Influence of leafroll on local grapevine cultivars in agroecological conditions of Central Anatolia region

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ABSTRACT: Leafroll is one of the most important virus diseases of vineyards of Central Anatolia region. Grapevine leafroll associated viruses GLRaV-1 and GLRaV-3 are the most frequently encountered viruses related with leafroll disease of grapes. The effect of leafroll disease on yield and fruit quality was studied. The disease caused in the average 31.22% decrease of berry weights and 28.72% reduction in total soluble solids, while titratable acidity was increased by 24.39%. The reaction of local cultivars to mixed infections of GLRaV-1 and GLRaV-3 was evaluated and Parmak, Kara dirmit, Kara gevrek and Gül üzümü cultivars were considered to be the most sensitive local cultivars to GLRaV-1 and GLRaV-3.

Keywords: Central Anatolia region; GLRaV-1 and GLRaV-3; influence; *Vitis vinifera* L.

Grapevine leafroll disease is considered to be the most economically important virus associated disease of grapevines**,** accounting for approximately 10–70% of the losses of grape production (Martelli 1986; Goheen 1988; Walker et al. 2004). Grapevine leafroll occurs wherever grapevines are grown and are associated with undesirable viticultural effects which include delayed ripening of fruits, reduced yield, altered fruit pigmentation, and reduced accumulation of sugar (Goheen 1988). Nine serologically distinct viruses from the family *Closteroviridae* were reported to be associated with leafroll disease and named grapevine leafroll associated virus GLRaV types 1–9 (Martelli et al. 2002; Alkowni et al. 2004; Peake et al. 2004). Many reports show that the most widespread viruses associated with leafroll disease worldwide are GLRaV-1 and GLRaV-3 (Walter, Martelli 1997; Stimili 2002; Gomez Talquenca et al. 2003; Gugerli 2003; Kominek et al. 2003; Santos et al. 2003; Martin et al. 2005; Rakhshandehroo et al. 2005).

GLRaV-1 and GLRaV-3 are also the most widespread viruses in Turkey (Yilmaz et al. 1997; Çiğşar et al. 2002; Akbaş et al. 2007). In this study we report the susceptibility of local cultivars to these viruses and their effect on quantity and quality of yield in the agro-ecological conditions of Central Anatolia region.

MATERIAL AND METHODS

Field inspection and serologic assays

The field study and collection of samples (clusters and 1 year old canes) were conducted during 2000–2003 in the commercial vineyards in the nine major grapevine growing areas. The symptoms of leafroll disease were evaluated on local cultivars in agro-ecological conditions of Central Anatolia region during the surveys. The cluster samples taken from leafroll infected vines were used in quality and quantity tests. Leafroll infection was determined by double antibody sandwich enzyme linked immunosorbent assay (DAS-ELISA) by using 4 different antisera of GLRaV-1, 2, 3 and 7. Leafroll types were not taken into consideration for infected cluster samples used in evaluation of disease impacts on quality and quantity. However, due to the fact that GLRaV-1 and GLRaV-3 were reported to be widespread leafroll viruses in the region (Akbaş et al. 2007), GLRaV-1 and GLRaV-3 infected cane samples were kept for utilization of determination of local cultivars susceptibility. Antisera were obtained from Agritest (Bari-Italy). These viruses were assayed using the manufacturer's protocol for DAS-ELISA. Positive and negative controls were included for each virus on the plate. Optical den-

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sity (OD) at 405 nm was read on an ELISA plate reader (TECAN-Sunrise). The sample was scored positive if both wells had an OD greater than triple the negative control.

Quality and quantity tests

The quality analysis consisted of total soluble solids and titratable acidity. A refractometer was used for total soluble solids measures and values were expressed as percent. Titratable acidity (g/l) was measured as tartaric acid by titration of grape juice with sodium hydroxide (0.1N) to pH 8.1 endpoint. The effect on quantity was evaluated as the weight of 100 berries (g). The berries were sampled from healthy and infected grapevines in the same row and vineyards.

All data were subjected to analysis of variance (ANOVA) by using SPSS procedures and the means were compared using Duncan's (1955) multiple range test (P = 0.05). Abbott's formula was used to determine % influence of leafroll among cultivars.

