Erwerbs-Obstbau (2023) 65:2143–2152
https://doi.org/10.1007/s10341-023-00982-w

ORIGINAL ARTICLE / ORIGINALBEITRAG

Responses of Potential Rootstock Hybrids (*Vinifera* × *American*) to Drought and Salt Stress

Damla Yüksel Küskü[1] · **Gökhan Söylemezoğlu**[2]

Received: 18 March 2023 / Accepted: 9 October 2023 / Published online: 3 November 2023

Abstract

Abiotic stress factors such as drought, salinity, and high temperatures have been increasing their impact in recent years with global warming. As in all areas of agriculture, viticulture is also faced with these negative effects. When the 10-year data are analyzed, it is seen that there is an intense loss in production and vineyard areas in Turkey. In the face of this decrease in vineyard areas, it is inevitable to try to obtain high yields from the existing areas. The basis of these studies is the selection and breeding of rootstock varieties with high yield and resistance to both abiotic and biotic stress conditions. In this work, individuals resistant to drought and salinity stress among 100 'Karadimrit × 140 Ruggeri' and 'Boğazkere × 1103 Paulsen' hybrids, which were obtained by crossbreeding in 2007–2008 and are potentially drought and salt tolerant, will be identified and registered as rootstocks. The study was carried out between 2018 and 2020 in order to identify those with potential. The results obtained according to the leaf water potential (LWP, Ψ) measurement data used to measure the stress occurring in the plant as a result of controlled drought and salt applications varied between –1.45 and –2.45 MegaPascal (MPa) in drought applications, –1.40 and –2.15 MPa in salt applications, and –1.45 and –2.30 MPa in polyethylene glycol (PEG) applications. As a result of the study initiated for rootstock breeding, it has been determined that 12 individuals out of 100 hybrids are more resistant to all stress conditions and are potential rootstocks.

Keywords Leaf water potential · Simple sequence repeats · Drought · Salinity · *Vitis*

Introduction

Anatolia (Arroyo-Garcia et al. 2006), which is one of the areas where *Vitis vinifera* L. was first cultivated, has a very rich grapevine gene potential that emerged with natural hybridization over time. According to the statistics for 2021 in Turkey, the total vineyard area is 390,221 hectares, and the total grape production is 3,670,000 tons (TÜİK 2022). When the statistics for the years 2011–2021 are compared, there has been a decrease of 14.58% in production and 17.42% in vineyard areas between the years. This decrease in agricultural lands, non-agricultural use of agricultural lands, climate change, global warming, and unconscious irrigation, which has been increasingly felt in the last quarter century, will adversely affect the Mediterranean basin, including Turkey, in the next 30 years. The decrease in underground water resources and lakes due to the selection of wrong agricultural product patterns, the drying up of ponds, barrenness, and salinization increase to the maximum level. This decrease in vineyard areas necessitates sustainable viticulture in the future with resistance to biotic and abiotic stress conditions and the selection of rootstocks and varieties with high yields.

Although *Vitis vinifera* L. has rich genetic diversity, it is very susceptible to phylloxera. For this reason, cultivation is carried out by grafting on American grape rootstocks in Turkey as well as all over the world. The rich genetic diversity seen in *Vitis vinifera* L. is not present in American grapevine rootstocks (Serra et al. 2014). Due to increasing stress factors, it is not enough for American grapevine rootstocks to be resistant only to phylloxera; other biotic (downy mildew [*Plasmopara viticola*], powdery mildew [*Uncinula necator*], gray mold [*Botrytis cinerea*], etc.) and abiotic stressors (drought, salinity, calcification, air pollution, etc.)

✉ Damla Yüksel Küskü
damla.yuksel@bilecik.edu.tr

[1] Department of Landscape and Ornamental Plants, Vocational High School, Bilecik Seyh Edebali University, Bilecik, Turkey

[2] Department of Horticulture, Faculty of Agriculture, Ankara University, Ankara, Turkey

are also required to have high resistance against the elements.

The term ‘vine breeding studies’ in viticulture refers to research done to select individuals that are productive, high quality, resistant to diseases and pests, and well adapted to environmental conditions in order to combine the desired characters in a variety (Ergül 1997).

Research on plant breeding is done to create new cultivars with superior traits for coping with environmental stress and climate change circumstances (Habash et al. 2009). The most popular technique in grapevine breeding projects is hybridization (combination) breeding, with the goal of developing varieties that are resilient to biotic and abiotic stress conditions while still producing high-quality products. In the second half of the 19th century, this technique—which involves fusing two or more features from distinct genotypes into a single individual—became very important and started to be applied consistently. Genetic information started to be employed in grapevine breeding and selection at this time in developed winemaking nations like France, Italy, and Germany. These decisions were based on scientific principles. The first hybrids between *Vitis labrusca* and *Vitis vinifera*, known as ‘Isabella’ and ‘Catawba’, were created between 1816 and 1819. Between *Vitis aestivalis* and *Vitis vinifera*, the ‘Norton’ variety was bred in around 1830. In Massachusetts in 1849, *Vitis labrusca* and *Vitis vinifera* hybrids gave rise to the ‘Concord’ variety (Di Gaspero et al. 2012). Similar experiments for the creation of new grape varieties utilizing the conventional hybridization (combination) breeding approach began in our nation in 1973 (Uslu et al. 1995).

The adverse environmental conditions created by global climate change on earth are increasing their impact day by day (Soltekin et al. 2021). High–low temperatures, drought, soil salinity, and adverse conditions such as lime, radiation, and soil and atmosphere pollution are the main abiotic stresses that significantly limit yield and quality in crop production (Lawlor and Cornic 2002). Oxidative damage occurs in all plants under these stress conditions, and the mechanisms developed by species and varieties vary in order to withstand these stress conditions or to escape from stress. Some plants are more affected by abiotic stress conditions, while others are resistant to these conditions. These differences can occur between species as well as between different varieties of the same species (Söylemezoğlu et al. 2010). The degree of damage to the plant due to abiotic stress factors varies according to the type of plant, its adaptation to the environment, and its degree of tolerance to stress, and these factors are among the main factors that determine the best growth of different plants (Bray 2007). The biggest cause of agricultural product losses in the world is abiotic stress, which causes about 50% of the average yield to decrease (Bray 2002).

Considering that it is not possible to open new areas to agriculture, it is inevitable that the most effective way to increase production is plant breeding. However, as a result of the decrease in genetic diversity in many plant species, it has become very difficult to develop varieties with desired characteristics. The variation required for breeding work is obtained from wild relatives, registered varieties, and local varieties. For this reason, it is necessary to meticulously screen the materials in question and transfer the appropriate genes to culture varieties using the developed techniques. However, success in plant breeding primarily depends on effective, accurate, and fast selection.

