The sanitation of autochthonous grapevine cultivars (Kallmet and Vlosh) from viral infections using *in vitro* techniques

Elektra Papakosta
Center of Transfers of Agricultural Technologies, Vlore, Albania

Abstract

The use of the "pure" plant material constitutes a necessity for development of the contemporary horticulture, determined by the phytosanitary normative. We studied some autochthonous grapevine cultivars (Vlosh and Kallmet) in order to evaluate the phytosanitation state diagnosed with the serological method of DAS-ELISA for the presence of viruses such as: GFLV, GFcV, GLRaV-1, GLRaV-3. The results of the serological testing the cultivars resulted infected with the virus GLRaV-3 (*Grapevine leafroll-associated virus*). In order to improve the grapevine cultivars from the viral infection we applied the *in vitro* technique: *In vitro* meristem tip culture and *in vitro* heat therapy, by going through all its phases such as inoculation, propagation, rooting, and *in vivo* acclimatization. For each of the studies cultivar were isolated 30 meristem tips 0,4-0,6 mm in size which were excised from young 4 -5 cm long shoots of infected plants from the apical stems and after 40 days in the vegetative room we received 18 explants Vlosh and 14 explants Kallmet. Infected plants were put in a growth chamber at 38°C, with a 16 h photoperiod (5000 lux). The new plants were put in the *in vitro* thermotherapy chamber; the Vlosh plants stayed there for 3 months and Kallmet plants 4 months in temperature 38^{0}C in therapeutic treatment. After the adoption *in vivo* acclimatization these plants underwent the sanitation evaluation which proved the phytosanitation purity of the cultivars 3/5 Vlosh grapevine and 2/3 Kallmet grapevine.

Keywords: *meristematic culture, the sanitized in vitro, thermotherapy in vitro. grapevine leafroll-associated virus, Kallmet, Vlosh.*

Introduction

The preparation and implementation of the scientific guidelines of the proliferation material is very important for the production of the grapevine seedlings, as a necessary condition to realize a high quality fruity culture as a determined task from the phytosanitary regulations.

The phytosanitary degradation of the grapevine fruit cultures makes it necessary to implement the program of genetic and phytosanitary improvement, in order to preserve the autochthonous germoplasm in fruit cultures. The sanitation controls in grapevine have evaluated a high rate of infection where the plants manifest symptoms of the disease from viruses (Myrta *et al.,* 1994). The proper recognition of the viral agents and the species that undergo sanitation techniques determine the selection of the more efficient techniques: *in vitro* and *in vivo* therapies of the apical meristems. The programs of cloned and sanitary selection of the local varieties with national importance for the creation of the primary resources are the only alternative to recuperate the autochthonous germoplasm in fruits culture and grapevines.

Materials and Methods

The plant material of the grapevine (Kallmet and Vlosh) was selected in the collection of the autochthonous the Experimental Base in Shamogjin, the Center for Transfer of the Agricultural Technologies, Vlore during April, 2018 and later the plants were diagnosed for the presence of viruses and other pathogens similar to viruses.

Diagnosing with the serological technique DAS- ELISA for virus presence

The plants of cultivars Kallmet and Vlosh were diagnosed with the serologic method of DAS- ELISA for the virus presence (Martelli *et al*.,1997; 2002) (Prota, 1996).

Grapevine fan leaf virus (GFLV)

Grapevine fleck virus (GFkV)

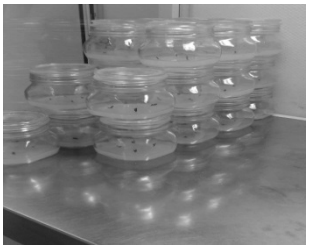
Grapevine leaf roll-associated virus (GLRaV-1, GLRaV-2, GLRaV-3)

After diagnosing, the plants of cultivars Kallmet and Vlosh resulted infected with the GLRaV-3 virus and we performed the sanitation *in vitro* technique.

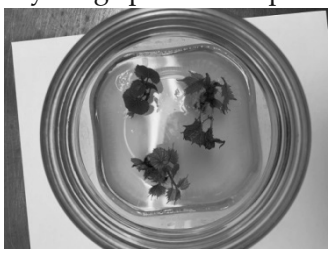
The sanitation of the infected plants with viruses (time period May, June, July, August, September) with the *in vitro* technique (Savino *et al.,* 1990; Barba *et al.,* 1992; Bottalico *et al.,* 2004) and combined among them.