Evaluation of local cultivar susceptibility

Fifty scions, each of the local grapevine cultivars Emir, Gül üzümü, Kalecik karası, Kara dirmit, Kara gevrek and Parmak and some standard cultivars Ata sarısı, Ergin çekirdeksizi and Uslu, were collected from pathogen tested mother plants, in which GLRaV-1 and GLRaV-3 had not been previously detected serologically. The scions were bench-grafted with buds taken from vines infected with GLRaV-1 and GLRaV-3 to determine their susceptibility to leafroll disease. The inoculum used for bud grafting was taken from commercial vineyards, whose infection status is well known.

Grafted cuttings were individually planted in containers and placed in a bottom-heated (27°C) rooting bench filled with perlite. Two months later only the rootlings which survived scion buds were planted in the pots. The inoculated bud, if growing, was excised later in the season. The grafted vines were planted in a fumigated (sterile) soil and were grown at least 2 years under the greenhouse conditions. Several buds from each cane of the virus infected material were also rooted in pots.

Periodic observations began 15 months after grafting. For each plant, the number of leaves with leafroll symptoms was calculated over the 2-year period and symptom intensity was graded on a scale of one to four, where: 0 = plants without symptoms, 1 = plants with mild leafroll, 2 = plants with leafroll, 3 = plants with leafroll and leaf coloration, 4 = plants with severe leafroll and leaf coloration (Téliz et al. 1980).

RESULTS

Virus symptoms observed in vineyards and their confirmation by ELISA

During the period of the survey, leafroll symptoms were observed on the grapevines through-

Table 1. The data of the quality and quantity criteria of leafroll infected cultivars

Cultivars	Berry weight (g)		Total soluble solids (%)		Acidity (g/l)	
	infected	healthy	infected	healthy	infected	healthy
Burdur dirmiti	113.8 ± 2.71^{CDb}	263.6 ± 3.58^{Aa}	16.3 ± 1.21^{Ab}	19.3 ± 1.31^{Ba}	8.2 ± 0.52^{BCa}	5.5 ± 0.54^{Bb}
Ekşi kara	102.6 ± 7.35^{Db}	164.9 ± 2.90^{CDa}	11.8 ± 1.15^{Bb}	17.8 ± 0.51^{Ba}	9.3 ± 0.62^{ABa}	8.0 ± 0.52^{Ab}
Gemre	117.0 ± 3.33^{CDa}	155.2 ± 1.02^{Da}	13.3 ± 0.72^{ABb}	17.9 ± 0.81^{Ba}	8.4 ± 0.61^{BCa}	6.4 ± 0.09^{ABb}
Göküzüm	129.7 ± 2.85^{BCDa}	173.0 ± 3.51^{CDa}	13.2 ± 0.35^{Bb}	18.4 ± 0.88^{Ba}	8.85 ± 0.6^{ABCa}	7.6 ± 0.04^{Aa}
Kara dirmit	115.0 ± 1.98^{CDb}	192.4 ± 2.60^{BCDa}	14.5 ± 0.58^{ABb}	18.8 ± 0.88^{Ba}	10.5 ± 0.79^{Aa}	6.5 ± 0.4^{ABb}
Kara gevrek	164.6 ± 1.90^{ABCb}	216.0 ± 4.65^{ABCa}	12.78 ± 0.74^{Bb}	19.7 ± 0.40^{ABa}	8.1 ± 0.84^{BCa}	6.1 ± 0.59^{ABb}
Yumru karası	188.7 ± 1.29^{Aa}	217.6 ± 5.11^{ABCa}	8.3 ± 4.11^{Cb}	16.7 ± 0.22^{Ba}	7.5 ± 0.37^{BCa}	6.6 ± 0.58^{ABa}
Oküz gözü	169.6 ± 1.59^{ABb}	233.3 ± 2.08^{ABa}	11.9 ± 2.63^{Bb}	22.3 ± 2.25^{Aa}	7.2 ± 0.73^{Ca}	4.6 ± 0.00^{Bb}

Means in a column followed by different capital letters or in a row by different small letters are significantly different (Duncan's test, α = 0.05)

out each province. The symptoms observed were typical for leafroll and included reddening of the leaves between major veins in red cultivars, yellowing of the leaves between major veins in white cultivars and downward rolling of the leaves in all cultivars (Fig. 1). No symptoms were observed on the grapevines in spring and summer, prior to harvest (the period May to August). However, first symptoms were generally seen at the end of the vegetation period in autumn (during mid August to the end of October), especially on red cultivars such as Kara dirmit, Kara gevrek, Gemre, Burdur dirmiti and white cultivars such as Emir and Parmak. These observations were verified serologically.

