values of all cultivars

Days before frost tests	Daily average temperatures (°C)					
	1st. year			2nd. year		
	Nov. 2014	Jan. 2015	Mar. 2015	Nov. 2015	Jan. 2016	Mar. 20
30	20.20	5.20	5.30	16.80	7.90	15.60
29	16.10	10.60	5.30	17.30	6.90	16.90
28	15.80	12.10	5.50	17.10	3.50	18.80
27	14.00	8.30	4.80	19.10	3.10	15.70
26	12.60	4.40	-0.30	20.80	6.70	12.70
25	11.90	5.40	-0.50	19.60	-0.10	15.10
24	12.60	6.60	3.70	18.30	-1.60	16.30
23	12.60	1.80	5.80	15.50	-3.30	11.40
22	12.20	3.10	5.00	14.00	2.50	9.30
21	11.70	5.50	11.10	13.60	8.60	7.10
20	10.80	10.30	11.20	14.20	13.70	10.10
19	10.50	9.50	9.50	14.10	16.50	11.70
18	8.60	4.30	10.80	13.00	14.60	12.50
17	8.60	13.20	8.70	12.70	7.50	11.20
16	10.60	4.90	9.80	13.90	3.60	14.20
15	12.10	1.10	9.80	14.10	5.90	11.00
14	14.00	2.80	9.80	12.80	13.20	13.40
13	12.80	3.30	10.10	12.50	15.70	18.80
12	13.20	0.80	9.90	11.70	15.10	16.40
11	14.20	1.00	7.10	10.00	9.10	12.90
10	13.60	3.90	13.60	9.80	5.30	12.20
9	13.40	1.20	15.10	12.60	13.30	10.40
8	12.30	-0.30	9.70	12.30	11.70	9.70
7	10.40	-3.80	8.90	11.10	2.70	10.80
6	14.90	-4.00	9.00	11.10	0.00	11.40
5	14.40	-1.70	10.80	12.10	-1.50	11.60
4	15.60	2.30	6.80	12.20	2.80	12.20
3	9.70	9.90	8.50	12.50	3.00	11.80
2	8.30	4.90	9.10	12.60	1.20	11.10
1	8.00	5.10	9.00	11.80	-0.20	13.30
Periodic Aver. Temp. (°C)	12.52	4.39	8.10	13.97	6.25	12.85
Aver. LT_{50} (-°C)	12.32	18.27	16.20	11.72	15.19	12.17

3.2. Grouping the olive cultivars based on cold-hardiness

Statistical grouping of olive cultivars based on their frost tolerance was conducted considering the interactions between the periods and LT_{50} values and mean LT_{50} values by logistic regression analysis are presented in the Figure 1 ($p<0.01$). The cultivars were separated into 3 categories including high, moderate, and low tolerance following frequency distribution through these data. The Figure shows that 8 cultivars had high frost tolerance, 22 cultivars had moderate frost tolerance, and 10 cultivars had low frost tolerance.

The cultivars with high frost tolerance were 'Butko', 'Memeli', 'Otur', 'Gemlik', 'Yün Çelebi', 'Satı', 'Sinop no 5', and 'Kara Yaprak', respectively. The cultivars with moderate frost tolerance were determined as follows; 'Sarı Ulak', 'Sinop no 1', 'Marantelli', 'Görvele', 'Ayvalık', 'Samsun Tuzlamalık', 'Çakır', 'Erkence', 'Saurani', 'Kan Çelebi', 'Eşek Zeytini (Tekirdağ)', 'Çilli', 'İzmir Sofralık', 'Samsun Yağlık', 'Domat', 'Saçaklı Otur', 'Eşek Zeytini (Ödemiş)', 'Sinop No 4', 'Nizip Yağlık', 'Memecik', 'Tekirdağ Çizmelik', and 'Patos'. The cultivars with low frost tolerance were 'Edincik', 'Sinop no 6', 'Çekişte', 'Mavi', 'Kiraz', 'Kilis Yağlık', 'Çelebi (İznik)', 'Trabzon Yağlık', 'Uslu', and 'Girit Zeytini'.

It was seen as the result of freezing tests applied in six different periods that the olive cultivar with the highest frost tolerance was 'Butko' (mean LT_{50}, -16.94°C) originated from Artvin province. The cultivars 'Otur' (mean LT_{50}, -16.25°C) and 'Satı' (mean LT_{50}, -15.60°C), which were grouped with highest frost tolerance, were also originated from Artvin province. It was observed that the cultivars adapted to this region where winters are more severe compared to other regions with olive cultivation were good choices for the circumstances of the Blacksea Region.

The cultivar ‘Gemlik’ which is one of the most important olive cultivars in Turkey was ranked as the fourth (mean LT_{50}, -15.71°C) in terms of frost tolerance. The cultivar which was originated from Gemlik district of Bursa province has been known to be well adapted to the regions with highly cold winter. The olive cultivar Gemlik is also cultivated with different names (as ‘Erdek Yağlık’, ‘Tekirdağ Siyah Salamuralık’, ‘Sinop No 2’, ‘Samsun Ufak Tuzlamalık’) in Marmara and Black Sea regions. This indicates that the cultivar Gemlik can be a beneficial option for these regions or similar oneswith severely cold winter.

