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Ancient Grape *Vitis vinifera* L. cv 'Ekşi Kara' in Anatolia

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ABSRACT

Many grape breeding programs have been conducted in certain institutions as cross breeding between superior genotypes or clonal selection of local accessions of *Vitis vinifera* L. varieties in Turkey. 'Ekşi Kara' is an ancient grapevine cultivar intensively grown in Konya due to its well-adaptation to ecology. Thus, it has been promising with its unique characteristics peculiar to similar ecologies. This variety is robust and very fruitful in comparison with many other *V. vinifera* L. varieties, and it has been commonly consumed as table grapes, seeded appetizer when dried naturally or molasses 'pekmez'. Consumption of its binned or fresh leaves is also a traditional and global marketing way. The sex of the flowers is functionally female, and need a pollinator, for a satisfactory berry set. 'Gök Üzüm', another ancient and autochthonous grape variety is suggested as a prima pollinizer. The clonal selection studies have been continuing since 2010 in commercial vineyards around its geographic origin. The ampelographic description of 'Ekşi Kara' was performed according to OIV (International Bureau of Grapes and Wine) descriptors using a total of 144 criteria, among which 63 were the basic and 81 were complementary identifier. The basic data of the cultivar was collected for clonal selection. There were no distinctive ampelographic differences between vary named grapevines in growing location.

1. Introduction

Grapevine has been cultured worldwide for a very long time and its long history of domestication has led to the diffusion of many biotypes and cultivars. This has resulted in a great genetic variability in germplasm (Vignani et al., 2002). Anatolia is the centre of origin and genetic diversity of *Vitis vinifera* L. and it has many different grape cultivars, many of which possess desirable characteristics. Anatolian peninsula has been the cradle of cultivated grapevine (*Vitis vinifera* L.) which has presented itself with numerous cultivars (Gokbayrak and Soylemezoglu, 2010). Grapevine cultivation in Konya province come from ancient times. The Hittite rock relief dates 8th century BC located in the Ereğli town of Konya, and grape clusters in King Warpalawas' hand to the God Tarhunzas indicate that he brings about fertility (Bier, 1976).

Nowadays Turkey has about 6.5% of the world area of vineyards, and meet 5.6% of grape production with about 2000 grapevine genetic stocks (Kara, 2014). In grapes, ampelographic methods are important in grapevine taxonomy (Galet, 1988), as well as in varietal identification. Local and indigenous varieties are threatened with extinction and difficult to find, since only a few individuals remain (Zaki et al., 1996).

Konya has 9906 ha area of vineyards, and 61535 tons grape production, usages 51% seeded raisin, 48% seeded table grapes, 1.5% wine, and 0.5% seedless raisin. Vineyard culture comes from ancient times and yield is up to 918 kg da-1. The main income in some villages of Hadim, Bozkır and Güneysınır towns is solely from vineyards as table grape, raisin, local products, and canned grape leaves for local and national market. Although many new cultivars have been introduced into this location in the last decades, almost no one has survived (Kara et al., 2016).

'Ekşi Kara' is one of the most important grapevine (*Vitis vinifera* L.) cultivars grown on the Taurus Mountain in Middle Anatolia. It is registered in the Turkish Register of Grape Varieties and represented in the cultivation areas by some biotypes, each of which need cross pollination (Kara, 2015).

The word "ampelography" by its derivation from ampelos-vine-and graphe-writing-means the description of vines (Bioletti, 1938). The ampelographic study of Turkish autochthonous varieties has a long history. A number of scientific investigations have been done (Oraman, 1937; İştar, 1959; Kara, Z., 1990; Sabir et al.,

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2009; Ates et al., 2011; Atak et al., 2014; Kara et al., 2016). In the last decades, grapevine phenotyping researches have been carried out by the application of various approaches (Marinon et al., 2009; Garcia-Muñoz et al., 2011; Bodor et al., 2013; Herzog et al., 2014; Susaj et al., 2014; Mdinaradze et al., 2015). However, characterization and identification methods are still evolving.

The idea was to virtually assemble all accessions maintained in the worldwide existing collections to face genetic erosion. The establishment of the Vitis International Variety Catalogue (VIVC) have been constructing since 1984. The activities aim to equip the prime names of VIVC with reliable genetic proles combined with the validation of their identity by ampelography (Maul and Töpfer, 2015).