Rootstock breeding in Turkey has not yielded successful results until today, and it is seen as a big deficit. In this geography, which has a very rich grape gene potential, it has become inevitable to give importance to grape breeding studies in order to get yield from the areas negatively affected by global warming. This study, which can be seen as a start for rootstock breeding, aims to select individuals with high resistance to drought and salt stress in the short term and to use these individuals as material in future studies. In the long term, it is based on the aim of realizing Turkey’s first rootstock registration.

Materials and Methods

Plant Material

As plant material in the research, cuttings from vines obtained from the crossing of ‘Karadimrit’ and ‘Boğazkere’ grape varieties, and ‘1103 Paulsen’ and ‘140 Ruggeri’ American rootstocks, which are known to be resistant to drought and lime. Plants were obtained from the breeding plot in the Kalecik Viticulture Research and Application Station of the Ankara University Faculty of Agriculture. After the hybridization breeding carried out in 2007–2008, healthy development of the plants was ensured, and applications were carried out to determine their resistance to abiotic stress factors (salt, drought, and PEG) and promising F1 plants were determined (Çakır 2011). As a result of the observations carried out on 600 hybrid vines in the breeding plot in the 2018 vegetation period, 100 individuals with strong shoot yield and development were selected for the purpose. Up to the planting date, the collected cuttings were preserved in cold storage. Cuttings were prepared with two buds and planted in polyethylene seedling bags. The planted cuttings were transferred to the temperature and humidity controlled greenhouse and their healthy development was ensured.

Drought Applications

Drought stress was carried out on the same plants in two stages. The first stage was carried out with no water for 15 days to control ('Boğazkere', 'Karadimrit', '1103 P', and '140 Ru') and F1 plants. Drought stress, which lasted for 15 days, started with the last irrigation of the plants, and the first leaf water potential (LWP) values were measured 24 hours after irrigation. The measurements were repeated on the 7th and 15th days, and the responses of plants to drought stress were observed. The results of the LWP measurements made on the 1st, 7th, and 15th days were recorded with the MPa value. PEG was given to the plants as a second application to measure drought resistance. PEG stress was started after it was observed that the plants, which were subjected to drought stress and then regularly irrigated, were relieved of the stress. For 100 milliliter (mL) of water per plant, 12% PEG 6000 was applied (Ueda et al. 2004). The first PEG application was made 24 h after the last irrigation (day 0). The first LWP measurement was performed 24 h after the application (day 1). The 2nd, 3rd, and 4th LWP measurements were made on the 5th, 10th, and 15th days and recorded.

Salt Applications

Salt applications for 100 mL of water per plant (120 millimolar (mM) NaCl and 12 mM $CaCl_2$) (Tattersall et al. 2007), were carried out with irrigations every 5 days. The first salt application was made 24 h after the last irrigation (day 0). The first LWP measurement was performed 24 h after the application (day 1). The 2nd, 3rd, 4th, and 5th LWP measurements were made on the 5th, 10th, 15th, and 20th days. The same applications and measurements were carried out in the control plants.

LWP Measurements (Ψ, MPa)

LWP, at 7:00 in the morning, was measured with an MPa value using a PMS Instrument Company brand pressure bomb (Xanthopoulos 2004, 2006).

Hybrid Parent Diagnosis

The simple sequence repeats (SSR) method was used to determine the parents of the individuals that were found to be resistant as a result of the applications, due to the random selection of 100 hybrids with strong growth in the breeding plot. For each F1 genotype to be used in hybrid parent diagnosis, samples were taken from shoot tips and half- or fully-opened young leaves just below, and DNA isolation was performed (Lefort et al. 1998). A Nanodrop ND-1000 spectrophotometer was used for DNA quality and quantity measurements. Polymerase Chain Reaction (PCR) of 15–200 ng DNA, 10 pmol forward primer, 10 pmol reverse primer, 0.5 mM total dNTP, 1 µL Taq DNA polymerase (SolisBioDyne), 1.5 mM MgCl2, and 3 µl buffer (10 × buffer) was carried out in 15 µL. After PCR, the PCR products of the loci were checked on a 2% agarose gel, and the capillary electrophoresis step was performed in the amplified samples. Microsatellite loci of VVS2, VVMD5, VVMD7, VVMD27, VrZAG62, and VrZAG79 (This et al. 2004), which are accepted as the standard set, were used in grapes. The base sequences of the primers are given in Table 1. The AATİ Fragment Analyzer Genetic Analysis System was used for capillary electrophoresis. Genotypes were diluted with the loading solution at certain ratios according to the PCR product amounts. Then, the peaks of each locus were visualized. Comparisons were made with the control groups according to the obtained allele sizes, and it was concluded to which of the 'Karadimrit × 140 Ru' and 'Boğazkere × 1103 P' hybrids the hybrid individuals belonged.

Statistical Analysis

The trials for drought application and salt application were planned according to the randomized plot design and were set up with cuttings belonging to the same grapevines in order to carry out the applications simultaneously but separately for both applications. Plant materials (5 × 1) for drought stress application and plant materials (5 × 1) for salt stress application in 100 hybrid genotypes were rooted and turned into plants.

The numerical values obtained as a result of the research were evaluated using one-way analysis of variance (ANOVA) in the Minitab 18 statistical program. The Tukey test was used to determine the significance level of the differences, and $p < 0.05$ was considered statistically significant. Results are expressed as mean ± standard error of the mean.

Table 1 Base sequences of primers of simple sequence repeat loci

No.	Locus	DNA primary sequences (5' ... 3')
1	VVS2-F	cagcccgtaaatgtatccatc
	VVS2-R	aaattcaaaattctaattcaactgg
2	VVMD5-F	ctagagctacgccaatccaa
	VVMD5-R	tataccaaaaatcatattcctaaa
3	VVMD7-F	agagttgcggagaacaggat
	VVMD7-R	cgaaccttcacacgcttgat
4	VVMD27-F	gtaccagatctgaatacatccgtaagt
	VVMD27-R	acgggtatagagcaaacggtgt
5	ZAG62-F	ggtgaaatgggcaccgaacacacgc
	ZAG62-R	ccatgtctctcctcagcttctcagc
6	ZAG79-F	agattgtggaggagggaacaaaccg
	ZAG79-R	tgcccccattttcaaactcccttcc

Results and Discussion

LWP Measurement Results

For the three abiotic stress applications, LWP of genotypes were measured in MPa with the help of a pressure bomb at certain intervals. LWP of genotypes were measured in MPa for the three abiotic stress treatments with the use of a pressure bomb at predetermined intervals. The acquired MPa values were categorized, as shown in Table 2, by providing four scale values in certain data intervals (Çakır 2011). According to the table, those with the least water loss and a value greater than or equal to –1.55 MPa are in the 1st class; those with a value between –1.60 and –1.75 MPa are in the 2nd class; those with a value between –1.80 and –1.95 MPa are in the 3rd class; and those with a value equal to –2.00 MPa are either the younger ones or were accepted as the 4th class. When drought application measurements, PEG application measurements, and salt application measurements were examined, it was seen that there was a decrease in LWP with increasing drought and salt stress. Some hybrids, on the other hand, could not withstand the increased stress and dried up (‘D’).

