საქართველოს მეცნიერებათა ეროვნული აკადემიის მოამბე, ტ. 4, №3, 2010
BULLETIN OF THE GEORGIAN NATIONAL ACADEMY OF SCIENCES, vol. 4, no. 3, 2010

Plant Growing

Pathogen Testing and Certification of Grapevine

Levan Ujmajuridze*, Lali Mosiashvili*

Institute of Horticulture, Viticulture and Oenology, Tbilisi

(*Presented by Academy Member Tengiz Urushadze*)

ABSRACT. To improve the quality and the sanitary status of Georgian autochthonous standard varieties of grapes clonal selection was carried out together with the ELISA test (enzyme linked immunosorbent assay) for screening of major viruses. The result showed that 12% of 170 samples were infected with nepoviruses (ArMV + GFLV), 18% of 200 samples with closterovirus ($GLRaV_{1+3}$). All tested samples out of 80 were virus free on maculavirus (GFkV). *© 2010 Bull. Georg. Natl. Acad. Sci.*

Key words: *grapevine, sanitary selection, viruses, enzyme-linked immunosorbent assay (ELISA), certification.*

Introduction

Grapevine (*Vitis vinifera* L.) is one of the oldest and most widespread cultivated crops. The International Council for the Study of Virus and Virus-like Diseases of the Grapevine recognizes over 70 infectious agents affecting grapevine (viruses, viroids and phytoplasmas) [1]. The technical annex of the Council Directive 68/193/EEC [2] - interprets the absence of Complex of infectious degeneration Grapevine Fanleaf Virus (GFLV) and Arabis Mosaic Virus (ArMV)); Grapevine Leafroll Viruses: Grapevine Leafroll-associated Virus 1 ($GLRaV_1$) and Grapevine Leafroll associated Virus 3 ($GLRaV_3$); Grapevine Fleck Virus (GFkV) (only for rootstocks)

Viral infections can negatively influence the yield, berry color, resistance to biotic and abiotic stress, the length of the growing cycle, sugar content and acidity of the grapes, wine quality, etc. These infections are spread with virus contaminated vine scions and rootstocks among the plants and within viticulture regions. Therefore, use of infected material for grafting is prohibited under the grapevine certification program. Certification of grapevine is a powerful and effective tool to control these pathogens, which enables vineyards to economically and sustainably maintain quality and productivity. At the same time the world-wide sanitary deterioration of grapevine calls for the enforcement of preventive measures. Thus, clonal and sanitary selection of the grapevine is an important activity for improving the quality and quantity of the produce and best results can be achieved if those proceed together [3-5]. A healthy vine is fundamental to the successful beginning and sustainability of all grape vineyards. This indicates the necessity of activities for production of certified grapevine planting materials.

The production of grapevine planting material has a long history in Georgia, but certification program has not been fully established. Numerous commercial nurseries have intensively been producing noncertified planting material for local growers. The demand for certified planting material of unique Georgian wine varieties has grown steadily over the year on both domestic and international market. To improve the quality and sanitary status of Georgian autochthonous varieties and clones, mass positive selection has been initiated and carried out at the Institute of Horticulture, Viticulture and Oenology since 2007 together with screening for the most important grapevine viruses. Primary works in this field have been implemented through the project financed by Georgia National Scientific Foundation (GNSF). At the research and training center in Gori-Skra

Mother Plant Foundation Blocks of phylloxera and nematode resistance rootstocks and scions with unique Georgian grape varieties on 2 ha were established.

Materials and Methods

Grapevine certified materials are obtained through a fixed number of steps. At each of these steps plants are tested to verify the absence of pathogens and they are maintained and propagated under strict conditions to exclude recontaminations.

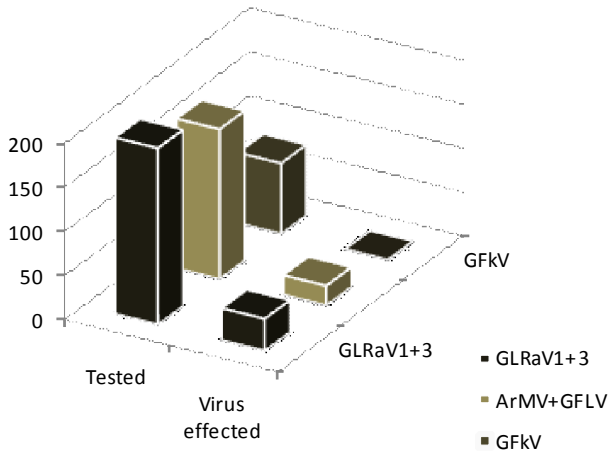
Several grapevine varieties and clones (Rkatsiteli CL48, Khikhvi CL, Saperavi CL 359, 430Chinuri CL 59/21, Goruli Mtsvane CL59/52, Tsitska CL 14/25, etc), growing in Georgia, were selected for screening of pathogens. Visual observation and random tests by enzyme linked immunosorbent assay (ELISA) were carried out at the ELISA laboratory of IHVO.

Totally 370 vine samples from *V. vinifera* and 80 samples of rootstock were tested for some major grapevine viruses: Grapevine Fanleaf Virus + Arabis Mosaic Virus (GFLV+ArMV); Grapevine Leafroll associated Virus$_{1+3}$ (GLRaV$_{1+3}$); Grapevine Fleck Virus (GFkV) (only for rootstock). For screening of GFLV + ArMV samples of young fully developed leaves were utilized during the spring season; but for GLRaV $_{1+3}$ and GFkV detection mature leaves were utilized during the fall season.

