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Clonal Preselection in Grape (*Vitis vinifera* L.) Varieties of Ekşi Kara and Gök Üzüm

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HIGHLIGHTS

- Pre-selection of local grape varieties, Ekşi Kara and Gök Üzüm varieties, was carried out.
- 17 Ekşi Kara and 2 Gök Üzüm clones were selected, which were confirmed to be clean by repeated health selection and tests.
- Clone comparison vineyard was established with selected clones grafted onto 110R grapevine rootstock.
- In the vineyard facility, pollinator Gök Üzüm were planted next to the clone of the functional female Ekşi Kara variety.

Abstract

Ekşi Kara (functional female flowers) and Gök Üzüm (hermaphroditic flowers) are the two most important autochthonous varieties of middle Anatolia. This clone selection study started with mass-selection in producer vineyards consisting of approximately 5000 vines by The International Organization of Vine and Wine (OIV) clonal selection procedure. Two-years genetic and sanitation were examined visually in population and 220 clone candidates were ampelography and fertilization biology and bud fertility determined for Ekşi Kara variety. The clone candidates were ranked at the level of sums, with weighted grading of three-year yield, growth, and quality records. Sanitation analyses of the superior clones were made. 17 clones in the Ekşi Kara grape variety were selected according to their superior scores in genetic selection and sanitation analyses. Eleven clones were selected by mass selection from Gök Üzüm carried out in a single location, and 2 clones were selected with genetic selection scores and health tests. *Grapevine fleck virus* (GfKV) was the most common

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(≅ 29%) in the samples tested, *Grapevine leafroll associated virus* 1+3 (GLRaV-1 + 3, ≅ 26%), *Grapevine virus A* (GVA, 12%), *Grapevine leafroll associated virus-2* (GLRaV-2, ≅ 3%), *Arabis mosaic virus* (ArMV) / *Grapevine fanleaf virus* (GFLV) (≅ 1%) are fallowed with indicated percentage. Although virus and bacterial infections are common in the vineyards, enough healthy clones were selected. 17 Ekşi Kara and 2 Gök Üzüm clones selected as pollinators were grafted onto the 110R rootstock for clone comparison in homogeneous conditions, and a "Clone Comparison Vineyard" was established in Selçuk University.

Keywords: Clonal selection, clone comparison, native grape varieties, phytosanitary tests

1. Introduction

Vine (*Vitis vinifera* L.) is widely cultivated all over the world. Today, it is the most important fresh fruit in the world (Stanimirović et al. 2018). The health effects of grape and grape products contribute significantly to their economic value (Jackson 2008).

The clone is the vegetative generation of a grapevine whose identity is precisely determined with its phenotypic characteristics and health qualities and remains stable until a new mutation occurs (Aurand 2017; van Leeuwen et al. 2019). Clone selection in viticulture is one of the first steps in the development of grape varieties and viticulture, which is of great interest in all viticultural countries in terms of productivity, quality, and sustainability (Rühl et al. 2003). According to van Leeuwen et al. (2019) clonal variation is important in terms of grapevine health and reacting to changing environmental conditions. Grapevine breeders want to use plant material as close as possible to the original selected clone to ensure similarity and preserve the flavor characteristics of the grape. Clonal selection is a two-step process, genetic and health selection, that takes genetic diversity into account within the purity of the varieties. This method eliminates the negative effects of mutational changes in the vineyard areas of the future, as well as prevents the repropagation of plants infected with viruses and related diseases (Rühl et al. 2003). Intra-variety genetic diversity can be explained by their polyclonal origin and accumulation of genetic mutation over time (Vondras et al. 2019). The application includes estimation of clones in the field, examination of their agronomic and oenological performance, health tests and clone identification processes. Healthy and more interesting clones are selected to maintain as long a continuity as possible. Clones are compared under homogeneous conditions to determine their quality grape production capacity, to provide certification and distribution to producers (Loureiro et al. 2011).