In different studies, it has been seen that the limits of the scales on which stress measurements are grouped are set at different values. According to Cohen et al. (2005), those with a LWP value greater than –1.40 MPa are over-watered, those with a value between –1.40 and –1.70 MPa are well-watered, those with a value between –1.70 and –2.00 MPa have low water stress, those with a value between –2.00 and –2.30 MPa have medium water stress, and those with a value less than –2.30 MPa were evaluated as showing severe water stress.

Four alternative water regimes were used to the ‘Chardonnay’ and ‘Cabernet Sauvignon’ grape types in the study looking at connections between LWP of predawn and midday in *Vitis vinifera*, and measurement data were obtained. While the values given as averages were between –0.02 and –0.62 MPa for ‘Chardonnay’ at dawn measurements, it was found between –0.26 and –0.75 MPa for the ‘Cabernet Sauvignon’ grape variety. In the midday measurements, values were between –0.86 and –1.81 MPa for ‘Chardonnay’ and between –1.29 and –1.71 MPa for the ‘Cabernet Sauvignon’ grape variety. As a result of the restrictions in the irrigation regime and the increase in temperature during the day, it was observed that the LWP of the plants decreased (Williams and Araujo 2002).

Table 2 Leaf water potential (Ψ) scale values (Çakır 2011)

Scale values (MPa)	
1. Class	$\Psi \geq -1.55$
2. Class	$-1.60 \leq \Psi \leq -1.75$
3. Class	$-1.80 \leq \Psi \leq -1.95$
4. Class	$-2.00 \leq \Psi$
Dried	D

Plants with a value between 0.00 and –0.20 MPa are classified as unstressed, those between –0.20 and –0.40 MPa as low-moderate stressed, those between –0.40 and –0.60 MPa as moderate-severe stressed, and those with a value less than –0.60 MPa as severely stressed in a study where LWP measurements were made on the vine at dawn and midday. Plants are considered to be under minimal stress if their LWP value is more than –1.00 MPa, low stress if it is between –1.00 and –1.20 MPa, medium stress if it is between –1.20 and –1.40 MPa, high stress if it is between –1.40 and –1.60 MPa, and severely strained if it is below –1.60 MPa (Girona et al. 2005; Korkutal et al. 2019).

In the study carried out to create irrigation programs according to LWP measurements, ‘Flame Seedless’ and ‘Italia’ grape varieties were used, and four different applications were carried out. These irrigations were created using unirrigated witness plants (I4) and three distinct noon LWP threshold values (I1: I = –1.0 MPa; I2: I = –1.3 MPa; I3: I = –1.6 MPa). Midday LWP in ‘Flame Seedless’ ranged from –1.0 to –1.3 MPa; in the ‘Italia’ variety, irrigation produced the best yield when –1.3 MPa. The results showed that limited irrigation should be done in the studied grape varieties and that this is necessary for the yield and water use potential to be at their maximum level (Bozkurt Çolak 2010).

The findings from the study, which aimed to demonstrate the significance of abrupt and extreme water stress on the vine and fruit composition of three wine grape types, revealed that the values of LWP at dawn and midday were nearly identical, with a fall of about –2.1 MPa. After this level (–2.1 MPa), both predawn and midday values converged at –3.7 MPa. This mean value was determined as the threshold for all leaf drying in the vines. In addition, it was determined that plant transpiration occurs when the LWP value is between 0 and –3.7 MPa. While the LWP was between –3.7 and –4.6 MPa, water was found in the xylem, but there was no transpiration from the leaves. Although all the leaves dried and fell due to sudden and excessive water stress, the vines did not die, and after re-watering, the vines started to recover from the auxiliary bud at the top of the shoots (Bahar et al. 2011).

It was found that the LWP measurement values obtained from the control application without irrigation gave lower results than the 25% and 50% restricted water applications in the study in which different irrigation regimes were applied to the ‘Touriga Franca’ grape variety in Portugal. When the results were examined, it was found that the LWP values decreased with the increase in water stress in the plants (Cabral 2017).

Table 3 Leaf water potential (Ψ) averages at the end of drought application (MPa)