ELISA-test assay: Standard double antibody sandwich ELISA (DAS-ELISA) [6] was performed with commercially available polyclonal antibodies, immunoglobulin G (IgG) and alkaline phosphatase-conjugated IgG (Bioreba, Reinach, Switzerland) according to manufacturer's instructions used for the detection of viruses.

For all testing, the coating antibodies, samples, controls and conjugate antibodies were incubated for 4 h at 30^{0}C or overnight at 4^{0}C. Results were read after adding the substrate (*p*-nitrophenyl-phosphate in 1M diethanolamine, pH 9.8) to the wells. Two wells were used for each sample. Absorbance at OD 405 nm was recorded on a computer programmed ELISA reader (BIOTEK -ELx800^{TM}). Samples were considered positive when the absorbance value was at least three times greater than the absorbance value of the healthy control. For precise calculation of results a more sophisticated method was used «mean value+3x standard division+10%». Samples that were close to the cut-off had been re-tested.

Sanitary Status of 450 Vines in Georgia



Results and Discussion

It is obvious that virus tested and healthy planting material is a major precondition for successful grapes production. ELISA test is a suitable method for routine detection, allowing large-scale testing for viruses for which antiserum is available. The correct time for testing and appropriate sample type ensures successful results.

As a result of our research it is clear that in Georgia the most spread viruses are closteroviruses GLRaV $_{1+3}$ (18%), which are transmitted by mealybugs and scale insects and can adversely affect vine growth. It can cause up to 60% yield reduction. It is followed by nepoviruses GFLV+ArMV (12.9%), which are transmitted by the nematodes living in the soil. Grapevine Fleck Virus (GFkV) is latent in European grapes and most American rootstocks and is associated with graft incompatibility for some rootstocks. This virus is a widespread and damaging disease in Europe, but the sanitary condition of the tested rootstocks in Georgia showed that all tested 80 rootstocks were virus free. The results obtained are summarized in Table.

The results showed that vines healthy enough can be found most of all tested. Harmfulness of viruses and

Table

Results of serological (ELISA) detection of major grapevine viruses

Virus Group	Viruses	Number of positive vines	Number of negative vines	% of positive vines
Nepovirus	GFLV+ArMV	200	134	18
Closterovirus	GLRaV $_{1+3}$	170	148	12.9
Maculavirus	GFkV	80	80	0

their effect on growth and fertility of grapevine in Georgia is still to be determined. The certification program for obtaining virus-free grapevine plants is being constantly developed due to the increasing number of cultivars and clones needed to be available as healthy material.

Conclusion

Virus tested and healthy planting material is a major precondition for successful grapes production. The control strategies of grapevine viruses are preventive. They are based on the identification and elimination of infected material to reduce disease incidence and minimize economic damage. The results showed a need for further assessment of virus status for the production of grapevine certified planting material.

Acknowledgements

This work was supported by the grant STEP BPG 05/10. We are grateful to the Science&Technology Entrepreneurship Program (STEP), GRDF and CRDF global team.

მემცენარეობა

ვაზის მცენარის პათოგენებზე ტესტირება და სერტიფიკაცია

ლ. უჯმაჯურიძე*, ლ. მოსიაშვილი*

** მებაღეობის, მევენახეობისა და მეღვინეობის ინსტიტუტი, თბილისი*

(წარმოდგენილია აკადემიის წევრის თ. ურუშაძის მიერ)

ქართული აბორიგენული სტანდარტული ჯიშების სანიტარიული ფონის შესწავლა-გაუმჯობების მიზნით ჩატარდა კლონური სელექცია და ვაზის ტესტირება ძირითად ვირუსულ დაავადებებზე იმუნოფერმენტული ანალიზის მეთოდით. კვლევამ აჩვენა, რომ ვაზის 170 ტესტირებული ნიმუშიდან 12.9% აღმოჩნდა პოზიტიური ნეპოვირუსის (ArMV+GFLV) მიმართ, ხოლო 200 ნიმუშიდან 18% პოზიტიურია კლასტეროვირუსის ($GLRaV_{1+3}$) მიმართ. GFkV-ზე ტესტირებულ ვაზის 80 ნიმუშიდან ყველა უვირუსოა.

REFERENCES

1. ICVG (2003), Recommendation for the certification of grapevine propagating material. September 17, 2003 by the General Assembly of the ICVG in the course of its 14th Meeting at Locorotondo, Italy. Available at: http://www.icvg.ch/data/recomm.pdf
2. The Council of the European Communities (1968), Council Directive 68/193/EEC of 9 April 1968 on the marketing of material for the vegetative propagation of the vine.Official Journal L 093, 17/04/1968 : 15-23.
3. *G.P. Marteli* (2006), Infectious diseases and certification of grapevine. Available at: http://resources. ciheam.org/om/ pdf/b29/00800231.pdf
4. *A. C. Goheen* (1989), Am. J. Enol. Vitic., 40: 67-72
5. *F. Mannini* (2003), Grapevine clonal and sanitary selection: the point of view of E.U. Selectors. In Extended abstracts 14th Meeting ICVG Locorotondo (Italy), 13-17 September, 2003.
6. *M.F. Clark, A.N. Adams* (1977), J. Gen. Virol., 34: 475-483.

Received September, 2010