- *In vitro* culture *of the meristematic stems*

Plant material-stems (4-5 cm) of Kallmet and Vlosh cultivars (5 plants for cultivars) were selected from infected plants and inoculated in the *in vitro* culture after disinfection with sodium hypochlorite NaOCl 10 % for 20 min, to perform the meristem culture (Gauther *et al.,*1982) as the non infected part from viruses (Quak, 1997). With the help of steriomicroscope we isolated the meristems with dimensions (0.4- 0.6 mm), for each cultivar 30 meristems and were put into a MS inoculation terrain (Murashige & Skoog, 1962) and later were put into yhe vegetative room in 24^0 C temperature, photoperiod 16 hours light and 8 hours dark, phosphorescent light 3500 lux. After 40 days in MS inoculation terrain (Alpi, 1983) the meristems were put into MS proliferation terrain in the vegetative room of plant growing. After 20 days, from each stem was formed a young sprout with proliferation (photo 1. A, B, C).



A. Inoculation 40 days



B. Proliferation of Kallmet

C. Proliferation of Vlosh

Photo1. A, B, C. *In vitro* culture of the grapevine

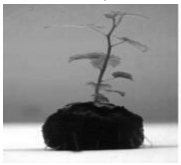
- *In vitro thermotherapy*

The grapevine *in vitro* cultivated offsprings, after the first phase of proliferation

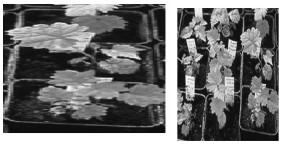
undergo the therapeutic treatment with the thermotherapy technique in the room of *in vitro* thermotherapy for a time period of 90 days for Vlosh cultivar and 120 days for Kallmet cultivar in controlled conditions, 38^{0}C temperature, photoperiod of 16 hours light and 8 hours darkness, phosphorescent lightening 5000 lux.

The plants were put into the rooting phase for 20 days with IBA hormonal treatment to develop the rooting system and later they were adopted in the greenhouse of *in vivo* acclimatization (photo 2). After 40 days in acclimatization, the adopted plants were analyzed individually with the DAS-ELISA method for verification of the presence of GLRaV-3 virus (Gribaudo I. *et al*., 2003).

A. Rooting of Vlosh



B. Acclimatization of Vlosh



C. Acclimatization of Kallmet

Photo 2. Rooting and acclimatization of the grapevine (Vlosh and Kallmet)

- *Phytosanitary verification*

After the plants acclimatization, (40 days) we realized the phytosanitary verification with ELISA (Enzyme Linked Immuno Sorbent Assay) serological testing for the presence or the absence of the viruses in the sanitized plants. The results of the sanitation were analyzed in percentage of the number of sanitized plants for the applied techniques with the apical meristem and thermotherapy.

Results and Discussions

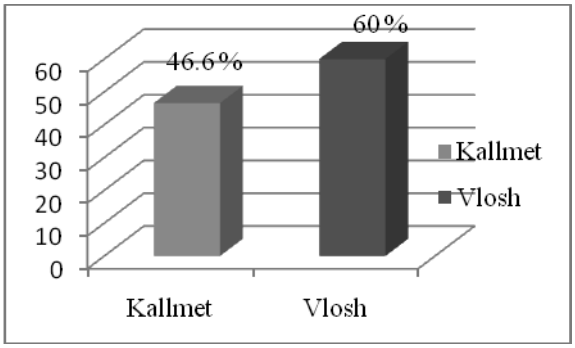
Based on the diagnosing results (table 1) with DAS-ELISA (Koening & Paul., 1982) with evidences were identified the infected plants for sanitation (Savino *et al*., 2002).

Table 1. The evaluation of the sanitary state of cultivars Kallmet and Vlosh

Cultivars	Number of plants	Virus GFLV	Virus GFkV	Virus GLRaV-1	Virus GLRaV-2	Virus GL-RaV-3
Kallmet	4	-	-	-	-	+
Vlosh	5	-	-	-	-	+

From the phytosanitary testing with the ELISA method, the grapevine cultivars resulted non infected from the GFLV and GFkV viruses but resulted positive from the GLRaV-3 virus. For the sanitation of the grapevine cultivars from the GLRaV-3 virus we applied the *in vitro* technique: the technique of the apical meristems and *in vitro* thermotherapy, by going through all its phases such as inoculation, proliferation, rooting, and acclimatization. For each cultivar we isolated 30 meristems from the apical buds and after 40 days in the vegetative room we got 18 explants (60%) Vlosh and 14 explants (46.6%) Kallmet (graph. 1).