Clonal selection, as a tool for grapevine improvement, has been increasingly used since the late 1950s. A collection of vines propagated from the same mother vine constitute a clone. Vines are propagated vegetatively; cultivars are not genetically homogeneous. Clonal selection takes advantage of the genetic variability within cultivars and their health status. Clonal selection is required to increase yield and quality of commercially important grape cultivars. The clonal selection process consists of examining clones of a cultivar in the field, studying their agronomic and oenological performances, health status and varietal identity (Walter, 1998). Clonal selection studies started as preliminary surveys of farmers' vineyards. For cultivars with greatest genetic diversity, clonal selection is a major issue in the production of quality wines (Keller, 2010; Van Leeuwen et al., 2013). Clonal selection in Turkey was started in the 1980s and still continue with different cultivars (Kader et al., 2005; Çelik et al., 2010; Martín et al., 2011).

The main aim of this study was the characterization of grape cultivar 'Ekşi Kara', from prehistoric times to the current grown in the *ex-situ* commercial vineyards at Hadim district of Konya province which is used for table, raisin, and local sweet products, and grape leaves (Fig. 1), and it is a leading cultivar in Central Anatolia, basically around the Konya Province (Kara, 2015) for the first step of clonal selection.

2. Material and Method

2.1. Plant Material and Edaphoclimatic Conditions

'Ekşi Kara' is an old Turkish autochthonous grapevine cultivar (*Vitis vinifera* L.), until now not ampelographically characterized in detail. The cultivar used to table grape (locally), natural dried, concentrated, and make red wine, and was native, ancient cultivar of the middle Anatolia regions of high level of Taurus Mountain in Turkey. Its long cultivation contributed to the creation of many synonyms (such as Erkek üzüm, Karaoğlan, Keçimen, İri Kara etc.) types, and variants which were characterized as mentioned by seeded and seedless bunches, and different berry long/width ratios. The related cultivars 'Kuş üzümü', 'Burdur Dimriti', and 'Kalecik Karası' (which was characterized by hens and chickens berry developments) and need to determine their phenotypic and genetic similarities using the ampelographic description and the molecular methods. Today, in Turkey, it was estimated that 'Ekşi Kara' was cultivated in approximately 15000 ha while its production exceeds 20000 tons of raisins.

At the beginning of the study, fifty vines were selected all about twenty years old, at the producer vineyard in Yağcılar village of Hadim town of Konya, is located on 1060 m a.s.l. of altitude and geographic location of 37°2'2515" N; 32°34'533" E. All were cultivated in the same way (trained as double cordon and spur pruned), and all received the same crop protection treatments. The soil in which they were grown has a sandy loam texture and an organic matter content of 4%. The phosphorus, calcium, organic ingredient, sand, clay, silt contents were 368.4 kg ha^{-1}, and 978 kg ha^{-1}, 12.9 kg ha^{-1}, 53.96%, 31.50%, 14.54% respectively, and pH was 7.65. The mean annual temperature of the area was 13.2 °C; effective heat summation is 1462 degree days (base temperature ≥10°C), mean annual rainfall was 331.19 mm, mean annual relative humidity was 54.5% (Meteoroloji, 2016).

2.2. Ampelographic Study

The study was carried out during three consecutive years (2013-2015) to reach accurate conclusions about the ampelographic characters of the 'Ekşi Kara' grapevine cultivar under climatic conditions of Konya on a representative sample, chosen randomly, constituted by 50 vines, 20 years old, planted in distances of 2 m x 3 m or 1666 vines ha^{-1}, and synonyms were evaluated in whole locations. Ampelographic characterization was carried out with 144 OIV descriptors (63 main, and 81 complementary) at different stages of the growth cycle, following a list of descriptors developed by the Organisation Internationale de la Vigne et du Vin (OIV, 2012). After the visual evaluation, measurements, and analysing all the qualitative and quantitative traits have been turned in scores as suggested by the OIV protocol (OIV, 2012).

Characterization of the young shoot, young leaf (Fig. 1) and flower characters was performed in the period May 15-25, each year. Young shoot and young leaf were evaluated for the form of tip, anthocyanin coloration of tip, density of prostrate hairs on tip and shape; young leaf upper surface color, etc., while the flower (Fig. 3) was evaluated for the flower type, node was inserts the first inflorescence and the number of inflorescences for shoot.