Quality and quantity tests

A summary of the quality and quantity test results and the results of variance analysis were shown in Table 1. Grape cultivars, which were tested positive for leafroll had significantly higher titratable acidity and lower weight in berries and total soluble solids than virus-free vines (Table 1).

Berry weight was found to be by 17.36–56.06% lower in leafroll infected cultivars when compared to uninfected cultivars. Total soluble solids were reduced by 17.87–44.88% in leafroll infected cultivars when compared to uninfected cultivars. Titratable acidity increased by 12.91–35.50% in leafroll infected cultivars when compared with uninfected cul-

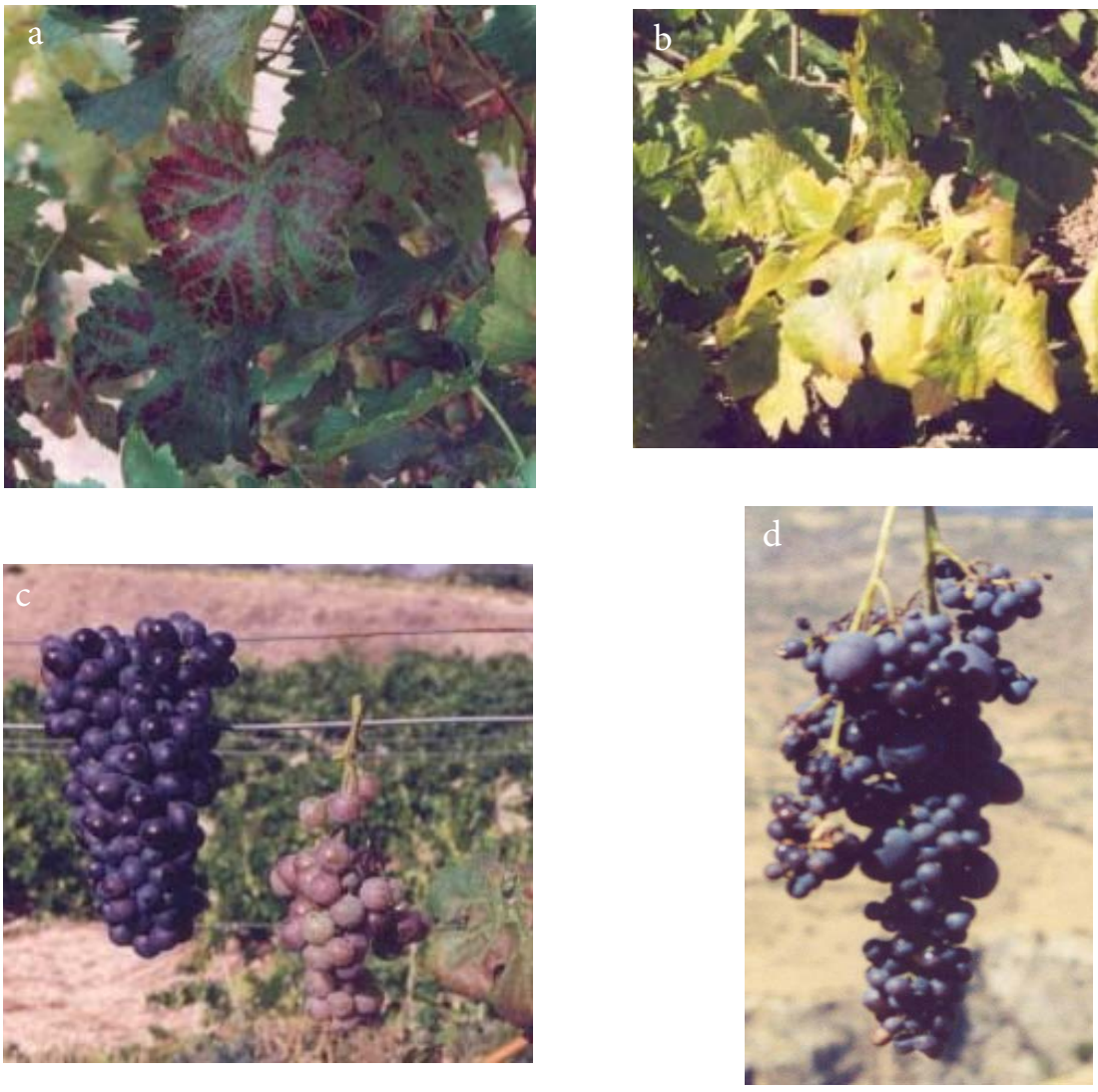

Fig. 1. Symptoms of leafroll disease on (a) leaves of Kara dirmit cultivar, (b) leaves of Parmak cultivar, (c) cluster of Kara dirmit cultivar and (d) cluster of Ekşi kara cultivar