Genotype	Ψ	Genotype	Ψ	Genotype	Ψ	Genotype	Ψ
1	–2.02 ± 0.12*	27	D	53	**–1.91 ± 0.06**	79	–2.24 ± 0.04
2	–2.06 ± 0.11	28	–2.35 ± 0.06	54	–2.16 ± 0.05	80	–2.27 ± 0.04
3	*–1.51 ± 0.05*	29	**–1.74 ± 0.07**	55	–2.02 ± 0.06	81	**–2.20 ± 0.05**
4	–2.09 ± 0.07	30	D	56	–2.01 ± 0.07	82	**–1.84 ± 0.04**
5	**D	31	–2.34 ± 0.07	57	–2.03 ± 0.07	83	**–1.78 ± 0.07**
6	D	32	–2.23 ± 0.08	58	**–1.95 ± 0.05**	84	–2.15 ± 0.05
7	**–1.71 ± 0.08**	33	–2.11 ± 0.07	59	–2.11 ± 0.02	85	**–1.94 ± 0.08**
8	**–1.93 ± 0.12**	34	–2.10 ± 0.14	60	–2.13 ± 0.04	86	**–1.90 ± 0.05**
9	–2.11 ± 0.05	35	–2.32 ± 0.06	61	–2.06 ± 0.04	87	**–1.74 ± 0.07**
10	–2.27 ± 0.10	36	**–1.82 ± 0.08**	62	–2.04 ± 0.06	88	**–1.78 ± 0.06**
11	–2.34 ± 0.11	37	**–1.94 ± 0.07**	63	**–1.83 ± 0.04**	89	**–1.71 ± 0.08**
12	D	38	–2.41 ± 0.07	64	**–1.86 ± 0.02**	90	**–1.76 ± 0.07**
13	**–1.62 ± 0.10**	39	**–1.75 ± 0.09**	65	–2.02 ± 0.06	91	**–1.77 ± 0.03**
14	**–1.85 ± 0.09**	40	**–1.76 ± 0.08**	66	**–1.69 ± 0.07**	92	**–1.69 ± 0.02**
15	**–1.92 ± 0.06**	41	–2.16 ± 0.07	67	**–1.67 ± 0.03**	93	**–1.91 ± 0.05**
16	–1.98 ± 0.12	42	–1.98 ± 0.10	68	**–1.69 ± 0.04**	94	**–1.69 ± 0.04**
17	–2.34 ± 0.04	43	**–1.70 ± 0.08**	69	–1.99 ± 0.04	95	**–1.60 ± 0.09**
18	–2.27 ± 0.09	44	–2.08 ± 0.06	70	–2.14 ± 0.10	96	**–1.59 ± 0.08**
19	–2.28 ± 0.12	45	**–1.68 ± 0.10**	71	**–1.70 ± 0.06**	97	**–1.67 ± 0.08**
20	–2.36 ± 0.07	46	**–1.57 ± 0.08**	72	–2.27 ± 0.03	98	–2.22 ± 0.06
21	–2.34 ± 0.05	47	**–1.62 ± 0.06**	73	**–1.75 ± 0.08**	99	**–1.74 ± 0.09**
22	–2.37 ± 0.03	48	–2.12 ± 0.09	74	**–1.72 ± 0.08**	100	D
23	–2.34 ± 0.04	49	–2.43 ± 0.08	75	**–1.75 ± 0.10**	B	–2.13 ± 0.08
24	–2.32 ± 0.08	50	–2.16 ± 0.07	76	D	K	–2.26 ± 0.08
25	–2.35 ± 0.04	51	**–1.89 ± 0.08**	77	D	1103 P	–2.00 ± 0.05
26	–2.29 ± 0.09	52	**–1.69 ± 0.07**	78	**–1.76 ± 0.06**	140 Ru	–1.87 ± 0.07
Average –1.98							

Italics 1st class, *bold* above average value
*$p < 0.05$ (Results are given as mean ± standard error of the mean)
**D: The genotype dried at the end of the treatments

It was observed that the LWP measurement values carried out in the middle of the day in all cultivars decreased as the amount of irrigation water decreased; that is, an increase in the stress level occurred. While the 'Sultani Çekirdeksiz' grape variety gave the lowest LWP value with –1.75 MPa, this value was found to be –1.60 MPa for 'Siyah Kişmiş' and –1.66 MPa for 'Crimson Seedless' (Soltekin 2019).

In this study in all genotypes, with the increase in stress duration and the number of applications, a decrease in the amount of water in the leaves was observed, and this was recorded with the decrease in the LWP. When the literature is examined, it has been seen that the studies are generally on the application of different irrigation regimes or on the creation of irrigation programs according to the results of LWP measurements made before dawn and in the middle of the day. This investigation demonstrated that the vines were experiencing a negative rise in LWP with the increase in stress. The values obtained as a consequence of measurements done at regular intervals in both drought and salt treatments were recorded. The stressors did not affect all hybrids in the same way, and the results of the measurements also showed how the hybrids responded to the stresses. In some hybrids, the plants could not withstand the increased stress period and the number of applications and dried up. In salt stress, which can be considered a water stress by negatively affecting both water stress and water intake, the water level in the leaves of the plants decreases, and the LWP decreases depending on this decrease. The results obtained from the study and the results of the literature showed parallelism.

LWP Measurement Results Under Drought Stress (MPa)

As a result of the drought applications that lasted for 15 days, the plants were stressed to a great extent. When the application was completed, 47% of the plants were in the 4th class, while 8% of them could not withstand the stress and dried up. The 3rd genotype was least affected by stress and took place in the 1st class. The average value

Table 4 Leaf water potential (Ψ) averages at the end of polyethylene glycol application (MPa)

Genotype	Ψ	Genotype	Ψ	Genotype	Ψ	Genotype	Ψ
1	**–1.70±0.03***	27	D	53	**–1.75±0.06**	79	–1.88±0.06
2	**–1.58±0.06**	28	**–1.77±0.08**	54	–2.19±0.07	80	–2.03±0.06
3	**–1.75±0.06**	29	–1.79±0.07	55	–2.09±0.09	81	**–1.64±0.08**
4	–1.80±0.05	30	D	56	–2.26±0.04	82	*–1.53±0.04*
5	**D	31	**–1.65±0.04**	57	–2.06±0.02	83	**–1.64±0.07**
6	D	32	**–1.74±0.04**	58	–1.83±0.03	84	**–1.58±0.07**
7	–1.89±0.04	33	*–1.49±0.04*	59	–2.04±0.04	85	–2.02±0.03
8	–1.81±0.04	34	*–1.49±0.04*	60	–2.08±0.03	86	–2.05±0.03
9	**–1.61±0.04**	35	**–1.58±0.04**	61	–1.95±0.07	87	**–1.71±0.06**
10	**–1.62±0.08**	36	**–1.57±0.06**	62	–1.84±0.06	88	**–1.77±0.07**
11	**–1.74±0.08**	37	**–1.58±0.05**	63	–1.88±0.06	89	–1.94±0.05
12	D	38	**–1.56±0.07**	64	–1.85±0.05	90	–2.13±0.04
13	**–1.57±0.04**	39	–2.09±0.05	65	–2.10±0.03	91	*–1.55±0.05*
14	–2.07±0.04	40	**–1.58±0.07**	66	–2.09±0.04	92	**–1.71±0.09**
15	**–1.76±0.07**	41	**–1.67±0.06**	67	–1.97±0.06	93	**–1.65±0.06**
16	*–1.52±0.06*	42	**–1.73±0.06**	68	–1.79±0.04	94	–1.84±0.02
17	–1.81±0.07	43	**–1.58±0.04**	69	–1.87±0.03	95	**–1.75±0.03**
18	–2.06±0.07	44	**–1.66±0.05**	70	–1.96±0.04	96	–2.00±0.03
19	D	45	**–1.56±0.04**	71	–1.86±0.06	97	–2.02±0.06
20	D	46	*–1.55±0.05*	72	–2.05±0.05	98	D
21	D	47	**–1.68±0.06**	73	–2.07±0.04	99	–1.82±0.04
22	*–1.50±0.06*	48	**–1.69±0.04**	74	–1.89±0.04	100	D
23	**–1.57±0.06**	49	**–1.63±0.07**	75	–2.10±0.05	B	–1.51±0.04
24	*–1.49±0.04*	50	**–1.73±0.08**	76	D	K	–1.35±0.08
25	**–1.71±0.06**	51	–1.79±0.07	77	D	1103 P	–1.54±0.04
26	**–1.71±0.04**	52	–1.91±0.08	78	**–1.78±0.04**	140 Ru	–1.49±0.04
Average –1.79							

Italics 1st class, *bold* above average value
*$p < 0.05$ (Results are given as mean ± standard error of the mean)
**D: The genotype dried at the end of the treatments

of the drought application LWP of 100 genotypes was found to be –1.98 MPa. As a result of taking the mean of recurrences, the number of genotypes above the mean value of –1.98 MPa was found to be 45. As a result of the application, the average values obtained for the genotypes are shown in Table 3.