The cultivars ‘Ayvalık’ (mean LT_{50}, -14.93°C), ‘Domat’ (mean LT_{50}, -14.18°C) and ‘Memecik’ (mean LT_{50}, -13.79°C) covering an significant portion of olive tree stock in Turkey were involved in the group with moderate tolerance. The cultivar ‘Ayvalık’ distributed mostly in Northern Aegean Region was ranked as the 13rd among all the cultivars, the cultivar ‘Domat’ as the 23rd, and the cultivar ‘Memecik’ which is adapted to climatic conditions of Southern Aegean as the 28th in terms of frost tolerance. The cultivar ‘Uslu’ which is one of the black table olive cultivars, was ranked as the 39th and showed a poor performance in terms of frost tolerance.

Frost tolerances of the cultivars examined in the project displayed significant changes depending on the sampling periods. It is known that frost tolerance is influenced by genetic predisposition of the plant and environmental factors, therefore it generally shows a time-dependent variance (Beck et al., 2004). Numerous researchers have suggested that cold acclimation increases frost tolerance of plants (Levitt, 1980; Palliotti and Bongi 1996; Travert et al., 1997; Thomashow, 1999; Mancuso, 2000; Kosova et al., 2007; Cansev et al., 2009). In line with the previous studies cold acclimated period increased the frost tolerance of the cultivars in current study. Indeed frost tolerance of each cultivar exhibited periodical differences. Additionally, the cultivars ‘Butko’, ‘Memeli’ and ‘Otur’ stood out as the cultivars generally with high tolerance considering all the periods. Frost tolerance of the cultivars ‘Girit Zeytini’, ‘Uslu’, ‘Trabzon Yağlık’ and ‘Çelebi (İznik)’, on the other hand, was found to be mostly low according to tests performed within 6 different periods. While frost tolerance of all these cultivars was low in the tests conducted in November, their frost tolerances were observed to increase significantly in January. ‘Gemlik’ was one of the most remarkable cultivars regarding the subject due to the fact that it had a LT50 value of -11.74 °C in the period of November during the 1st year of study but it had an almost doubled LT50 value (-22.42°C) in January.

Previously some data were examined regarding the frost tolerance of the cultivars ‘Uslu’, ‘Domat’, ‘Edincik’, ‘Samanlı’ and ‘Gemlik’ which were the cultivars examined in this project (Sütçü et al., 1994; Cansev et al., 2009). Sütçü et al. (1994) who started the first trials on this subject in Turkey reported that there was no significant differences between the cultivars they examined however, the olive cultivar ‘Domat’ was more tolerant to cold, the cultivars ‘Gemlik’ and ‘Edincik’ were moderately tolerant, and the cultivar ‘Uslu’ was susceptible. Another study (Cansev et al., 2009) also found that the cultivar ‘Domat’ was tolerant and the cultivars ‘Gemlik’ and ‘Uslu’ were moderately tolerant. Results of the present study are relatively accordance with the results from other studies. This was considered to associated with the materials, methods, and environmental differences among studies. In the study by Sütçü et al. (1994), 1-year-old offshoots with foliation were planted in fogging greenhouse for vitality test after their exposure to frost stress and their cold tolerance was determined by the rate of surviving leaves after 4 weeks. Even though they concluded that the cultivar ‘Domat’ which was moderately tolerant in the present study, was tolerant, there was no statistical differences between the cultivars and authers attributed this to the effects of environmental factors. Results of the study by Cansev et al. (2009) were based on freezing tests performed in 2 different periods as cold-acclimated and non-acclimated (January and July, respectively). In the present study freezing tests were carried out during 3 different periods starting from winter cold for two years. Thus, a greater number of tests were applied and further data were obtained from considered periods when winter cold starts, which are critical for olive plant.