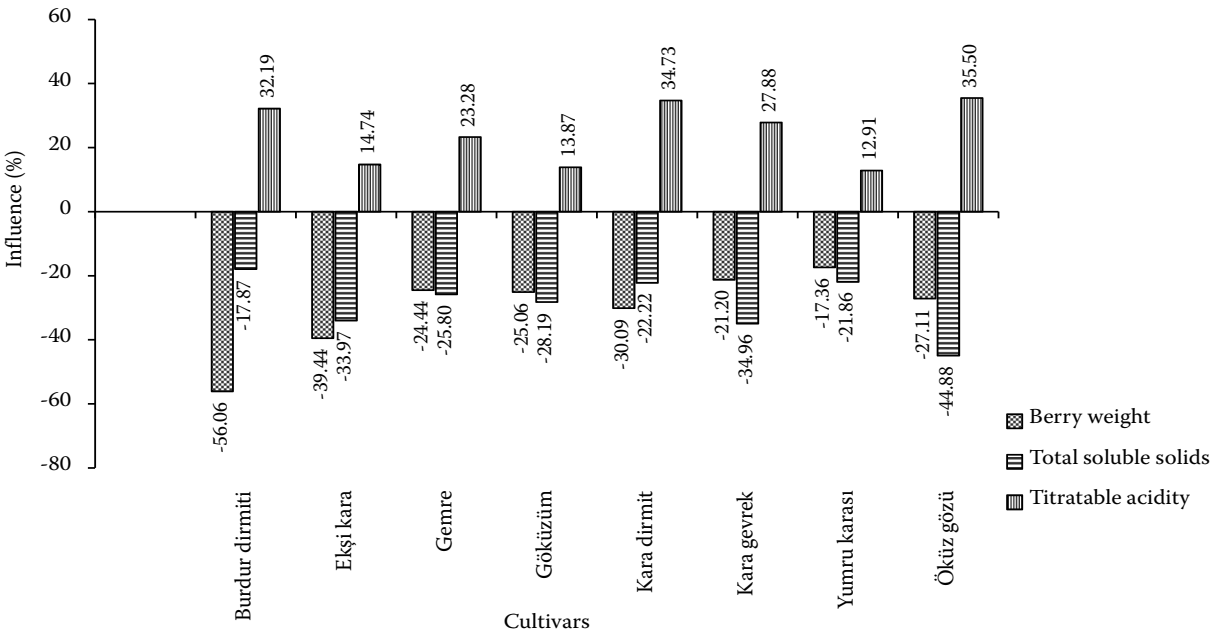

Fig. 2. The percentage of influence (according to Abbott's formula) on berry weight, total soluble solids and titratable acidity in infected and healthy vine cultivars

tivars. Clusters of infected vines displayed changing of the berry color from typical ripening color to none ripening or over ripening color. In white cultivars, dark green or dark yellow and in red cultivars very dark or pale red berry colors were accepted as abnormal. In addition to color alteration, it was observed that infected vines carried clusters consisting of berries with different ripening colors.

Influence (%) of leafroll infection on different cultivars is also calculated according to Abbott's formula and given in Table 2. A statistical analysis was performed on the data. The result of variance analysis is also showed in Table 2. Influence of leafroll on berry weight, total soluble solids and titratable acidity was found to be significant (Fig. 2).