Clonal selection in viticulture started in the nineteenth century in Germany and continued in other European countries such as France and Italy in the second half of the twentieth century. It started in Spain in the 1970s in La Rioja and Catalonia regions (Ibáñez et al. 2015). Since virus diseases, especially fanleaf, greatly affect the vine performance in the cold vineyard areas of Germany, the clone selection based on visual assessment and performance has done well there. Since viruses are accepted as the main factor in the reduction of vineyard areas, visual evaluation has been done by serological methods such as indexing of candidate clones since the 1970s, and enzyme-linked immunosorbent analysis in the mid-1980s. Since the early 1990s, all German clones have been subjected to virus tests and since 2013, all main blocks are managed according to the European Union legislation (European Union Council 14.02.2002, 23.06.2005 Commission Directive). This combined strategy has proven to be successful, and while many varieties were used in the mid-1950s, today vineyards are established almost entirely from clones (Eibach and Töpfer 2015).

Clonal selection is considered a crucial tool for genetic improvement. Improved overall performance of clones after purification has been confirmed by much evidence. In general, vigor of the plant increases all the time, but all other parameters are modified depending on the viruses. Healthy plants show higher physiological activity than those infected with GLRaV-3 and GLRaV-1 of the same clone. Grape quality was improved without any yield increase when purified from GLRaV-3, while yield and quality parameters increased without adversely affected when cleansed from GLRaV-1 (Mannini 1998).

Initially, the main purpose of clone selection was to obtain healthy plants and increase yield. Today, quality is considered as a goal to reduce yield in some cases (Martínez et al. 2006). The biggest bene-fit of using clones is to select the genotype within a particular variety, best adapted to a particular vine-yard region (soil, climate) and produce a product with a certain quality potential. Also, identical genotypes within a vineyard have the same behavior and growth stages, which facilitates the management and harvesting of the vineyard (Forneck et al. 2009).

The stable and uniform grape production character of modern viticulture requires virus-free planting material that can only be obtained through clonal selection process. Clone selection, which focuses on improving the characteristics of native grape varieties, improving planting material quality and health status, is carried out in three stages. 1) Selection of the first material from old vineyards and virus tests (ELISA); 2) Establishment of trial vineyards in the cultivar production site by vegetative generations of virus-free parent plants, 3) Final evaluation and registration of selected clones (Šikuten et al. 2018).

In this study, the selection of clone candidates made in the producer vineyards of the mass and individual clone selection (OIV procedure, Aurand 2017) studies of Ekşi Kara and Gök Üzüm varieties commonly grown in middle Anatolia Konya and Karaman provinces the genetic and health selection studies of the selected clones until the clone comparison are presented.

Within the scope of this study, "Clonal preselection in grape (*Vitis vinifera* L.) varieties Ekşi Kara" was carried out together with Selçuk University and Republic of Türkiye Ministry of Agriculture institutes with support 3 Selçuk University and 1 TAGEM projects.

2. Materials and Methods

The material of this study was generally identified (OIV 2009) and selected by mass-selection, Ekşi Kara and as pollinators of the variety Gök Üzüm variety populations, and clone candidates selected from these populations according to their phenotypic characteristics and sanitary status in the first phase of the mass-clone selection stage. Since the performance of a clone is determined by health conditions (Aurand 2017), the health status of the parent plants was monitored from the beginning of the vegetation in April and evaluated twice a year in June and September in wholesale selection studies.

At this stage, considering their exposure to abiotic (hail, frost, sunburn, nutritional disorders) or biotic (disease and pests) stress factors, approximately 5000 vines with superior performance from more than 30 years age vineyards were followed since they have a greater probability of carrying mutations.

The selection of the starting material is from 17 vineyards in total from Konya (14 vineyards) and Karaman (3 vineyards) belonging to producers with elevations varying between 800 m and 1500 m, taking into account the effects of environmental characteristics on the Ekşi Kara grape variety, as stated in The Organization Internationale de la Vigne et du Vin (OIV) Standard Protocol (Aurand 2017) done. The distribution of the selected clones according to the districts was Bozkır 2, Hadim 2, Güneysınır 6, Karaman Central district 7 clones. The Ekşi Kara variety requires an absolute pollinator, and this need is met most successfully with the Gök Üzüm variety in the region (Kara et al. 2016; Kara et al. 2017a). Both grape varieties are used for table, snack, dried and grape juice (Kara et al. 2016). Producer vineyards in Hadim district where all three features are used most intensively (Yağcı village, the vineyard area is 1000 m above sea level) was selected as the population for clone selection from Gök Üzüm variety.