'Boğazkere' and 'Karadimrit' cultivars were used as parents, and '1103 P' American rootstocks were in the 4th class, and '140 Ru' rootstocks were in the 3rd class with a value of –1.87 MPa.

LWP Measurement Results in PEG Stress (MPa)

As a result of PEG applications, 20% of the plants were in the 4th class, while 12% of them could not withstand stress and dried out (eight of the 100 hybrids included in the trial dried as a result of the drought applied without water for 15 days, and measurements could not be made because the PEG application was continued with the same plants). In total, eight individuals (8.0%) were least affected by stress (16, 22, 24, 33, 34, 46, 82, and 91) and were in the 1st class.

The average value of LWP for PEG application of 100 genotypes was found to be –1.79 MPa. As a result of taking the mean of recurrences, the number of genotypes above the mean value of –1.79 MPa was found to be 46. 'Boğazkere' and 'Karadimrit' cultivars and '1103 P' and '140 Ru' American vine rootstocks, which are used as parents, were in the 1st class with little effect from stress. As a result of the application, the average values obtained for the genotypes are shown in Table 4.

LWP Measurement Results Under Salt Stress (MPa)

As a result of salt applications, 7% of the plants were in the 4th class, and 7% of them dried up as they could not withstand the stress. In total, two genotypes were least affected by stress (61 and 83) and were in the first class.

The average value of LWP for 100 genotypes with salt application was found to be –1.75 MPa. As a result of taking

Table 5 Leaf water potential (Ψ) averages at the end of salt application (MPa)

Genotype	Ψ	Genotype	Ψ	Genotype	Ψ	Genotype	Ψ
1	–2.06 ± 0.06*	27	–2.02 ± 0.03	53	–1.83 ± 0.04	79	–1.98 ± 0.09
2	–2.07 ± 0.06	28	**–1.67 ± 0.06**	54	–1.75 ± 0.08	80	–1.80 ± 0.05
3	**–1.67 ± 0.03**	29	**–1.65 ± 0.03**	55	**–1.68 ± 0.03**	81	**–1.58 ± 0.07**
4	–2.03 ± 0.09	30	D	56	**–1.61 ± 0.06**	82	**–1.73 ± 0.06**
5	**D	31	**–1.65 ± 0.06**	57	**–1.62 ± 0.09**	83	*–1.51 ± 0.04*
6	–1.92 ± 0.06	32	**–1.74 ± 0.06**	58	**–1.67 ± 0.06**	84	**–1.69 ± 0.04**
7	**–1.74 ± 0.04**	33	–1.77 ± 0.06	59	**–1.68 ± 0.03**	85	**–1.74 ± 0.04**
8	**–1.63 ± 0.07**	34	**–1.71 ± 0.06**	60	–1.83 ± 0.06	86	**–1.64 ± 0.09**
9	–1.79 ± 0.06	35	**–1.68 ± 0.09**	61	*–1.47 ± 0.07*	87	**–1.65 ± 0.03**
10	**–1.72 ± 0.06**	36	**–1.69 ± 0.07**	62	**–1.73 ± 0.06**	88	–1.98 ± 0.04
11	–1.90 ± 0.08	37	D	63	**–1.57 ± 0.06**	89	**–1.71 ± 0.02**
12	–1.85 ± 0.08	38	–1.75 ± 0.03	64	**–1.67 ± 0.03**	90	–1.89 ± 0.10
13	**–1.63 ± 0.09**	39	D	65	**–1.65 ± 0.03**	91	**–1.66 ± 0.04**
14	–1.77 ± 0.07	40	**–1.66 ± 0.06**	66	–1.88 ± 0.07	92	–2.04 ± 0.08
15	–1.91 ± 0.06	41	D	67	–1.79 ± 0.06	93	**–1.67 ± 0.06**
16	**–1.66 ± 0.07**	42	**–1.69 ± 0.06**	68	**–1.63 ± 0.06**	94	**–1.65 ± 0.03**
17	**–1.68 ± 0.07**	43	**–1.74 ± 0.04**	69	**–1.73 ± 0.07**	95	**–1.61 ± 0.06**
18	**–1.71 ± 0.06**	44	–1.79 ± 0.04	70	–1.82 ± 0.03	96	**–1.72 ± 0.08**
19	–1.86 ± 0.04	45	–1.92 ± 0.06	71	–1.80 ± 0.09	97	**–1.68 ± 0.06**
20	–1.76 ± 0.04	46	–1.78 ± 0.04	72	–1.78 ± 0.07	98	**–1.66 ± 0.07**
21	–1.84 ± 0.05	47	**–1.62 ± 0.06**	73	–1.82 ± 0.06	99	–1.84 ± 0.06
22	**–1.69 ± 0.04**	48	–1.79 ± 0.04	74	**–1.70 ± 0.06**	100	**–1.74 ± 0.08**
23	**–1.69 ± 0.04**	49	D	75	–1.95 ± 0.06	B	–1.36 ± 0.07
24	–1.82 ± 0.06	50	–1.75 ± 0.03	76	**–1.65 ± 0.06**	K	–1.43 ± 0.06
25	–2.00 ± 0.03	51	**–1.59 ± 0.07**	77	–1.93 ± 0.09	1103 P	–1.47 ± 0.04
26	–1.79 ± 0.04	52	–1.87 ± 0.06	78	D	140 Ru	–1.41 ± 0.04
Average –1.75							

Italics 1st class, *bold* above average value
*$p < 0.05$ (Results are given as mean ± standard error of the mean)
**D: The genotype dried at the end of the treatments

the mean of recurrences, the number of genotypes above the mean value of –1.75 MPa was found to be 52. 'Boğazkere' and 'Karadimrit' cultivars and '1103 P' and '140 Ru' American vine rootstocks, which are used as parents, were in the 1st class with little effect from stress. As a result of the application, the average values obtained for the genotypes are shown in Table 5.

