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Survey for *Grapevine leafroll viruses (GLRaVs)* in Malatya region

Malatya bölgesi bağlarında Asma yaprak kıvrılma virüsleri (GLRaVs)'nin sürveyi

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ABSTRACT

Grapevine (*Vitis vinifera* L.) is one of the most important fruit crops in Turkey and viral diseases are one of the main problems of grapevine production. They decrease yield quantity and quality of crops as well as cause death of the plants in an interaction with other agents. Leafroll disease of grapevine, caused by a complex of viruses, is considered a serious threat to production across many grapevine growing regions in Turkey. This study was conducted to determine the current status of *Grapevine leafroll-associated viruses* (GLRaVs) in Malatya province. Grapevine plantations in Malatya province were surveyed for the presence of *Grapevine leafroll-associated viruses* (GLRaVs) -1, -2, -3, -4 and -6. Purposive sampling was done in vineyards of Arapgir, Darende and Battalgazi districts, which are the primary grapevine producing areas of Malatya during 2016-2017 growing seasons. A total of 241 samples were collected and tested by DAS-ELISA. Samples giving two fold of absorbance values (405 nm) of negative control were evaluated as positive. According to the results, 131 samples were found to be infected with GLRaV-1, whereas 48 samples with GLRaV-3 and -4 samples with GLRaV-4. None of the samples were reacted as positive against GLRaV-2 and GLRaV-6. Forty five samples were found to be infected with mix infection of GLRaV-1 and -3, and three samples were found to be infected with GLRaV-1 and -4 strain 6.

INTRODUCTION

There are approximately 41.047 acres of grapevine (*Vitis vinifera* L.) production areas in Malatya province, mainly in Darende, Arapgir and Battalgazi districts among 4.169.068 acres of grapevine production areas in Turkey (Anonymous 2018). Grape production is increasing in value in Malatya. Grapevine leafroll disease (GLD) is one of the most important diseases of grapevine with economic importance worldwide. *Grapevine leafroll-*

associated virus-1 (GLRaV-1) is the first identified virus related to leafroll viruses which are belong to *Closterovirus* and *Ampelovirus* genera. Martelli et al. (2012) reviewed that GLRaV-1, GLRaV-3, GLRaV-4, GLRaV-5, GLRaV-6, GLRaV-8, GLRaV-9, GLRaV-Pr and GLRaV-Car belong to *Ampelovirus* genus, whereas GLRaV-2 belongs to *Closterovirus* genus and GLRaV-7 is unassigned in the family. Leafroll symptoms are typical for these viruses but

vary according to the cultivar, the infecting viruses and their combinations (Krake 1993).

Leafroll disease is known to be occurring in Europe for a long time (Martelli and Boudon-Padieu 2006). One of the most widespread viruses is *Grapevine leafroll-associated virus-3* (GLRaV-3) among at least nine viruses associated with grapevine leafroll disease (Akbas et al. 2007, Almeida et al. 2013, Martin et al. 2005, Naidu et al. 2014, Pietersen 2006). Affecting quality and quantity of the yield of grapevine (Atallah et al. 2012, Cabaleiro et al. 1999), the disease has been reported from all major grapevine growing areas worldwide (Martelli 1986).

To date, there is only one research on grapevine viruses in Malatya, reporting no viral infection of grapevines. However, in recent years the growers have been complaining the virus-like symptomatology on local grapevine varieties. Despite its wide cultivation in Malatya province, little attention has been paid to sanitary status of vineyards. The lack of information on the sanitary status of grapevines of Malatya necessitates determining the causes of virus-like symptoms in Malatya's vineyards. The present study was conducted to identify GLRaV-1, -2, -3, -4, and -4 strain 6 and their incidences in vineyards using DAS-ELISA method.

MATERIALS AND METHODS

Surveys and sample collection

To perform a successful virus screening of grapevine viruses, the cane samples were collected in a sampling plan consisting of two subsequent growing seasons. Visual observations were carried out in the fields concerning the symptoms of GLRaV infections on grapevine. Fields and villages were selected at random manner, and then the grapevine cane cuttings were collected randomly from Battalgazi, Arapgir and Darende districts of Malatya province. Canes from symptomatic grapes were sampled from September through November in 2016 and 2017. Before storing the samples into in a freezer, the leaf symptoms of symptomatic plants were recorded.

Detection of GLRaVs by DAS-ELISA

DAS-ELISA test was applied as described by Clark and Adams (1977) for detecting GLRaV-1, GLRaV-2, GLRaV-3, GLRaV-4 strain 6, and GLRaV-6 and the testing was done following the manufacturers' instructions of the antisera producer (Bioreba AG, Reinach, Switzerland). For each of the viruses tested, the test was carried out with duplicate wells per sample. Positive and negative controls were included in each test. Canes scrapings were homogenized in

grinding buffer containing 0.1 M carbonate (pH 9.6), 0.2% nonfat milk powder, 2% polyvinylpyrrolidone (PVP) and 0.05% Tween 20 in extraction bags at 1:10 (w/v) dilution. Using an ELISA reader, the absorbance at 405 nm of each well was measured to judge positive reaction if the mean absorbance value was greater than twice from the negative control. If necessary, the weak and questionable reactions were verified by second run ELISA test.