The method for this clone selection study is mainly proposed by OIV (Aurand 2017). According-ly, clonal selection is most effective when the initial individuals constituting the starting population are preferably selected from vineyards established without the selected clones. Intra-variety variation in such vineyards is more likely, increasing the likelihood that seemingly superior individuals will be selected for the target traits of the clonal selection program. In addition, they must meet the desired requirements for other important

viticulture properties. In addition, selected individuals should be identified as the true type based on ampelographic and genetic studies. This first choice should be made with ampelographic and phenological considerations. Moreover, care should be taken to eliminate individuals affected by infectious diseases in selected clones. The second step of clone selection is the observation and protection of the vegetative lineage of the selected individuals. Selected clones that successfully completed the phytosanitary inspection may have come from various locations. Trial vineyards should be established for comparison with individually propagated clones, preferably in two area with different pedoclimatic properties. For comparison, this trial plot should contain one or more existing standard clones for reference. The test area should exhibit homogeneous soil and micro-climate conditions. The soil of the test area should not contain *Xiphinema* ssp, which acts as a vector for viral diseases. All clones of the experiment should be grafted onto the same clonal rootstock. The rootstock used for grafting should be suitable for local soil conditions and preferably one of the most frequently used rootstocks in this region. Each clone should have at least three replications and at least 5 vines per iteration. Evaluation should be done over a period of three to five years (Aurand 2017).

In the starting material, phytosanitary selection was visually performed at the stage of mass selection, negative traits were removed, diseased clone candidates were not selected (Loureiro et al. 2011) and a total of 220 clone candidates, apparently less susceptible to disease were selected as clone candidates.

Individual clone selection was carried out in two steps, genetic selection, and phytosanitary selection (Aurand 2017). In the genetic selection stage, in order to evaluate the genetic variations within the variety, clone quality and genetic characteristics, variants were monitored in their own environment in 17 different vineyards, their fruitfulness, yield, development and quality records were kept and their mathematical calculations (Stenkamp et al. 2009) were made according to the weighted grading method. In the weighted rating method Ibáñez et al. (2015), the criteria and relative scores used in the calculation of clone scores were determined based on birth rate (20%, OIV 153), yield (kg m-2, 40%, OIV 504), vegetative growth (g vine pruning weight-1, 10%), cluster weight (g cluster-1, 10%, OIV 502), berry weight (g 100 berry-1, 10%, OIV 503), The maturity index (°Brix, 5%, OIV 505 / total acidity (g L-1, OIV 506) values respectively (OIV, 2012).

Genetic potentials of selected clones were sorted by weighted grading method, and infections free were determined by sanitation tests, and a clone comparison vineyard was established by grafted clones onto the 110 R rootstock (Aurand 2017). In the next stage of the study, whether the genetic variability of the clones are spontaneous natural mutations fixed by vegetative propagation and their kin-ship relations will be examined.

2.1. Viral analyses

Ekşi Kara and Gök Üzüm clone samples were tested serologically with DAS-ELISA method in terms of ArMV / GFLV, GLRaV-1, -2, -3, GLRaV-4 strains -4 -5, -6, -9, -Ob, SLRSV, TBRV, RpRSV-ch, RpRSV-g, GVA and GFkV. DAS-ELISA tests were performed according to the "Double Antibody Sandwich" method (Clark and Adams 1977), that was used in accordance with the recommendations of the antibody and conjugate manufacturer company (Bioreba, Switzerland). The results were deter-mined by measuring the absorbance values of DAS-ELISA plates at 405 nm wavelength using Multiscan GO ELISA Reader (Thermo Scientific, USA). As a result of the measurement, samples reaching 2 times and above the negative control absorbance value were evaluated as positive for the tested virus / vi-ruses (Clark and Adams 1977).