According to the three years of data, leafroll caused the mean of berry weight to decrease the most in Burdur dirmiti and the least in Yumru karası, the mean of total soluble solids decreased the most in Oküz gözü and the least in Burdur dir-

Table 2. The percentage of influence according Abbott's formula of leafrool disease on berry weight, total soluble solids and titratable acidity

Cultivars	Influence (%)		
	berry weight	total soluble solids	acidity
Burdur dirmiti	56.06 ± 5.65^{a}	17.87 ± 7.41^{b}	32.19 ± 7.08^{a}
Ekşi kara	39.44 ± 13.96^{ab}	33.97 ± 5.18^{ab}	14.74 ± 1.03^{a}
Gemre	24.44 ± 9.09^{b}	25.80 ± 2.75^{ab}	23.28 ± 6.37^{a}
Göküzüm	25.06 ± 0.50^{b}	28.19 ± 5.33^{ab}	13.87 ± 5.39^{a}
Kara dirmit	39.09 ± 4.09^{ab}	22.22 ± 2.92^{ab}	34.73 ± 5.70^{a}
Kara gevrek	21.20 ± 6.89^{b}	34.96 ± 3.92^{ab}	27.88 ± 3.70^{a}
Yumru karası	17.36 ± 5.21^{b}	21.86 ± 4.31^{ab}	12.91 ± 6.28^{a}
Oküz gözü	27.11 ± 5.32^{b}	44.88 ± 17.38^{a}	35.50 ± 6.51^{a}
Average	31.22	28.72	24.39

Means in a column followed by different small letters are significantly different (Duncan's test, $\alpha = 0.05$)

Fig. 3. Symptoms of GLRaV-1 and 3 on Kara gevrek cultivar (a), Kara dirmit cultivar (b) and Parmak cultivar (c) 24 months after grafting

miti compared to the mean of weight and total soluble solids of healthy local cultivars in the region. On the other hand, titratable acidity increased the most in Oküz gözü and the least in Yumru karası (Table 2).

The susceptibility of local cultivars

The reaction of different cultivars to GLRaV-1 and GLRaV-3 was summarized in Table 3. The first symptoms associated with leafroll disease (GLRaV-1 and GLRaV-3) were observed on Parmak cultivar 19 months after grafting. These first symptoms on Parmak cultivar were mild leafroll of several leaves. Mild leafroll symptoms were also observed on Kara dirmit, Kara gevrek and Gül üzümü cultivars 20 months after grafting. In addition to these cultivars, after 22 months, mild leafroll symptoms occurred on several leaves of Ata sarısı, Emir and Kalecik karası. Meanwhile, leafroll symptoms began to increase on Parmak and Kara gevrek cultivars. Besides leafroll symptoms, color change began to occur on Parmak cultivar 22 months after grafting. In fact, during the observation it was noted that when the number of leaves with symptoms was low, the intensity of leaf reddening was also low. Twenty four months after grafting, susceptibility of Gül üzümü, Kara dirmit, Kara gevrek and Parmak cultivars were found to be high under greenhouse conditions of Central Anatolia region (Fig. 3). Moreover, Emir cultivar was also found to be susceptible, relatively. In contrast to these cultivars, Ata sarısı, Ergin çekirdeksizi and Uslu cultivars were found to be tolerant to disease. Cultivar Kalecik karası was also found to be mild tolerant to the disease, comparatively. These observations were confirmed by DAS-ELISA. Test results showed that virus con-

Table 3. The length of time after grafting when symptom expression was observed and the degree of severity

Cultivars	First symptom occurring time (months) and its severity					
	19th	20th	21st	22nd	23rd	24th
Ata sarısı	0	0	0	0	1	1
Emir	0	0	0	0	1	3
Ergin çekirdeksizi	0	0	0	0	0	1
Gül üzümü	0	1	1	1	2	4
Kalecik karası	0	0	0	0	1	3
Kara dirmit	0	1	1	1	2	4
Kara gevrek	0	1	1	2	3	4
Parmak	1	2	2	3	3	4
Uslu	0	0	0	0	1	1

0 – plants without symptoms, 1 – plants with mild leafroll, 2 – plants with leafroll, 3 – plants with leafroll and leaf coloration, 4 – plants with severe leafroll and leaf coloration

centration in susceptible cultivars were higher than tolerant ones. In other words, virus concentration decreased from susceptible to tolerant cultivars.

DISCUSSION

In this study, leafroll disease symptoms were not observed on local grape cultivars in spring during the surveys. However, in autumn, preliminary symptoms were observed on some red cultivars such as Kara dirmit, Kara gevrek, Gemre, Burdur dirmiti and some white cultivars such as Emir and Parmak. This observation is in partial agreement with FAO report of Turkey concerning grapevine viruses (Martelli 1987). He stated that no leafroll disease symptoms were observed during spring in any of the cultivars grown in the areas.