Characterization of the mature leaf features (Fig. 1), such as mature leaf shape, number of lobes, length of petiole, main veins lengths (N1, N2, N3, N4), length and width of tooth N2, length of upper and lower late-

ral sinuses, shape of lateral teething, etc., were performed in the period July 10-20, each year, in a representative sample of 10 intact mature leaves, taken from the first node over last bunch of shoot for each vine.

Characterization of the bunch characters (Fig. 3, shape, weight, length, width) were performed in the full ripening period (end of August), in a representative sample of kg bunches, at the full grape maturity, 2-3 days prior to harvest (IPGRI, 1997).

Characterization of the berries characters (shape, weight, skin color, number and seeds dimensions, etc.) were performed in a representative sample of 100 berries taken randomly form the middle part of bunches (OIV, 2009).

Characterization of chemical and technological characters of grape were based on data analysis of the must yield (ml 100 g fresh grape^{-1}), and sugar content (%) and total acidity content (g L^{-1}) in must, and was performed on a sample of 5 kg fully-ripen grape without pedicels, crushed and centrifuged at 3000 rpm, and was carried out at the Horticulture Lab of Agriculture Faculty of Selçuk University in Konya.

3. Results and Discussion

3.1. Ampelographic descriptions

Ampelographic descriptions were made OIV protocol (OIV, 2012) that 63 were the basic and 81 are complementary identifier used.

Young shoot and the shoot tips were investigated when they were approximately 10–30 cm in height, and the first-four distal leaves of young leaves were evaluated.

OIV 001 Opening of the shoot tip 5=fully open.

OIV 002 Distribution of anthocyanin coloration on prostrate hairs of the shoot tip 3=overall.

OIV 003 Intensity of anthocyanin coloration on prostrate hairs of the shoot tip 9=very high.

OIV 004 Density of prostrate hairs on the shoot tip 9=very high.

OIV 005 Density of erect hairs on the shoot tip 1=none or very low.

OIV 006 Attitude (before tying) 3=semi-erect.

OIV 007 Color of the dorsal side of internodes 2=green and red.

OIV 008 Color of the ventral side of internodes 1=green. OIV 009 Color of the dorsal side of nodes 3=red.

OIV 010 Color of the ventral side of nodes 2=green and red.

OIV 011 Density of erect hairs on nodes 1=none or very low.

OIV 012 Density of erect hairs on internodes 1=none or very low.

OIV 013 Density of prostrate hairs on nodes 1=none or very low.

OIV 014 Density of prostrate hairs on internodes 1=none or very low.

OIV 015-1 Distribution of the anthocyanin coloration on the bud scales 9=on the whole bud scale.

OIV 015-2 Intensity of the anthocyanin coloration on the bud scales 9=very strong.

OIV 016 Number of consecutive tendrils 1=2 or less.

OIV 0017 Length of tendrils 7=long (about 25 cm) 24.36±2.81 cm.

All characters of young shoots and young leaves (Fig. 1) come from *Vitis vinifera* L., which was also close to

'Kuş Üzümü' which was used for current (described by Kara, 1990).

3.2. Young leaf

OIV 051 Color of upper side of blade (4th leaf) 4=copper – reddish.

OIV 053 Density of prostrate hairs between main veins on lower side of blade (4th leaf) 9=very high.

OIV 054 Density of erect hairs between main veins on lower side of blade (4th leaf) 3=low.

OIV 056 Density of erect hairs on main veins on lower side of blade (4th leaf) 3=low.

OIV 055 Density of prostrate hairs on main veins on lower side of blade (4th leaf) 7=high.

3.3. Mature leaf

Descriptions were obtained between berry set and beginning of berry maturity and were conducted on leaves above the cluster within the middle of the shoot.

OIV 065 Size of blade 7=large 234.92±25.26 cm2.

OIV 067 Shape of blade 3=pentagonal.

OIV 068 Number of lobes 3=five (Fig. 1).

OIV 069 Color of the upper side of blade 7=dark green.

OIV 070 Area of anthocyanin coloration of main veins on upper side of blade 3=up to the 1st bifurcation.

OIV 071 Area of anthocyanin coloration of main veins on lower side of blade 2=only at the petiolar point.