When all the results are combined and analyzed, the genotypes identified as a result of the overlap of those individuals that displayed greater resistance than the other individuals and had values above the average (drought –1.98 MPa; PEG –1.79 MPa; salt –1.75 MPa) in all three treatments were found to be 3, 13, 36, 40, 43, 47, 82, 83, 87, 91, 93, and 95 (Fig. 1).

When the outcomes of the parents that had drought, salt, and PEG stress treatments are combined, it can be observed that the parents, like the hybrids, are also impacted by the drought stress of up to 15 days. While all parents were in the 1st class in PEG and salt stress, 'Boğazkere', 'Karadimrit',

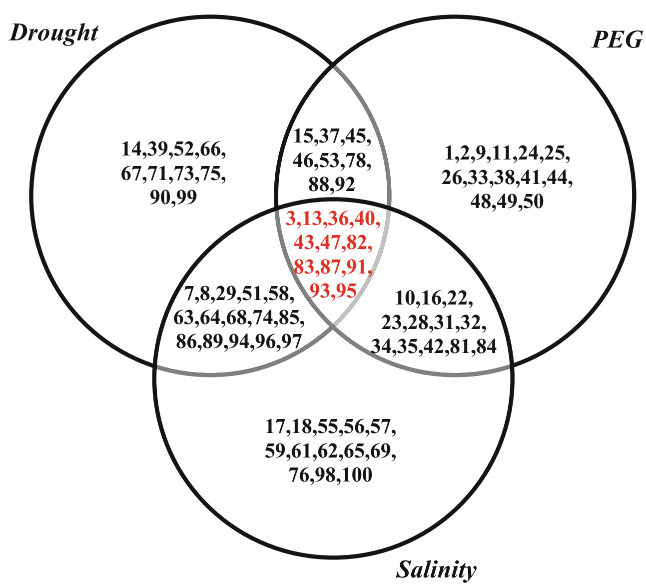

Fig. 1 Schematic representation of individuals with above-average values in all three stress treatments. *PEG* Polyethylene glycol

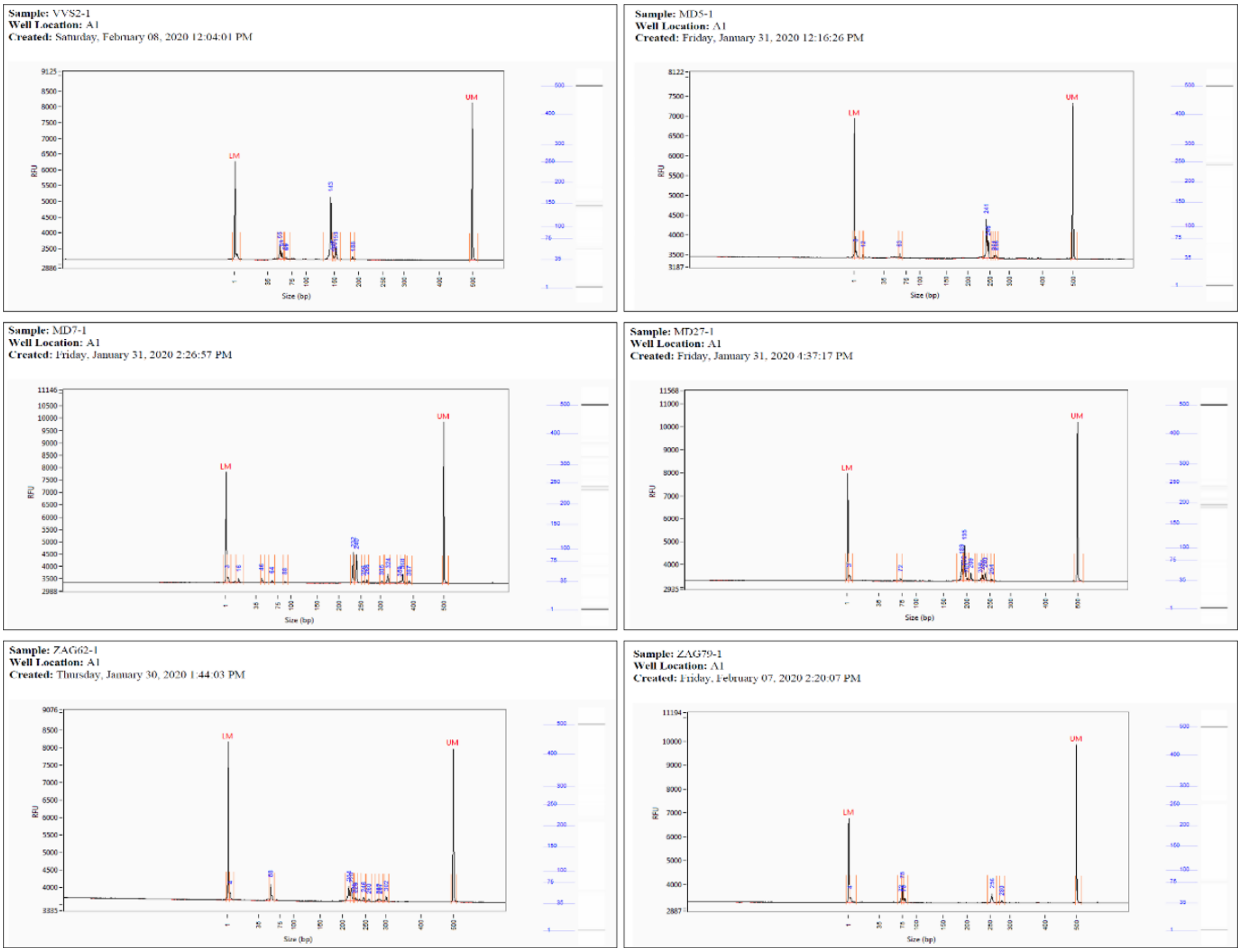

Fig. 2 Locus-allele profiles of different primers (VVS2, VVMD5, VVMD7, VVMD27, ZAG62, ZAG79) of the same genotype

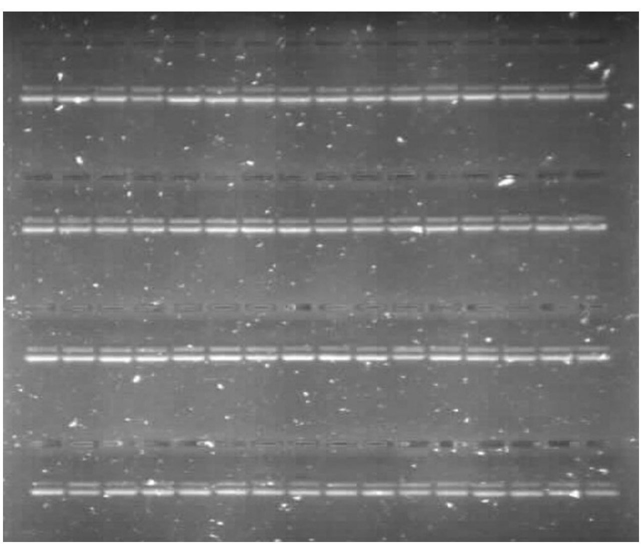

Fig. 3 Polymerase chain reaction gel image of ZAG62 locus

and '1103 P' were in the 4th class, and '140 Ru' were in the 3rd class in the drought application.