2.2. Bacterial analysis

Dormant shoots of Ekşi Kara and Gök Üzüm clones were analyzed for the presence of *Rhizobium vitis*. Shoot washing method for extraction of bacteria from dormant shoots was made according to Benlioğlu and Özakman (1998). Extracts obtained by shoot washing were planted in R&S (Roy and Sasser 1983). After growth, bacterial colonies were purified into KB (King et al. 1954) broths. DNA extraction was performed from typical colony-growing bacterial isolates in the KB broth (Abolmaaty et al. 2000), then the PGF / PGR primer pair was tested for the presence of *Rhizobium vitis* by PCR method (Szegedi and Bottka 2002).

2.3. Fungal analysis

Fungal disease factors of *Phaeoacremonium* spp., *Phaeomoniella chlamydospora*, *Cylindrocarpon* spp., *Stereum hirsitum*, *Phellinus igniarius*, Eutypa dieback (*Eutypa lata*), dead arm (*Phomopsis viticola*) were analyzed in dormant shoots of Ekşi Kara and Gök Üzüm clones. For this purpose, sections of 5 mm from dormant shoots were planted in a medium containing potato dextrose agar (PDA) after surface sterilization and incubated in 20-25 °C dark environment for 14 days, after which the morphological diagnosis of the growing cultures was made (Poyraz and Onoğur 2013). These factors are wound parasites, they infect the plant by entering from wound sites. They can spread transversely and longitudinally in the plant wood tissue. Since their mycelial development is slow, symptoms in the plant may appear too late. The most suitable growth temperature of the agents is in the range of 20-30 °C. Signs of infection of fungal woody tissue disease agents are the appearance of pallor of green parts, growth retardation and even drying symptoms. These symptoms can be seen on the whole or only part of the vine. The disease has two types of symptoms. In the first, the disease is chronic and mani-fests itself with the symptoms on the leaves. The second has an acute course and the vine dies suddenly.

Small pieces, about 5 mm in size, were removed from the Dormant shoot specimens. These pieces were first kept in 70% ethyl alcohol for 30 seconds, then in 3% calcium hypochlorite for 15 seconds and were taken on sterile blotting papers. After isolation, samples were taken into petri dishes containing potato dextrose agar (PDA) and malt extract agar (MEA) and incubated at 20-25 °C in the dark for 14 days.

At the end of this period, the diagnosis of the isolates, taking into account the colony colors, conidia and conidiophore structures, was made by Halleen et al. (2004) and Alaniz et al. (2007). Selected cultures were transferred to Eppendorf tubes containing 40% glycerol and placed at -20 ° C for long-term storage (Akgül et al. 2014). DNA of fungi was obtained by following the extraction protocol of Cenis (1992) during the molecular identification of these factors. Molecular identification of the iso-lates was carried out by PCR amplifications per-formed specifically to three different protected gene regions of fungi. For this purpose, primer pairs of the ITS (White et al. 1990), β-tubulin (Glass and Donaldson 1995) and translation elongation factor 1-α (EF 1-α) gene regions were used. Sequences of ITS, β-tubulin and EF1-α oligonucleotides and Real-Time PCR cycles at 95 ˚C: 10 min (95 C: 20 sec, 58 ˚C: 20 sec, 72 ˚C: 35 sec) and 35 cycles was carried out. With the melting analysis performed after RT-PCR amplification, non-specific amplifications such as primer dimers were eliminated, and it was deter-mined whether the amplified region was the target region. Sequence data of PCR products obtained from ITS, β-tubulin and EF1-α gene regions were obtained by receiving bidirectional genome sequencing service from a Sanger sequencing laboratory. Chromatogram files of sequence data were analyzed with ChromasPro 1.7.6 chromatogram analysis program. The identification of the fungi was determined by blastn analysis using the Nation-al Center for Biotechnology Information (NCBI) GenBank database of the consensus sequences obtained for each gene region.

3. Results

The results of the research were presented under two subheadings as genetic selection studies and sanitation tests.

3.1. Genetic selection

After 2 years of observation in the population of the mass-selection, 220 healthy clone candidates were selected and their yield, quality and development characteristics were recorded for 3 years. Clone candidates were ranked at the level of vineyards according to the weighted grading scores based on the average values of the records kept for 3 years (Table 1). At the end of 3 years, sanitation tests were performed in duplicate in clone candidates without visible signs of virus, bacteria, or fungal disease infection. As a result of this evaluation, a total of 17 clones from 9 vineyards were selected with their superior scores in weighted grading

and negative sanitation tests. In the weighted rating, scores of clone candidates ranged from 380 to 780 (Table 1). The difference in scores was due to the care and cultural practices applied to the clone candidates.