Graph 1. The number of explants obtained after 40 days



From each meristematic bud after the growing phase and elongation was formed a well formed shoots in giving other new sprouts by proliferation in MS terrain (Murashige & Skoog, 1962). The sprouts were kept in the vegetative rooms for 20 days. After the proliferation phase the shoots underwent the rooting phase in feeding terrain with auxine base (IBA) which stimulates the development of the rooting system. The plants with roots underwent acclimatization in *in vitro* state with technical parameters such as temperature and controlled lightening.

The sanitized plant materials with the techniques of the meristematic culture and *in vitro* thermotherapy as applied techniques and combined within them, were verified for the final sanitation state with the ELISA diagnosing technique. The samples (leaves from the apical stems and basal parts of each sprout) resulted negative in a number of plants (Table 3.) from the GLRaV-3 virus, thus sanitized plants of Kallmet (2 plants) and Vlosh (3 plants).

Table 3. The results of the *in vitro* Thermotherapy technique (4 months, 3 months)

Cultivars	Virus	Thermotherapy in vitro	Diagnosing DAS-ELISA	Sanitized plants
Kallmet	GLRaV-3	4 months	ELISA Negativ (2) ELISA Pozitiv (1)	2/3
Vlosh	GLRaV-3	3 months	ELISA Negativ (3) ELISA Pozitiv (2)	3/5

Sanitation of the grapevine plants resulted efficient with the technique of the apical meristem which as an aseptic tissue culture avoids the virus presence in plants but is better combined with *in vitro* thermotherapy (Mannini, 2001) as a thermo-therapeutic treatment with the heat air effect in elimination of GLRaV-3 virus.

Conclusions

The results of the study show the real possibility of the sanitation of Kallmet and Vlosh infected cultivars with viruses or pathogens similar to them. The combination of the technique of the apical meristems and *in vitro* thermo-therapy show the efficiency of this technique for sanitation. There are sanitation possibilities of application of the sanitation guidelines for other grapevine cultivars and other viral agents. Sanitation is an efficient technique with the goal to improve the sanitation and the creation

of the Primary Resources with importance in preservation of the autochthonous germoplasm of grapevine.

References

Alpi A., 1983. La coltura *in vitro* di meristemi apicali di specie arboree con particolari riferimenti alla vite.

Barba M., Martino L., Cupidi A., 1992. Il risanamento della vite: tre tecniche a confronto. *Vignevini*, 3:33-36.

Bottalico G., M. Saponari, A. Campanale, G. Mondelli, C. Gallucci, E. Serino, V. Savino, G.P. Martelli, 2004. Sanitanion of virus-infected olive trees. Journal of Plant Pathology, 86 (4 Special issue) 311.

Gautheret R.J., 1982. Plant Tissue Culture: the History. *Proc. 5th Int. Cong. Plant Tissue and Cell Culture:* 7-12.

Gribaudo I., Bondaz J., Cuozzo D., Gambino G. (2003). Elimination of GLRaV-3 from the wine grapevine 'Müller Thurgau' (Vitis vinifera L.) through somatic embryogenesis. *14th Meeting Int. Council for the Study of Virus and Virus.like Diseaeses of the Grapevine*, Locorondo BA,I, 240-241.

Koening R., Paul H.L., 1982. Variant of ELISA in plant virus diagnosis. *Journal of Virological Methods*. 5: 113-125.

Mannini F., 2001. Effetti del risanamento da virus sulle attitudini di cloni di vite. *Informatore Fitopatologico*, 4, 25-30.

Martelli G.P., Boscia D., Saltarelli P., 1997. Filamentous viruses of the grapevine: Closteroviruses. In: P.L. Monette (ed). *Recent Research Development in Plant Pathology.* Filamentous Viruses of Woody Plant. Research Singpost, Trivandrum.

Martelli G.P., 2002. Le principali virosi della vite oggi. *Informatore Fitopatologico*, 52, 4, 18-27.

Myrta A., Di Terlizzi B., Digiaro M (1994). Occurrence and distribution of Sharka in Albania. Phytopathologia Mediterrania, 33: 59-62.

Murashige T., Skoog F., 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.*, 15: 473-497.

Prota U., 1996. Virus e virosi della vite. Da *"Virus floematici e malattie della vite"*, Locorotondo (BA), 3-4 Ottobre: 203-218.

Quak F., 1997. Meristem culture and virus-free plants. *Plant Cell, Tissue and Organ Culture.* In Y.P.S. Bajaj, : 598-615.

Savino V., Boscia D., D'Onghia A.M., Martelli G.P., 1990. Effect of heat therapy and meristem tip culture on the elimination of grapevine leafroll-associated closterovirus type III. *Proc. 10 th Meet*. ICVG,Volos , Grecia: 433-436.