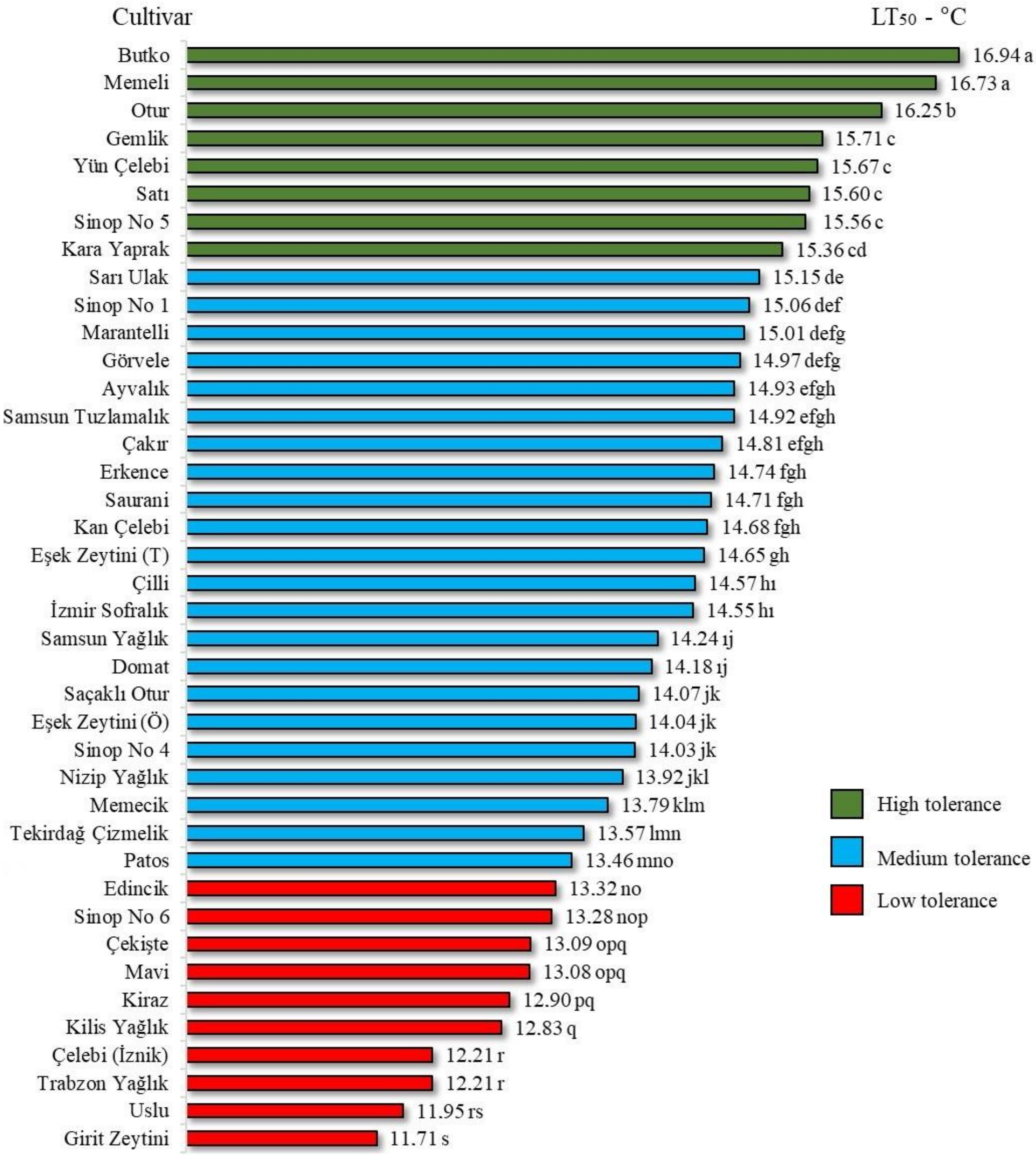

Figure 1. Grouping olive cultivars in terms of the frost tolerance

4. Conclusions

According to the results, cold-hardiness of olive cultivars significantly changed in terms of both genetic and climatic factors. The cultivars were clustered in three groups with high , medium and low frost tolerance. The data revealed that the cultivars 'Butko', 'Memeli', 'Otur', 'Gemlik', 'Yün Çelebi', 'Satı', 'Sinop No 5', and 'Kara Yaprak' had high frost tolerance. The cultivars 'Sarı Ulak', 'Sinop No 1', 'Marantelli', 'Görvele', 'Ayvalık', 'Samsun Tuzlamalık', 'Çakır', 'Erkence', 'Saurani', 'Kan Çelebi', 'Eşek Zeytini (Tekirdağ)', 'Çilli', 'İzmir Sofralık', 'Samsun Yağlık', 'Domat', 'Saçaklı Otur', 'Eşek Zeytini (Ödemiş)', 'Sinop No 4', 'Nizip Yağlık', 'Memecik', 'Tekirdağ Çizmelik' and 'Patos' had moderate frost tolerance. The cultivars 'Edincik', 'Sinop No 6', 'Çekişte', 'Mavi', 'Kiraz', 'Kilis Yağlık', 'Çelebi (İznik)', 'Trabzon Yağlık', 'Uslu' and 'Girit Zeytini' were fall into the group with low frost tolerance.

This study revealed that frost tolerance of olive cultivars displayed both genetic and seasonal variations. It was found that cold-acclimation was considerably significant for olive, exposure to low temperatures for a period of time substantially elevated frost tolerance. But this situation was not stable and could rapidly become reversed when temperature reached to the point at which adapdation did not ensure. In addition, as stated by many researchers, electrolyte leakage method has been found to be a fast and efficient screening method to determine frost tolerance of cultivars. To the best of our knowledge this is the first detailed study screening the local olive genetic resourses for frost tolerance and clustring the cultivars according to their cold-hardiness.

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