Initially, 11 clone candidates were selected from the Gök Üzüm variety. Two clones with negative weighted rating and second sanitation tests were selected before the clone comparison stage.

Table 1. Scaled Rating Scores of Ekşi Kara Selected Clones *

Clone No	Place of vineyards	Birth rate	Berry weight	Cluster weight	Yield	Maturity index	Vegetative growth	Total
1	Hamzalar B	180	15	90	360	45	90	780
16	Yağcı H	140	135	70	200	35	30	610
63	Sarıhacı G	100	75	50	200	15	10	450
67	Sarıhacı G	60	105	30	200	15	90	490
72	Sarıhacı G	60	75	70	280	35	30	550
73	Sarıhacı G	140	75	50	200	25	70	560
103	Damlapınar K	180	15	90	280	45	70	680
106	Damlapınar K	100	15	50	200	15	30	410
114	Damlapınar K	100	105	50	120	25	10	410
127	Damlapınar K	100	105	70	280	25	70	650
136	Damlapınar K	60	75	70	280	35	50	570
138	Damlapınar K	100	135	30	200	15	90	570
148	Damlapınar K	180	105	90	360	15	10	760
153	Alanözü G	180	45	10	120	35	30	420
155	Alanözü G	140	15	30	200	45	30	460
182	Hamzalar B	60	75	90	280	45	10	560
197	Kalınağıl H	60	75	90	120	25	10	380

B: Bozkır, H: Hadim, G: Güneysınır, K: Karaman central district, *: The values given in the table are the average score values formed according to the class ranges created for the characteristics examined. The differences between the total scores were quite high as each vineyard was evaluated within itself. The vineyards in which the clone candidates which got a low total weight rating score were not watered. Training and other cultural practices also caused differences in the total score of the clone candidates, as they differed significantly according to vineyards.

The local producers take cuttings from the vineyards that they find better in terms of yield and development characteristics and establish their vineyards by rooting them or grafting them into vine rootstocks. With this method, we can talk about applying a rough positive mass-selection. In Ekşi Kara and Gök Üzüm varieties, the vines that constitute the vineyard population in which the clone selection study was carried out and the clone candidates selected among them do not come from the selected clones as origin. In other words, it is accepted that the Ekşi Kara and Gök Üzüm vineyard populations, which are the basis of clone selection, may be of polyclonal origin.

Since there is a mixture of varieties at different levels in each vineyard, and in the observations made in the near harvest period, it has been evaluated that the differences in the berry shape and parthenocarpic fruit set ratios may be intra-variety variations. Therefore, the ampelographic descriptions of the cultivars (Kara et al. 2016; Kara et al. 2018) and the fertilization biology of the Ekşi Kara variety (Kara et al. 2017a) and bud fertility (Kara et al. 2017b) were examined. In a similar study (Muganu et al., 2019), the morphological characteristics of the Romanesco variety in Italy were characterized in five growth periods. Ampelographic identification was analyzed using 50 OIV morphological descriptors.

It was understood that the flower type of the Ekşi Kara variety was functional female, the pollen vital-ity did not exceed 3% under the producer conditions, and foreign pollination was necessary to set seeded berry. The producers gave importance to weed cleaning to direct the honeybees to the vine during the flowering period, where honeybees were used effectively for pollen transportation.

It was understood that the differences observed in berry shape and size were due to the pollinator variety and therefore due to pollen, and it was not possible to seeded fruit set in all clone candidates when foreign pollination was prevented by closing the inflorescences. It was understood that the size of the cluster, as well as the berry size, and as a result, the yield changed directly depending on the fertilization biology (Kara et al. 2017a).

To determine whether the differences in the birth rate were clonal or not, the bud productivity of the selected clone candidates was examined. At the end of this study, the difference in the birth rate depends on the primary bud damage, in other words, the summer shoots developing on the canes may be from primary, secondary or tertiary growth cones, and their birth rates naturally differ according to the positions of the shoots and the location of the vine-yards, as a result, yield, maturity index and vegetative growth potency values (Kara et al. 2017a).

