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THE STUDY OF VIRAL DISEASES IN GEORGIAN VINE GRAFTED NURSERIES

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ABSTRACT

Grapevine infections are one of the most viral diseases affecting grapevines throughout the world. The impact on vine health, crop yield and quality is difficult to assess due to a high number of variables, but significant economic losses are consistently reported over the lifespan of a vineyard if intervention strategies are not implemented. Georgia is one of the oldest wine producing regions. The main goal of the research was to study the spreading of grapevine viral diseases for further selection of virus-free material in Georgia. Survey for the detection of viral agents was conducted from grapevine varieties: Rkatsiteli, Rkatsiteli Pink and Qisi located in vineyards grafted nurseries in the east part of Georgia region of Mtskheta in Jighaura and Mukhrani. Grapevine cultivars were tested for 6 types of viruses: Grapevine Fanleaf Virus(GFLV), Grapevine Fleck Virus (GFkV), Grapevine Leaf Roll Virus-1 (GLRaV-1), Grapevine Leaf Roll Virus-2 (GLRaV-2), Grapevine Leaf Roll Virus-3 (GLRaV-3) and Grapevine Virus A (GVA) by means of Triple and Double Antibody Sandwich-Enzyme linked Immunosorbent Assay (DAS-ELISA, TAS –ELISA) using monoclonal and polyclonal antibodies. The results showed that different types of viral infections were accumulated in 36.09% of tested samples. Among viral pathogens tested GLRV-2 (0.50%), GLRaV-3 (1.21%) and GVA (3.66%) were in minority. GLRaV-1, GFLV and GFkV virus presence, respectively: 8.04%, 12.68% and 19.26% in collected samples was relatively high comparing to others. It should be noted that in the same samples were found mixed infections (2 or 3 types of viruses), particularly 9.8% of patterns were containing GFLV and GFkV viruses. Regular sampling and testing of all vines has provided virus free propagation material in Jighaura and Mukhrani grafted nurseries in Georgia.

INTRODUCTION

Viral infections are distributed in all regions of the world, more than 60 viruses and viroid's have been reported to infect grapevine (Martelli *et al*., 2009) and number of new virus-like sequences and novel viruses increase (Nakaune *et al*., 2008; Sabanadzovic *et al*., 2011),. Grapevine is a highly valuable agricultural commodity that is host to the largest number of viruses of any crop plant (Martelli, 1998–2004). Plant viruses are mostly spread via vegetative propagation, although insect and nematode vectors are responsible for the spread of some grapevine viruses. Grapevine viruses can be unrecognized causes of low yields and poor plant growth as well as grapevine decline. Viral diseases are the most dangerous grapevine infection affecting wine, juice, and table grape cultivars, as well as rootstocks (Naidu, 2008).

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The grapevine response to infection depends on several factors such as cultivar, vegetative season, environmental conditions and viral isolate (Martelli, 2009). Vine showing decline symptoms supported a mixed infection included some different genomes (Rwahnih *et al*., 2009) Viral infection is rapidly growing, Crop losses caused by viral infections vary from moderate to high, affecting more than 80% of the crop depending on the virulence of the virus isolate, the susceptibility of the grapevine variety, and environmental factors (Bovey *et al*., 1990).

Two methods to propagate virus free vegetativly plants are meristem tip culture and thermotherapy. Those methodologies enable us to produce healthy plants from a single individual in a short period of time regardless of location or season of the year. It was discovered that Chemotherapy inhibits viral replication and induces plant resistance, but ant metabolites are capable of blocking the virus nucleic acid synthesis (Fazio *et al*., 1987).

AIM AND OBJECTIVE

Grapevine viral disease has a significant impact on grape-growing regions worldwide, resulting in significant losses. Among all viruses associated with GFkV, GFLV, GLRaV-3, GLRaV-1 is the most noticeable and widely distributed in different regions of the world, Evidently, this virus has been introduced in most grape growing regions by exchange and propagation of infected plant material and subsequent local spread by vegetative propagation and insect vectors (Cabaleiro *et al*., 2013).