RESULTS

Field surveys

Typical GLRaV symptoms were observed frequently and identified with reasonable confidence in the majority of the surveyed vineyards of Malatya. The field symptoms were best observed late in the autumn. In general, GLRaV infected plants were exhibited distorted red and mottled leaves (Figure 1), poor fruit setting with irregular ripening. They were also less vigorous than normal vines. Leafroll symptoms were more frequently observed in the surveyed red-berried grapevine varieties.

DAS-ELISA tests

Of the 241 grapevine samples tested by DAS-ELISA against GLRaV viruses, 183 (75.9%) proved to be infected by at least one or multiple viruses. The present survey demonstrated the high levels of infections of some GLRaVs that were previously unreported in Malatya. Viruses detected by DAS-ELISA and their prevalence in grapevine samples of commercially grown vineyards are shown in Table 1. Based on our results, 131 grapevine samples were found to be infected with GLRaV-1, 48 samples with GLRaV-3 and 4 samples with GLRaV-4 strain 6. None of the samples were reacted as positive against GLRaV-2 and GLRaV-4 strain 6. GLRaV-1 was the most prevalent virus with the 54.35% infection rate and followed by GLRaV-3 and GLRaV-4 respectively with the 19.91% and 1.65%. Forty five samples were found to be infected with mix infection of GLRaV-1 and -3, and three samples were found to be infected with GLRaV-1 and -4 strain 6. Two years successive survey yielded close infection rates of vineyards (Table 1).

DISCUSSION

The present survey revealed for the first time the occurrence of GLRaV-1, -3 and -4 strain 6 on grapevines of Malatya province. The high level of infections indicates the probable use of infected materials as primary source in producing propagation material. In grapevine producing areas, GLRaVs are proved to be introduced mostly by propagation of infected planting material and exchanging of infected sources. The insect vectors and vegetative

Table 1. Viruses detected by DAS-ELISA in the investigated grapevine samples of Malatya Province

Samples (2016)									
Districts	Tested		Viruses/incidence (%)						
	No	Infected %	GLRaV-1	GLRaV-2	GLRaV-3	GLRaV-4 strain 6	GLRaV-6	GLRaV-1+3	GLRaV-1+4 strain 6
Arapgir	71	100	42/59.15	-	27/38.02	2/2.8	-	27/38.02	1/1.40
Battalgazi	39	82.05	32/82.05	-	-	-	-	-	-
Darende	11	0	-	-	-	-	-	-	-
Samples (2017)									
Districts	Tested		Viruses/incidence (%)						
	No	Infected %	GLRaV-1	GLRaV-2	GLRaV-3	GLRaV-4 strain 6	GLRaV-6	GLRaV-1+3	GLRaV-1+4 strain 6
Arapgir	56	100	33/58.92	-	21/37.5	2/3.57	-	18/32.14	2/3.57
Battalgazi	42	57.14	24/57.14	-	-	-	-	-	-
Darende	22	-	-	-	-	-	-	-	-
TOTAL	241	183/75.9	131/54.35		48/19.91	4/1.65		45/18.67	3/1.24

propagation have been considered the main reason of the subsequent local spread (Cabaleiro and Segura 2006, Martelli and Boudon-Padieu 2006, Sharma et al. 2011, Tsai et al. 2012). However, the growers in Malatya province are not aware of precautions to control and manage the virus dissemination. In surveyed areas, GLRaV-1 was most frequently encountered virus associated with leafroll disease of grapes. Mixed field infections of GLRaV-1 and -3 were also frequent. All of the samples showing reddening and rolling of red varieties were detected as positive in DAS-ELISA tests (Figure 1).



Figure 1. Typical Grapevine leafroll symptoms on local red cultivars of *Vitis vinifera*

GLRaV symptoms were observed in most of the surveyed vineyard areas. Typical symptoms of GLD were more frequent in autumn. Our observations are in agreement with FAO report of Turkey concerning grapevine virus diseases. In report, it has been stated that the GLD symptoms are not visible during spring in any of the cultivars grown in the areas surveyed in Turkey (Martelli 1987). Because of significant crop losses, grapevine leafroll disease has an important impact on grape-growing regions worldwide. Among GLRaVs, GLRaV-3 is the most noticeable and widely distributed in different regions of the world, including Europe, South and North America, Middle East, Northern and Southern Africa, Asia, and Oceania (Cabaleiro and Segura 2006, Charles et al. 2006, Martin et al. 2005, Pietersen 2006, Pio Ribeiro et al. 2004, Sharma et al. 2011). In contrast, GLRaV-1 was the most common virus in our study.