Hybrid Parent Diagnosis Results

A parental diagnosis was performed on 12 hybrid individuals that were determined to be resistant according to LWP measurements of individuals treated with drought, salt, and PEG.

DNA Isolation

On 12 F1 plants and their parents, DNA purity analysis and DNA isolation were carried out, and the resistance of the plants was measured. In order to determine whether there is amplification in the studied loci in the pure DNAs obtained, all PCR products representing that locus were first checked on a 2% agarose gel. Allele sizes at each locus were determined as peak data by the fragment analysis program

Table 6 Allele sizes of genotypes at six loci (bp)y

Microsatellite Loci												
Genotype	vvs2	vvs2	vvmd5	vvmd5	vvmd7	vvmd7	vvmd27	vvmd27	vrzag62	vrzag62	vrzag79	vrzag79
K	*143*	*145*	*238*	*242*	*249*	*249*	*186*	*195*	*188*	*190*	*247*	*249*
140 Ru	*137*	*143*	*248*	*270*	*231*	*257*	*190*	*206*	*196*	*214*	*243*	*259*
B	*151*	*155*	*234*	*240*	*249*	*255*	*180*	*195*	*194*	*204*	*247*	*249*
1103 P	*137*	*147*	*238*	*238*	*233*	*257*	*204*	*208*	*196*	*214*	*251*	*263*
3	143	143	242	270	249	249	195	206	188	196	247	259
13	147	155	234	238	249	257	195	208	196	204	249	263
36	143	145	242	248	231	249	186	206	190	196	247	259
40	143	143	242	248	249	249	186	190	190	196	249	249
43	143	145	242	242	249	249	190	195	188	196	249	259
47	143	143	242	248	231	249	186	206	190	196	247	259
82	143	143	242	242	249	257	186	206	190	196	249	249
83	143	145	242	248	231	249	186	206	188	196	249	249
87	143	145	242	242	231	249	190	195	190	196	247	259
91	137	143	242	248	249	257	195	206	188	196	247	259
93	137	145	242	270	231	249	186	206	188	196	247	247
95	143	143	238	242	231	249	186	206	190	214	249	259

Italics values belong to the parents, **bold** values belong to hybrids

in the AATI capillary electrophoresis system. Some images of allele profiles in capillary electrophoresis are presented in Fig. 2. After the PCR stage, the agarose gel produced unambiguous band pictures, indicating effective PCR optimization and successful allele type detection at all loci (Fig. 3).

Genetic Analysis

The allele sizes of six SSR loci in the study are presented in Table 6 as base pairs (bp). In addition, parents ('Karadimrit', 'Boğazkere', '140 Ru', and '1103 P') were analyzed together with the samples.

The results obtained were compared with the allele sizes of the parents, and it was determined which genotypes were 'Karadimrit × 140 Ru' and which 'Boğazkere × 1103 P' hybrids.

Fragment sizes of 96 samples can be determined in each analysis.

In order to illustrate the variations of up to 2 bp that may occur in loadings made at various times, the band sizes of the grape varieties 'Karadimrit' and 'Boğazkere' as well as the American vine rootstocks '140 Ru' and '1103 P' were taken from the VIVC (*Vitis* International Variety Catalogue) database.

According to the analysis results, it was found that 12 out of 100 selected F1 plants were more durable than the others. It was observed that one of the genotypes found to be resistant was 'Boğazkere × 1103 P', and 11 of them were 'Karadimrit × 140 Ru' hybrids. It was concluded that 'Karadimrit × 140 Ru' hybrids showed higher resistance to increased stress conditions, and this should be considered in breeding studies.

Conclusion

It has become very difficult to cultivate agricultural lands that have decreased and become inefficient due to global warming, which we have seen more and more in recent years around the world. In our country, agricultural areas are becoming inefficient, and it is inevitable that the effects of salinity, calcification, and drought will cause heavy losses in agricultural areas due to global warming. In addition, factors such as the conversion of agricultural lands to rental areas, the fact that traditional agriculture does not satisfy the farmers on low-yield lands, and wrong agricultural practices cause the abandonment of agriculture. Considering the current conditions, it has become necessary to use superior varieties and rootstocks against abiotic and biotic stress conditions in viticulture, as in all areas of agriculture. In parallel with the world, population density is increasing rapidly in our country, and the fact that the increasing population does not face food shortages means that we can obtain high-yield and quality products with the use of resistant rootstocks in existing agricultural areas. For this reason, there is a great need for rootstock breeding studies in our country. This study was aimed at determining the genotypes resistant to drought and salinity in order to enable cultivation in vineyard areas where stress factors are intense. It is planned to use 12 genotypes (3, 13, 36, 40, 43, 47, 82, 83, 87, 91, 93, and 95) that show superior properties against drought, PEG, and salt stress as source material for further studies. The study is seen as a pioneer in rootstock breeding studies in Turkey.

Acknowledgements This research consists of a part of the PhD thesis of the first author, Damla YÜKSEL KÜSKÜ, Department of Horticulture, Institute of Science and Technology, Ankara University. For its support of the projects '19L0447005' and 'Obtaining Drought and Salt Resistant Rootstocks from Cross-Species Hybrids (*V. vinifera* × *V. rupestris*)' we would like to thank the Ankara University Scientific Research Projects Coordinatorship of the University.

Conflict of interest D. Yüksel Küskü and G. Söylemezoğlu declare that they have no competing interests.