3.2. Sanitation analyses results

Sanitation analyses were performed in three stages as virus, bacteria, and fungi and two replications. Clone candidates selected in the first stage were tested for the viral diseases listed in Table 2. Health selection was performed by sanitation tests on 94 Ekşi Kara and 11 Gök Üzüm clone candidates, which were superior in weighted rating scores among 220 clones and had no visible signs of virus, bacteria, or fungal disease. All the dormant shoot samples of selected clones were tested for certification based ArMV / GFLV, GLRaV-1 + 3, GLRaV-2, GLRaV-4 strains, SLRSV, TBRV, RpRSC-ch, RpRSC-g, GVA and GfKV. Dormant shoot samples of the same selected clones were tested for the pres-ence of bacterial disease agent *Rhizobium vitis* and fungal disease factors *Phaeoacremonium* spp., *Paeomoniella chlamydospora*, *Cylindrocarpon* spp., *Stereum hirsitum*, *Phellinus igniarius*, *Eutypa lata*, *Phomopsis viticola* and *Rosellinia nealaria*. 51 clones were found healthy because of tests for viral, bacterial, and fungal diseases. Sanitation tests were repeated in 51 Ekşi Kara and 11 Gök Üzüm clone candidates before proceeding to the second stage of the clone selection study. According to highly weighted rating points and second sanitation tests results; 17 Ekşi Kara and 2 Gök Üzüm clones were selected.

Table 2. Virus test results in Ekşi Kara selected clones

Virus	Tested clone candidates	Infected clone candidates
ArMV/GFLV*	94	1
GLRaV-1+3**	94	24
GLRaV-2	94	3
GVA	94	11
GfKV	94	27
GLRaV-4,5,6,9 and Ob***	94	0
SLRSV	94	0
TBRV	94	0
RpRSC-ch	94	0
RpRSC-g	94	0
Total	94	43

*: Samples infected with ArMV and/or GFLV

**: Tables may have a footer. Samples infected with GLRaV-1 and/or GLRaV-3

***: Tables may have a footer. Samples infected with at least one of the GLRaV-4 strains -4, -5, -6, -9, -Ob.

In the 17 vineyards where clone selection was studied, no clean vineyards were found in the virus tests based on DAS-ELISA analyses. In the first stage, 43 (46%) of 94 clone candidates which had no symptoms of virus, bacteria or fungal diseases and had high scores in weighted grading were infected with at least one of the tested viruses. GfKV, one of the viral diseases, was found most common in the tested samples (27/94), followed by GLRaV-1 + 3 (24/94), GVA (11/94), GLRaV-2 (3/94), ArMV / GFLV (1/94). GLRaV-4 strains -4, -5, -6, -9, -Ob, SLRSV, TBRV, RpRSV-ch and RpRSV-g infections were not detected. In the region where we work, *Vitis rupestris* hybrid vine rootstocks, which form a lot of bottom shoots, were widely used. Producers

preferred to obtain saplings by grafting their bottom shoots with the green grafting method. This situation caused the spread of viral diseases in the region.

In a similar study, Çelik et al. (2019) reported the contamination rate of 80.5% and the most common viruses as GLRaV-1, GfKV and GLRaV-3 in the virus tests performed on selected clones of the Kalecik Karası variety. In another similar study, Vončina et al. (2019), by testing 9 viruses (ELISA) in 1116 vines in 14 autochthonous Croat grape varieties from 51 vineyards in the Dalmatian region (ArMV, GFLV, GFkV, GLRaV-1, GLRaV-2, GLRaV-3, GLRaV-7, GVA and GVB) had confirmed the existence of 8 viruses. Contamination rates were as GLRaV-3 (79.6%), GVA (61.4%), GLRaV-1 (40.8%), GFkV (19.9%), GFLV (19.6%), GLRaV-2 (4.1%), ArMV (3.2%) and GVB (3.1%) respectively, and total of 93 vines (8.3%) free of all viruses tested.