OIV 072 Goffering of blade 3=weak (Fig. 1).

OIV 073 Undulation of blade between main or lateral veins 9=present (Fig. 1).

OIV 074 Profile of blade in cross section 2=V-shaped.

OIV 075 Blistering of upper side of blade 5=medium.

OIV 076 Shape of teeth 3=both sides convex (Fig. 1).

OIV 077 Size of teeth in relation to blade size 3=small.

OIV 078 Length of teeth compared with their width 7=long 537.22 cm.

Fig. 1
Young shoot and young leaves, and mature leaf

OIV 079 Degree of opening/overlapping of petiole sinus 7=overlapped; 0.75±0.07.
OIV 080 Shape of base of petiole sinus 3=V-shaped (Fig. 1).
OIV 081-1 Teeth in the petiole sinus 1=none (Fig. 1).
OIV 081-2 Petiole sinus base limited by vein 1=not limited (Fig. 1).
OIV 082 Degree of opening/overlapping of upper lateral sinuses 1=open (Fig. 1).
OIV 083-1 Shape of base of upper lateral sinuses 1=U-shaped (Fig. 1).
OIV 083-2 Teeth in the upper lateral sinuses 1=none (Fig. 1).
OIV 084 Density of prostrate hairs between main veins on lower side of blade 1=none or very low.
OIV 085 Density of erect hairs between main veins on lower side of blade 5=medium.
OIV 086 Density of prostrate hairs on main veins on lower side of blade 1=none or very low.
OIV 087 Density of erect hairs on main veins on lower side of blade 5=medium.
OIV 088 Prostrate hairs on main veins on upper side of blade 3=low.
OIV 089 Erect hairs on main veins on upper side of blade 1=none or very low.
OIV 090 Density of prostrate hairs on petiole 1=none or very low.
OIV 091 Density of erect hairs on petiole 1=none or very low.
OIV 093 Length of petiole compared to length of middle vein 1=much shorter (Fig. 1).
OIV 094 Depth of upper lateral sinuses 7=deep; 0.91±0.07 (Fig. 1).
OIV 601 Length of vein N1 5=medium (about 135 mm); 134.2±0.09 mm.
OIV 602 Length of vein N2 5=medium (about 105 mm); 111.3±0.04 mm.
OIV 603 Length of vein N3 5=medium (about 75 mm); 82.3±0.05 mm.
OIV 604 Length of vein N4 9=very long (about 55 mm and more); 51.9±0.3 mm.
OIV 605 Length petiole sinus to upper lateral leaf sinus 3=short (about 50 mm); 5.84±0.88 cm.
OIV 606 Length petiole sinus to lower lateral leaf sinus 3=short (about 45 mm); 49.8±0.07 mm.
OIV 607 Angle between N1 and N2, measured at the first ramification 7=large (about 56°-70°) 63.8±4.93°.
OIV 608 Angle between N2 and N3, measured at the first ramification 7=large (about 56°-70°) 58.3±5.14°.
OIV 609 Angle between N3 and N4, measured at the first ramification 5=medium (about 46°-55°) 51.7±5.81°.
OIV 610 Angle between N3 and the tangent between petiole point and the tooth tip of N5 7=large (about 56°-70°) 63.1±5.64°.
OIV 611 Length of vein N5 9=very long (about 55 mm and more).
OIV 612 Length of tooth of N2 3=short (about 10 mm); 10.1±0.01 mm.
OIV 613 Width of tooth of N2 5=medium (about 14 mm); 14.8±0.03.
OIV 614 Length of tooth of N4 3=short (about 10 mm); 10.4±0.01 mm.
OIV 615 Width of tooth of N4 5=medium (about 14 mm); 14.6±0.01 mm.
OIV 616 Number of teeth between the tooth tip of N2 and the tooth tip of the first secondary vein of N2 including the limits 5=medium (about 5-6) 6±1.
OIV 617 Length between the tooth tip of N2 and the tooth tip of the first secondary vein of N2 7=long (about 56-70 mm) 62.4±0.06 mm.
OIV 618 Opening/overlapping of petiole sinus 7=overlapping (about 25 mm) (Fig. 1).