Grapevine is one of the most important cultivated fruit species in Georgia. There were several reasons to undertake a survey of viruses spreading in grapevine: Grapevine plantations are widespread in all regions of Georgia but distribution of vine viral diseases is unknown. Several viral symptoms such are: early redness, leaf roll, less vigorous growth, streaks of leaves or flowers and fleck of leaf, necrosis were found in vineyards of Georgia. It seemed that many plants were internally infected with different types of viruses. Grapevine viral infection was not examined and respectively there was not any regulations of viral diseases in vineyards of Georgia. Center of planting materials production was founded in Georgia in 2008, it is known that screening and monitoring viral disease in new established grafted nurseries is very significant. Viruses screening is basic of viral diseases management which allow us to produce healthy and certified planting materials (Fuller *et al*., 2016). The present investigation was carried out to study the spreading of grapevine viral diseases for further selection of virus-free materials in Georgia.

Grapevine cultivars (Rkatsiteli, Rkatsiteli Pink and Qisi) from east part of Georgia region of Mtskheta in Jighaura and Mukhrani grafted nurseries were tested for 6 types of viruses: Grapevine Fan leaf Virus (GFLV), Grapevine Fleck Virus (GFkV), Grapevine Leaf Roll Virus-1 (GLRaV-1), Grapevine Leaf Roll Virus-2, (GLRaV-2), Grapevine Leaf Roll Virus-3 (GLRaV-3) and Grapevine Virus A (GVA).

MATERIALS AND METHODS

In summer and early autumn of 2012-2014 years 410 collected grapevine samples were tested. Samples were collected from the grapevines planted in the east part of Georgia region of Mtskheta in Jighaura and Mukhrani respectively. During the same season adult leaves were collected from 130 grapevines of Rkatsiteli pink, 120 grapevine of Qisi and 160-Rkatsiteli.
The GLRaV-1, GLRaV-2, GLRaV-3, GVA and GFLV infections were confirmed by Double Antibody Sandwich (DAS)-ELISA using commercial Kits (BIOREBA AG, Switzerland) and The GLRaV-2, GFLV infection by Triple Antibody Sandwich Enzyme Linked Immuno Sorbent Assay (TAS)-ELISA using commercial Kits (SEDIAG S.A.S, 3, Bd de Beauregard -21600 Longvic-France) according to the manufacturer's Instructions: leaves were crushed ((w/v) 1:5)in extraction buffer (pH 8.2) containing 2% polyvinylpyrrolidone (PVP MW 24,000) 0.02% NaN_3 and 0.05% Tween 20. Absorbance was determined at 405/450 nm on ELX800 Microplate Reader (Bio-Tek Instruments, Winooski, VT) and Samples with absorbance values greater than or equal to three times of the average of the kit negative control samples were considered infected.

RESULTS

This research was focused on observation spreading of viral diseases in three types Grapevine cultivars: Rkatsiteli, Rkatsiteli Pink and Qisi from Jighaura and Mukhrani grafted nurseries. (See Figure 1.)

Fig. 1. Part of region Mtskheta in Jighaura and Mukhrani Grafted Nurseries grafted nurseries in Georgia (a,b)

Grapevine varieties were tested for 6 types of viruses: GLRaV-1, GLRaV-2, GLRaV-3, GVA, GFkV and GFLV The result showed that viral diseases characterized several spreading depending on grapevine cultivars. Visual survey some of collected Rkatsiteli samples showed viral symptoms (See Fig. 2)

Fig. 2. Grapevine Cultivars Rkatsiteli Associated with Grapevine Fan Leaf Virus Symptoms(a,b)

According the ELISA result of Rkatsiteli sample contained of GFLV, GFkV and GLRaV-1 viruses, with respective percentage of: 20%, 15% and 7.5%, whereas the presence of GLRaV-2, GLRaV-3 and GVA was not detected. (See Table I)

Table 1. Result of Viruses Testing in Grapevine (Rkatsitheli)

Grapevine	Virus Varieties	Sample Tested	Positive Sample	
Rkatsitheli			Number	%
	GFLV	160	32	20
	GFkV	160	24	15
	GLRaV-1	160	12	7.5
	GLRaV-2	160	-	-
	GLRaV-3	160	-	-
	GVA	160	-	-

Survey of grapevine cultivar Rkatsiteli Pink showed that in case of Rkatsiteli, GFkV infection was characterized as highly spreading (22.3%) in Georgian grafted nurseries whereas distribution of GLRaV-3, GLRaV-2 was low, respectively: 2.3%, 1.73%. Presence of GVA was not found in cultivar Rkatsiteli Pink. (See Table II). On the next stage of the study of grapevine varieties, 120 samples of Qisi from Georgian region of Mtskheta grafted nurseries were tested for the above listed viruses. It was revealed that distribution of GFkV infection was most spreading (21.6%).