References

Arroyo-Garcia R, Ruiz-Garcia L, Boulling L, Ocete R, López M et al (2006) Genetic evidence for the existence of independent domestication events in grapevine. Mol Ecol 15(12):707–3714

Bahar E, Carbonneau A, Korkutal I (2011) The effect of extreme water stress on leaf drying limits and possibilities of recovering in three grapevine (Vitis vinifera L.) cultivars. Afr J Agric Res 6(5):1151–1160. https://doi.org/10.5897/AJAR10.778

Bozkurt Çolak Y (2010) Akdeniz Bölgesinde Flame Seedless ve Italıa Sofralık Üzüm Çeşitlerinde Yaprak Su Potansiyeline Göre Sulama Programlarının Oluşturulması. Dissertation, University of Çukurova

Bray EA (2002) Abscisic acid regulation of gene expression during water-deficit stress in the era of the Arabidopsis genome. Plant Cell Environ 25:153–161. https://doi.org/10.1046/j.1365-3040.2002.00746.x

Bray EA (2007) Plant response to water-deficit stress.

Cabral IL (2017) Influência de diferentes dotações de rega sobre o rendimento e qualidade na casta Touriga Franca na região do Douro. Faculdade de Ciências da Universidade do Porto, Porto

Çakır A (2011) Bağcılıkta Abiyotik Stres Koşullarına Yönelik Melezlemelerden Kuraklık ve Tuz Stresine Toleranslı Ümitvar Tiplerin Elde Edilmesi. Dissertation, University of Ankara

Cohen Y, Alchanatis V, Meron M, Saranga Y, Tsipris J (2005) Estimation of leaf water potential by thermal imagery and spatial analysis. J Exp Bot 56(417):1843–1852. https://doi.org/10.1093/jxb/eri174

Di Gaspero G, Copetti D, Coleman C, Castellarin SD, Eibach R, Kozma P, Lacombe T, Gambetta G, Zvyagin A, Cindrić P, Kovács L, Morgante M, Testolin R (2012) Selective sweep at the Rpv3 locus during grapevine breeding for downy mildew resistance. Theor Appl Genet 124(2):277–286. https://doi.org/10.1007/s00122-011-1703-8

Ergül A (1997) Asmanın sitolojik yapısı ve özel amaçlar açısından asma ıslahının esasları. University of Ankara

Girona J, Mata M, Del Campo J, Arbones A, Baartra E et al (2005) The use of midday leaf water potential for scheduling deficit irrigation in vineyards. Irrig Sci 24:115–127. https://doi.org/10.1007/s00271-005-0015-7

Habash DZ, Kehel Z, Nachit M (2009) Genomic approaches for designing durum wheat ready for climate change with a focus on drought. J Exp Bot 60(10):2805–2815. https://doi.org/10.1093/jxb/erp211

Korkutal I, Bahar E, Carbonneau A (2019) Effects of early water stress on grapevine (Vitis vinifera L.) growing in cv. Syrah. Appl Ecol Environ Res 17(1):463–472. https://doi.org/10.15666/aeer/1701_463472

Lawlor DW, Cornic G (2002) Photosynthetic carbon assimilation and associated metabolism in relation to water deficits in higher plants. Plant Cell Environ 25:275–294. https://doi.org/10.1046/j.0016-8025.2001.00814.x

Lefort F, Lally M, Thompson D, Douglas GC (1998) Morphological traits microsatellite fingerprinting and genetic relatedness of a stand of elite oaks (Q. robur L.) at Tuallynally, Ireland. Silvae Genet 473(176):5–6

Serra I, Strever A, Myburgh PA, Deloire A (2014) The interaction between rootstocks and Cultivars (Vitisvinifera L.) to enhance drought tolerance in grapevine. Aust J Grape Wine Res 20:1–14. https://doi.org/10.1111/ajgw.12054

Soltekin RO (2019) Bazı Sofralık Üzüm Çeşitlerinde Su Stresinin Omca Gelişimi, Verimi ve Üzüm Kalitesi Üzerine Etkileri. Dissertation, University of Ege

Soltekin O, Altındişli A, İşçi B (2021) İklim değişikliğinin Türkiye'de bağcılık üzerine etkileri. Ege Univ Ziraat Fak Derg 58(3):457–467. https://doi.org/10.20289/zfdergi.882893

Söylemezoğlu G, Güneş A, Çelik H, İnal A, Yaşa Z et al (2010) Amerikan asma anaçlarında bor ve tuz stresine tolerans mekanizmalarının stres ile ilgili fizyolojik parametreler ve antioksidan enzimler ile belirlenmesi (Unpublished). TUBİTAK Project number: 106 O 061. 211. Ankara

Tattersall EAR, Grimplet J, DeLuc L, Wheatley MD, Vincent D et al (2007) Transcript abundance profiles reveal larger and more complex responses of grapevine to chilling compared to osmotic and salinity stress. Funct Integr Genom 7(4):317–333. https://doi.org/10.1007/s10142-007-0051-x

This P, Jung A, Boccacci P, Borrego J, Botta R (2004) Development of a standard set of microsatellite reference alleles for identification of grape cultivars. Theor Appl Genet 109:1448–1458. https://doi.org/10.1007/s00122-004-1760-3

TÜİK (2022) https://data.tuik.gov.tr/Kategori/GetKategori?p=tarim-111&dil=1. Accessed 17 Mar 2022

Ueda A, Kathiresan A, Inada M, Narita M, Nakamura T et al (2004) Osmotic stress in barley regulates expression of a different set of genes than salt stress does. J Exp Bot 55(406):2213–2218. https://doi.org/10.1093/jxb/erh242

Uslu D, Samancı H, Demiray T, Gökçay E (1995) Melezleme yoluyla sofralık yeni üzüm çesitlerinin elde edilmesi. Bilimsel Arastırma ve İncelemeler Yayın No 56. Atatürk Bahçe Kültürleri Merkez Arastırma Enstitüsü, Yalova

Williams LE, Araujo FJ (2002) Correlations among Predawn Leaf, Midday Leaf, and Midday Stem Water Potential and their Correlations with other Measures of Soil and Plant Water Status in Vitis vinifera. J Am Soc Hortic Sci 127(3):448–454. https://doi.org/10.21273/JASHS.127.3.448

Xanthopoulos G (2004) Sap Pressure (Plant Water Potential) Measurement: Method. Euro-Mediterranean Wildland Fire Laboratory, a "wall-less" Laboratory for Wildland Fire Sciences and Technologiesin the Euro-Mediterranean Region, p 23

Xanthopoulos G, Maheras G, Gouma V, Gouvas M (2006) Is the Keetch-Byram drought index (KBDI) directly related to plant water stress? Ecol Manag 234(1):27. https://doi.org/10.1016/j.foreco.2006.08.043