3.4. Woody shoot

Ten canes were analysed after leaf fall.
OIV 101 Cross section 1=circular.
OIV 102 Structure of surface 2=ribbed. OIV 103 Main color 2=brownish.
OIV 104 Lenticels 1=absent.
OIV 105 Erect hairs on nodes 1=absent.
OIV 106 Erect hairs on internodes 1=absent.

3.5. Inflorescence

OIV 152 Insertion of 1st inflorescence 2=3rd and 4th node.
OIV 153 Number of inflorescences per shoot 2=1.1 to 2 inflorescences.
OIV 155 Shoot: fertility of basal buds (buds 1 - 3) 5=medium.

Flower type was functional female and need to pollination for fruit set. Open pollination effects fruit set, and berry shape, hens and chicken rates. Honey bee (Fig. 2) and some other bees were visits the flowers during bloom

time. Covered inflorescences were not fruit set, and some seedless berry can develops. Hens and chicken berry development was also belonging to weather (rainfall) conditions during the bloom (Fig. 3). In the area the bunch size, density and length, and all berry characters highly dependent of pollination, which was also differ by yearly weather conditions. The ampelographic descriptions, agronomic parameters and phenology were influenced by whether condition (Garcia-Muñoz et al., 2011).

Fig. 2
Inflorescence, pollination by bees

3.6. Bunch

The clusters were measured at maturity, and berry characteristics were obtained from ripe berries located in the middle of the bunch (Fig. 3).
OIV 202 Length (peduncle excluded) 7=long (about 200 mm); 234.38±3 mm.
OIV 203 Bunch: width 5=medium (about 120 mm) 129.3±22.9 mm.
OIV 204 Density 5=medium.
OIV 206 Length of peduncle of primary bunch 1=very short (up to about 30 mm) 21.45±16.56 mm.
OIV 207 Lignification of peduncle 7=more than the middle.
OIV 208 Shape 2=conical (Fig. 3).
OIV 209 Number of wings of the primary bunch 2=1-2 wings (Fig. 3).

3.7. Berry

OIV 220 Length 5=medium (about 18 mm); 18.74 ±1.18 mm.
OIV 221 Width 5=medium (about 18 mm); 16.17±1.04 mm.
OIV 222 Uniformity of size 1=not uniform (Depends on pollination, Fig. 3).
OIV 223 Shape 4=narrow ellipsoid (Depends on pollination, Fig. 3).
OIV 225 Color of skin 6=blue black (Fig. 3).
OIV 226 Uniformity of skin color 2=uniform (Fig. 3).
OIV 227 Bloom 5=medium.
OIV 228 Thickness of skin 5=medium.
OIV 229 Hilum 1=little visible.

Fig. 3
Fruit set differences, hen and chickens berry development

OIV 231 Intensity of flesh anthocyanin coloration 1=none or very weak.
OIV 232 Juiciness of flesh 2=medium juicy.
OIV 233 Must yield 5=medium (about 65-75%) 73.3%±5.77.
OIV 235 Firmness of flesh 2=slightly firm 0.24±0.04 kg. OIV 236 Particular flavor 1=none.
OIV 238 Length of pedicel 3=short (about 7 mm) 7.84±0.54 mm.
OIV 240 Ease of detachment from pedicel 2=easy (0.150-0.249) 0.15±0.06 kg.
OIV 241 Formation of seeds 3=complete (Fig. 4). OIV 242 Length of seeds 3=short 7.07±0.06 (Fig. 4).
OIV 243 Weight of seeds 5=medium (about 40 mg) 35.30±1.79 mg.
OIV 244 Transversal ridges on dorsal side of seeds 1=absent (Fig. 4).