Table 2. Result of Viruses Testing in Grapevine (Rkatsitheli Pink)

Grapevine	Virus Varieties	Sample Tested	Positive Sample	
Rkatsitheli Pink			Number	%
	GFkV	130	29	22.3
	GLRaV-1	130	14	10.76
	GFLV	130	11	8.46
	GLRaV-3	130	3	2.3
	GLRaV-2	130	2	1.73
	GVA	130	-	-

Table 3. Result of Viruses Testing in Grapevine (Qisi)

Grapevine	Virus Varieties	Sample Tested	Positive Sample	
Qisi			Number	%
	GFkV	120	26	21.6
	GVA	120	15	12.5
	GFLV	120	9	7.5
	GLRaV-3	120	2	1.66
	GLRaV-1	120	7	5.8
	GLRaV-2	120	2	1.66

Table 4. Result of Viruses Testing in Grapevine Varieties

Grapevine Varieties	Virus Mix Infection	Samples Tested	Positive Samples	
			Number	%
Rkatsiteli	GFLV/ GFkV	160	31	19
Rkatsiteli Pink	GFLV/ GFkV	130	-	-
Qisi	GFLV/ GFkV	120	9	7.5

It should be noted, that among the all tested samples, GVA (12.5%) was found only in Qisi (see Table III). It is well known that simultaneous infection by multiple viruses can significantly increase severity of symptoms. Studies have shown that grapevines carried mixed infections and often times the symptoms could be caused by more than one virus. Furthermore symptoms can be influenced by seasonal and climatic conditions (See Fig.3).

Fig. 3. Grapevine Cultivars Associated with Mix (GFLV/GFkV) Infection Symptoms (a, b)

Cultivars Rkatsiteli and Qisi showed mixed virus infections (2 types of viruses in the same samples) in particular the combination of GFLV/ GFkV viruses were spreading in grapevine cultivars Rkatsiteli (19%), Qisi contained only 7.5 % of doubleinfections. Despite that Rkatsiteli Pink showed GFLVand GFkV infections severally, double virus combination GFLV/ GFkV had not found (see Table IV).In conclusion all tested grapevine samples 36. 09% were infected.

GFkV, GFLV, GLRaV-1 virus were characterized high spreading respectively: 19.26%, 12.68%, 8.04%, and comparatively low distribution was found among viral pathogens tested GVA (3.66%) GLRaV-3 (1.21%) and GLRV-2 (0.50%).

Conclusion

As a result of this survey, it can be concluded that, GFk V (together) virus distribution was the highest among the all listed viruses in all tested grapevine varieties, Grapevine fleck virus is not known to cause symptoms except in Vitis rupestris. When samples are tested negative this may have been due to low virus titer in response to warm weather, crown gall, physical injury, fungal trunk diseases, nutrient deficiencies, herbicide injury or other pathogens that could not be detected with the available tests. GFLV distribution related to the scientific date in this direct, GFLV can be widespread in some vinicultural regions, in the vineyard, GFLV is transmitted from grapevine to grapevine by the ectoparasitic nematode *Xiphinema index* (8). Many grapevine varieties and rootstocks infected by the GFkV are symptomless although infection may be associated with graft incompatibilities. GFkV is a virus found only in the phloem (part of the vine's vascular system) and is transmitted through propagation and grafting.

Plant Infection depends on several factors, it is known that different environmental and micro-climatic conditions can influence grape physiology, and plant with different physiology statuses differently react to infection with several types of viruses.(11,12) Differences in the soil conditions might influence the presence of the virus vectors and affect virus presence. (6)

Our research team is planning to use specific methods for virus quantitation based Real Time PCR protocols. The reliable method (Real Time PCR) of the virus quantitation in field samples will allow further studies of the influence of the viral infections of grapevines and competitions between viral isolates in mixed infections. Regular sampling and testing of all vines will be useful for screening for genetic resistance in different grapevine accessions and rootstock materials. Infected plants were labelled for successive analysis that will provide virus free propagation material in Jighaura and Mukhrani grafted nurseries in Georgia. This study has provided a backdrop direction of virus control programs which could be developed more efficiently in Georgia. A greatly expanded program, in order to provide elite, virus free propagation material in registered nurseries in the east region of Georgia has emerged as a cornerstone of virus control in this area, in part as a result of the data obtained in the study presented here. It is recommended to establish Certification schemes in Georgia. These schemes contribute to the improved productivity and sustainability of the viticulture sector and the use of high-health material is encouraged for vineyard establishment and vine replacement.

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