Despite that ELISA is not as sensitive as nucleic acid-based techniques; its scalability makes it popular for routine testing by industry for the detection of GLD associated viruses in grapevines used for propagation (O'Donnell 1999, Ward et al. 2004). Because that the first antiserum was

produced against Closterovirus-like particles (Gugerli et al. 1984) several groups have produced their own polyclonal antisera or monoclonal antibodies to develop ELISAs to detect GLRaV's, especially GLRaV-3 (Goszczyński et al. 1995, Gugerli et al. 1990, Ling et al. 2000, 2001, Teliz 1987, Zee et al. 1987). In a previous study carried out by Çiftçi et al. (2015) a total of 42 grapevine samples were tested against GLRaVs. However, none of the samples was reacted as positive.

Akbas et al. (2007) collected samples from nine major grapevine-growing areas in Central Anatolia. 622 samples were collected from vines and were tested for Grapevine leafroll-associated virus 1, 2, 3 and 7 (GLRaV-1, -2, -3 and -7). They found out that 27 of 41 cultivars and 95 of 622 samples (15.27%) were found to be infected at least one virus and GLRaV-1 (8.36%) was found to be the most frequently encountered virus associated with leafroll disease of grapes, followed by GLRaV-3 (5.78%), GLRAV-7 (3.86%) and GLRAV-2 (2.41%). Akbas et al. (2009) reported that GLD is one of the most important virus diseases of vineyards of Central Anatolia region. In this research, GLRaV-1 and GLRaV-3 were the most frequently encountered viruses related with leafroll disease of grapes at Central Anatolia. Çağlayan (1997), conducted a survey for the presence of GLRaVs, *Grapevine virus A* (GVA) and *Tomato black ring virus* (TBRV) in the grapevine growing districts of Hatay province reporting the presence of TBRV, GLRV I and III and GVA. Erilmez and Kaya (2016) conducted a study to compare the sensibility of different methods (DAS-ELISA and RT-PCR) which are used to detect the agents of viral diseases in grapevine. PCR analysis confirmed DAS-ELISA results.

Prior to this study, no data was available reporting GLRaV infections and their incidence in vineyards of Malatya. Our survey has demonstrated the presence of three viruses, i.e. GLRaV-1, -3 and -4. Although GLRaV-4 strain 6 was less encountered in surveyed areas, it is not less important than the detected other viruses. To reduce the incidence and spread of GLRaVs in Malatya and other grapevine growing areas of Turkey, the implementation of a certification program is prerequisite to ensure the production and delivery of sanitarily improved grapevine propagative materials.

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ÖZET

Asma (*Vitis vinifera* L.) ülkemizde yetiştiriciliği yapılan en önemli meyvelerden birisi olup üretimini sınırlayan faktörlerin başında virüs hastalıkları gelmektedir. Virüs hastalıkları üzüm üretimini kalite ve kantite bakımından azaltmanın yanı sıra diğer hastalık etmenleri ile beraber bulunmaları durumunda asmanın kurumasına kadar ulaşabilen ciddi sonuçlar doğurabilmektedir. Bir virüs kompleksinin neden olduğu Asma yaprak kıvrılma hastalığı ülkemiz asma yetiştiriciliği üzerinde önemli bir tehdittir. Bu çalışma, Malatya ilinde yetiştirilen asmaları etkileyen Asma yaprak kıvrılma hastalığı ile ilişkili virüsler (*Grapevine leafroll-associated virus*, GLRaV)'ini tespit etmek amacı ile yürütülmüştür. Bu amaçla Malatya ilindeki bağlar gezilerek etmenin GLRaV-1, -2, -3, -4 ve -6 virüsleri araştırılmıştır. 2016-2017 yıllarında Arapgir, Darende ve Battalgazi ilçelerini kapsayan survey çalışmalarında güdümlü örneklemeler yapılarak asma numuneleri toplanmıştır. Toplanan 241 örneğin tamamı DAS-ELISA metodu ile testlenmiştir. Testlerde negatif örneğin iki katı ve yukarısında absorbans (405 nm) değeri veren numuneler enfekteli kabul edilmiştir. Yürütülen test sonuçlarına göre toplam 131 numunenin GLRaV-1 ile 48 numunenin GLRaV-3 ile ve -4 örneğin ise GLRaV 4 ile enfekteli olduğu tespit edilmiştir. Örneklerin hiçbirinde GLRaV-2 ve GLRaV-6 enfeksiyonuna rastlanmamıştır. Testlenen 45 numunenin GLRaV-1 ve -3 ile, üç numunenin ise GLRaV-1 ve -4 ile karışık enfeksiyon halinde bulunduğu tespit edilmiştir.

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