3.8. Phenology

OIV 301 Time of bud burst 3=early, end of March.
OIV 302 Time of full bloom 5=medium, first week of June.
OIV 303 Time of beginning of berry ripening (veraison) 5=medium, first week of July.
OIV 304 Time of full physiological maturity of the berry 5=medium, end of August.
OIV 305 Time of beginning of wood maturity 3=early first week of June.
OIV 306 Autumn coloration of leaves 2=reddish.
OIV 351 Vigor of shoot growth 5=medium.
OIV 352 Growth of lateral shoots 5=medium.
OIV 353 Length of internodes 3=short (about 9 cm) 9.05±1.78 cm.
OIV 354 Diameter of internodes 3=small (about 8 mm) 7.13±0.59 mm.
OIV 401 Abiotic resistance; Resistance to iron chlorosis 9=very high, dark green leaves.
OIV 402 Abiotic resistance: Resistance to chlorides (salt) 9=very high, completely green leaves.
OIV 403 Abiotic resistance: Resistance to drought 7= high.
OIV 452 Leaf: degree of resistance to Plasmopara 7=high (low, relative humidity in vegetation period).
OIV 452-1 Leaf: degree of resistance to Plasmopara (leaf disc test) NA.
OIV 453 Degree of resistance to Plasmopara (leaf and cluster) 7=high - 9=very high.
OIV 455 Leaf: degree of resistance to Oidium 7=high.
OIV 455-1 Leaf: degree of resistance to Oidium (leaf disc test) NA.
OIV 456 Degree of resistance to Oidium (leaf and cluster) 7=high.
OIV 458 Leaf: degree of resistance to Botrytis 7-9=height or very high.
OIV 458-1 Leaf: degree of resistance to Botrytis (laboratory analysis) NA.

Fig. 4.
Berry and seeds characteristics

OIV 459 Degree of resistance to Botrytis (leaf and cluster) 7=high - 9=very high.
OIV 460 Degree of resistance to Eutypa dieback (laboratory analysis) 1=very little.
OIV 461 Leaf: degree of tolerance to Phylloxera (leaf gall) 7=high.
OIV 462 Root: degree of tolerance to Phylloxera (root gall) 1=very low.

3.9. Production

OIV 501 Percentage of berry set 1=very low (up to about 10%) - 7=high (about 60%), depends on fertilisation (Fig. 3).
OIV 502 Single bunch weight 5=medium (about 500 g); 407.84±72.12 g.
OIV 503 Single berry weight 3=low (about 3 g); 3.39±0.53 kg.
OIV 504 Yield per m2 5=medium-7=high, depends on fertilisation (Fig. 3).

3.10. Character of grape must

OIV 505 Character of grape must 7=high (about 21%), 19.86%±0.23.
OIV 506 Total acidity of must 5=medium. OIV 508 Must specific pH 7=high; 3.68±0.02.

4. Discussion

Grapevine cultivars are often spread via vegetative propagation and this leads to the diffusion of numerous genetically identical copies of a specific plant. During this process, somatic mutation could occur and this results in a plant characterised by unique genomic traits that could lead to a unique phenotype (Myles et al., 2011).

The occurrence of morphological differences, which was indeed frequent among generative characters, was not rejected at the vegetative characters because of environmental influences on the expression of several fruit traits. It has to be considered, however, that morphological parameters (especially those that refer to leaves) were influenced by environmental conditions and the age of the tissues, and this could determine phenotypic variation, also without genetic diversity (Barth et al., 2009). Ampelographic descriptors of fruit set, clusters, and berries were depend on the clusters that come from which bud (primary, secondary and/or tertiary), pollination and the weather conditions during the blooming and also fertilization of vinestocks. In order to restrict this aspect, the ampelographic data have been collected by the same operators and on the same plants under similar cultural condition. However, certain environmental pressures on the different accessions cannot be excluded. It was clear, however, that morphological or ampelographic data, although less subjective than in the past, may have an important role to play if supported by molecular analysis (Regner et al., 2000). Furthermore, ampelographic characters might usually be insufficient in the differentiation of closely related genotypes due to ecological factors and vine growth stages. Nevertheless, ampelographic characters are needed when describing the accessions in a gene bank to detect close agronomic mutations (Ortiz et al. 2004). Based on the outcome of clonal study have been working since 2013 to achieve more objectivity and limited sensitivity to environmental factors.

5. Conclusion

This research produced an available ampelographic characterization of the Turkish grape variety grown in the Konya germplasm repository. The accession distinctive features were determined. In this way, the scientific base for the development of an identification software has been prepared. ‘Ekşi Kara’ and its synonyms were investigated in upper Göksu Valley (major, minor and neglected cultivars), the only ‘Ekşi Kara’ proved to be unique genotypes, revealing the occurrence of synonyms with cultivars from the same region as well as from neighbouring areas or from the Middle Taurus Mountain. Further development of ampelographic and genetic databases will greatly contribute to the ancient cultivars and accessions nationwide, under cultivation or in collections, thus increasing overall the accurate identification of varieties.

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