Another important observation is that as leafroll symptoms were observed on foreign vine cultivars, they were not observed on the local vine cultivars at the mid of vegetation period and before pre-harvest period in the region. At these periods, though some local varieties showed mild symptoms, virus titration of these cultivars was found to be lower than the foreign origin vines. From this data, the conclusion can be reached that our local cultivars are not so much sensitive to leafroll compared with foreign cultivars such as Alicanthe Bouchet, Alphonse Lavallée, Pinot Noir, Semillon etc. in our agro-ecological conditions. Nevertheless, Gugerli (2003) notified that symptoms are not easily interpreted in general and are unsatisfactory for the determination of the sanitary status of grapevine. However, Martelli (1986) stated that only sensitive cultivars show leafroll symptoms in the early season.

Emir, Parmak (white cultivars), Gemre, Gül üzümü, Kara dirmit, Burdur dirmiti and Kara gevrek (red cultivars) were found to be the most fairly affected cultivars by the disease based on visual inspection and serological assays in Central Anatolia region (Akbaş et al. 2007). The clearest symptoms were also observed on these cultivars in the end of period. Burdur dirmiti, Gemre, Gül üzümü, Parmak, Kara dirmit and Kara gevrek were considered to be the most susceptible local cultivars. Mild symptoms were seen on Ak dirmit, Hesap ali, Kalecik karası and Nazlı cultivars. Virus titration was also found to be low in these cultivars. These results suggest that mild symptoms in these cultivars cannot be attributed to the low virulence of the viral strains, but probably to host tolerance.

Grapes tested as positive for leafroll had significantly lower berry weight and total soluble solids than uninfected grapevines, and were higher in acids than healthy vines in the mean of local cultivars in the region, compared to healthy vines. These data are comparable with data reported by Kovacs et al. (2001), who demonstrated that GLRaV-3 induced 5% reduction of berry weight and 5% and 9% increase in titratable acidity in Vidal blanc and St. Vincent cultivar, respectively. The reduction between 20–51% in yield due to leafroll infection was also reported in various cultivars (Simon et al. 2003; Walker et al. 2004; Tomazic et al. 2005).

In cultivar susceptibility assays, indexing onto the local cultivars gave valuable outcomes. Two years after inoculation Parmak, Kara gevrek, Kara dirmit, Gül üzümü and Emir cultivars were decided to be the most susceptible local cultivars because they expressed symptoms earlier than other local cultivars and they were more severely affected. However Ergin çekirdeksizi, Uslu and Ata sarısı cultivars showed very mild symptoms. Therefore, these cultivars were considered to be tolerant to the disease. In addition, Kalecik karası was also thought to be mild tolerant to virus in agroecological conditions of the Central Anatolia region by verification with serological tests. Likewise, it was reported in some studies that cultivar reactions are not only related to the cultivar, but also to agro-ecological conditions (Egger et al. 1985; Gomez Talquenca et al. 2003). Egger et al. (1985) also stated that symptom development is often complicated by the presence of various biological and abiological stress factors. In addition, the reaction of tested cultivars was similar to those field symptoms.

This study conclusively demonstrates a considerable decrease of quality and quantity and describes different reaction on local grapes in Central Anatolia agro-ecological conditions in Turkey.

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Vliv svinutky révy vinné na místní kultivary révy v agroekologických podmínkách Střední Anatolie

ABSTRAKT: Svinutka je jednou z nejvýznamnějších virových chorob na vinicích Střední Anatolie. Viry svinutky révy vinné GLRaV-1 a GLRaV-3 jsou nejčastějšími viry spojenými s touto chorobou vinných hroznů. Byl studován vliv svinutky na výnos a kvalitu plodů. Choroba způsobila v průměru 31,22 % pokles váhy bobulí a 28,72 % snížení celkové rozpustné sušiny, zatímco titrační kyselost se zvedla o 24,39 %. Byly vyhodnoceny reakce místních kultivarů na kombinaci dvou infekcí (GLRaV-1 a GLRaV-3) a Parmak, Kara dirmit, Kara gevrek a Gül üzümü byly posouzeny jako lokální kultivary s nejvyšší citlivostí na GLRaV-1 a GLRaV-3.

Keywords: Střední Anatolie; GLRaV-1 a GLRaV-3; vliv; *Vitis vinifera* L.

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