Vine is one of the plant species most susceptible to viral infections that cause many complex diseases. The effects of viruses on grapevine performance are generally considered to be potentially severe, but factors affecting the grapevine response such as mixed infections, viral species, environment, grape variety and rootstock, vineyard management, etc. are complex. However, diseases such as infectious degenerations caused by Nepoviruses are highly harmful and significantly affect plant viability and yield. More complex are the effects of members of the genus Ampelovirus, Closterovirus and Vitivirus, which are factors of leaf curl, leaf discoloration - spots and wrinkled woody tissue. Vines infected with these species usually produce sufficient crops, so growers are unaware of the true damage, especially in qualitative parameters. Grape vines generally offer better growth and increased yield; there-fore, cultural practices (green pruning, cluster thinning, wider spacing etc.) must be adjusted to cope with these improved performances. Vines are also affected by "small" virus diseases (e.g., speckle, vein mosaic, rupestris stem pitting, etc.), the effect of which is still uncertain. Their presence should not be overlooked, as the synergistic negative effects of these agents with other major viruses cannot be ruled out. Viruses are dangerous and difficult to eliminate pathogens whose presence in vines must be prevented using clean propagation material (Mannini and Digiaro 2017). Therefore, hygienic selection is the most economical strategy to reduce the presence of viruses in the propagation material and to limit their prevalence in newly established vineyards through the production of clean stocks from which high-quality planting material is obtained. Clean stock selection requires efficient therapy methodologies and careful screening of selected clones of scion and rootstock material for economically important viruses (Golino et al. 2017).

Šikuten et al. (2018) reported that due to the lack of clonal and sanitary selection in the past, native varieties in Croatia have a high level of intra-varietal variability and virus infections. Researchers were able to select enough virus-free clone candidates at the first stage of selection, despite the high level of virus infection they detected, as well as the high level of intra-varietal variability in native cultivar populations.

Lemos et al. (2020) reported that when they examined 30 "Tempranillo" clones in two regions for a period of two years, high variation was observed in terms of total phenols and antiradical activity, anthocyanin content was significantly affected by environmental conditions, and location tests enabled the recognition of elite grapevine clones. They also reported that the genetic variability exhibited by selected clones could be an important resource in the short / medium term to respond appropriately to the changing climate by selecting clones that best adapt to new conditions.

According to Gonçalves and Martins (2019), conserving intra-variety genetic diversity is a crucial strategy for preserving traditional viticulture and facing future challenges (Carbonell-Bejerano et al. 2019).

3.3. Establishing the clone comparison vineyard

Clone comparison vineyards established in the second stage of clone selection also form the field gene banks (FGB) of the selected clones. Although clonal repositories require less space, are easy to manage and cost-effective, FGB are needed to pre-serve genetic diversity. In the 1980s, procedures were developed for the maintenance of FGB germplasm collections (Rajasekaran and Mullins 1979).

To compare these selected clones together, all clones were grafted onto the virus-free 110R rootstock. The clone comparison vineyard was established in Selçuk University Vineyard research plot, where there were

three replicates of each clone and 6 vines for each repeat. The clone comparison vineyard was planned to be a Gök Üzüm clone next to each Ekşi Kara vine to allow pollination (Figure 1). The sanitary condition is usually initially assessed by visual inspection of the vineyards and the presence of various viruses by DAS-ELISA analyses. This test is generally considered definitive if one is positive, but when the result is negative it does not rule out an infection. Directive 2005/43 / EC of the European Union on the marketing of vine propagation material, GFLV, ArMV and GLRaV- 1 and GLRaV-3 to ensure that it is not included in the grapevine seedlings from each member country (Rizzo et al. 2015).

To protect the Ekşi Kara and Gök Üzüm clones, which were found to be free from viral, bacterial, and fungal diseases, cleanly and to prevent contamination, own rooted saplings were produced and planted one by one in the greenhouse for protection. At this stage, a study plan was prepared to determine the kinship relations of the selected clones. In a previous study, Roach et al. (2018) reported that many clones with differences in basic viticulture and oenological characteristics were formed in the Chardonnay cultivar with the accumulation of somatic mutations during the asexual reproduction process over centuries, the genetic diversity under-lying these differences was largely unknown, how-ever, Pinot noir and Gouais blanc. They determined that the Chardonnay genome exhibited features indicative of inbreeding.

Mannini et al. (2002) reported that serious and costly sanitation protocols were established world-wide to reproduce only clones free of harmful vi-ruses. In the study, virus-free clones performed best overall, whereas increased vegetative growth and/or yield associated with healthy vines may have ad-verse side effects on grape quality in cooler cli-mates, suggesting that cultural practices in the vineyard must be adapted to the changing abilities of the clones to cope with this. suggested.

In a similar previous study, Cirami et al. (1993) evaluated the field performance of selected clones of Cabernet Sauvignon for 30 years by examining yield and juice composition values with 9 reference clones. They suggested testing the clones in single vine plots with 10-20 replicates and in the vineyard areas where they will be planted for more precise statistical discrimination.

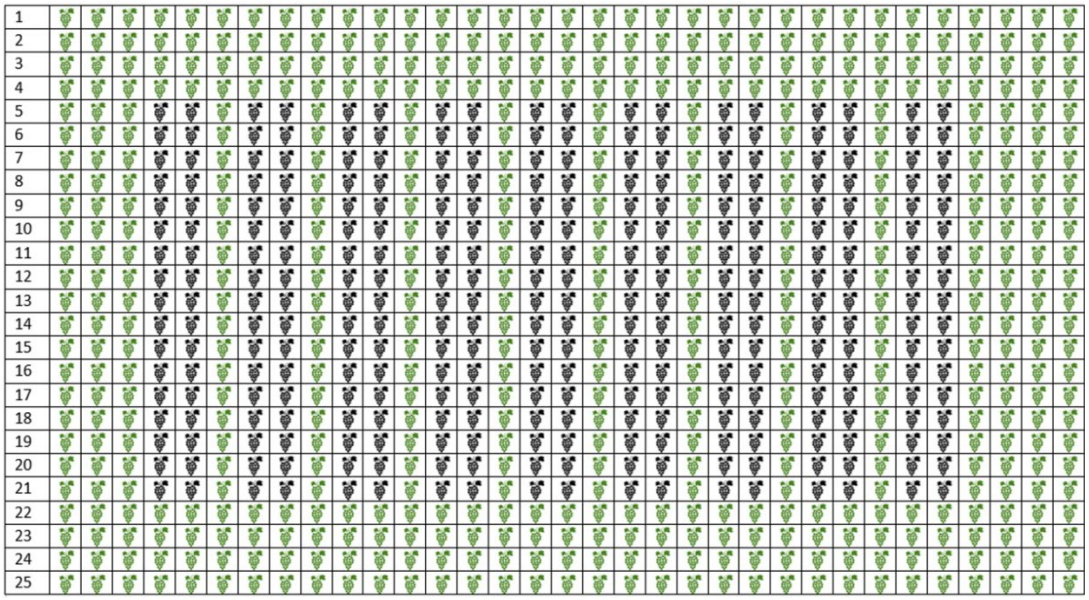

Figure 1. Ekşi Kara Clone Comparison vineyard planting plan. The green clusters indicate Gök Üzüm clones and the black clusters indicates Ekşi Kara clones. The Gök Üzüm clone was planted as an edge affect for all sides. In the middle area clones were placed in order of 18 vine from each clone

4. Conclusions

During the mass-selection stage of the Ekşi Kara grape variety, ampelographic description was made and the vineyard population of clone selection consisting of 5000 vines in 17 different vineyards varying between 800 m - 1500 m above sea level was determined. As a result of the 2-year yield, development and quality observations made in the population, 220 clones were selected, and the stage of single selection was initiated. Single clone selection was carried out in two stages, genetic selection, and health selection.

In the genetic selection phase, yield, quality, and growth values were determined in the clone candidates and the clone candidates were ranked separately according to their average weighted grading scores.

Considering the repeated sanitation tests in 51 Ekşi Kara and 11 Gök Üzüm clone candidates selected by genetic selection, 17 Ekşi Kara and 2 Gök Üzüm clones were selected for the third stage studies. Clone comparison vineyard was established in Selçuk University (38°03'50''N, 32°50'11''E) to compare the selected clones at the same location and on the same rootstock.

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