GRAPEVINE CERTIFICATION AND THE IMPORTATION OF GRAPEVINES INTO THE MEMBER COUNTRIES OF THE NORTH AMERICAN PLANT PROTECTION ORGANIZATION (NAPPO)

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NAPPO is the Regional Plant Protection Organization (RPPO) for the North American countries of Canada, the United States of America, and Mexico. As a RPPO under the International Plant Protection Convention, NAPPO has the mission of coordinating the efforts of the three countries to protect their plant resources from the entry, establishment, and spread of regulated plant pests, while facilitating intra/interregional trade. Each country within North America retains its sovereignty in establishing and administering Plant Protection matters and issues. In carrying out its mission, NAPPO develops regional standards for phytosanitary measures (RSPM). These standards are approved by the member countries and serve as guidelines.

NAPPO has developed part 1 of a regional standard addressing the importation of grapevines into a NAPPO member country from other countries. The Standard describes the requirements for the importation of grapevines by the member countries, and the movement of grapevines between them. Grapevine pests specifically dealt with in the Standard are viruses and virus-like agents, viroids, phytoplasmas, and bacteria. The scope of the Standard does not include non-pest related items such as varietal trueness-to-type, and quality grades and standards. These issues, although very important to viticulturists and nurseries, are outside NAPPO's phytosanitary mandate.

The Standard has been developed to provide for equitable and orderly trade of grapevine propagative material while assuring that the probability of the introduction of regulated pests is reduced to an acceptable level. The Standard outlines a program for managing viruses and virus-like agents, viroids, phytoplasmas and bacteria, achieved through a combination of prohibitions, restrictions, and certification approaches.

The Standard is divided into two sections with an appendix of economically significant pests and acceptable testing methods. The General Requirements section addresses the pest risk analysis and pest risk management measures. A pest risk analysis is required to determine the risks associated with importing grapevines from another country. Appropriate pest management measures such as prohibition and import restrictions are then applied as required. These measures are explained in the Standard.

The Specific Requirements section of the Standard identifies components of a comprehensive phytosanitary certification program. A certification program is an effective way of controlling pests within a country or area. It may also sufficiently mitigate the pest risks associated with importing foreign grapevines to allow importation with some restrictions. A certification program must be well defined and managed in order to be effective. The parameters of a certification program and requirements of participants and administrators must be clear. The issues addressed in this section include program administration, terminology, testing, eligibility, the nomenclature of certification levels, horticultural management, isolation and sanitation requirements, inspection and re-testing, documentation, identification and labelling, quality assurance, non-compliance and remedial measures, and criteria for post entry quarantine. This section may be used as a guideline for the evaluation of a foreign certification program or the establishment of a new certification program.

There are two basic types of grapevine certification programs within North American. The Canadian Plant Protection Export Certification Program (PPECP) for Grapevine Nursery Stock was primarily developed to meet foreign import requirements. However, much of the plant material produced under the program is used within Canada. The PPECP is a voluntary program administered by the Canadian Food Inspection Agency, Canada's National Plant Protection Organization. The program deals with phytosanitary certification issues only. It does not consider non-pest items such as varietal trueness-to-type, and quality grades and standards. These items are negotiated between the buyer and seller. Domestic or imported varieties that have been fully tested through the Centre for Plant Health in Sidney, British Columbia, Canada are eligible for the PPECP. The PPECP explains the eligibility, approval, certification, inspection and testing requirements. Grapevines produced under the PPECP are eligible for export to the United States, and other countries.

The primary grapevine certification program used in the United States is slightly different. These grapevine certification programs are administered at the state level. Some states have voluntary certification programs. Other states require mandatory registration and licensing before grapevine material may be sold within that state. These certification programs strive to prevent the spread of regulated or harmful pests and promote the elimination of specific grapevine diseases that are spread by vegetative or cultural practices. Propagative material originates from approved testing facilities. Nursery registration, inspection and testing ensure the quality of grapevines produced within those programs. Variety trueness-to-type requirements are often included. Grapevines produced under some state certification programs are eligible for export to Canada and Mexico.

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EPPO CERTIFICATION SCHEME FOR GRAPEVINE

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The EPPO Panel on Certification of Fruit Crops was created in 1985 and is constituted of experts coming from different EPPO member countries interested in the production of healthy planting material. This Panel has the aim to produce certification schemes for fruit crops which are of importance to the Euro-Mediterranean region. So far, 11 certification schemes have been officially approved by the Organization and published. They cover pome and stone fruits, small fruits, citrus, and also grapevine. The EPPO certification scheme for grapevine is based on filiation, and describes the steps to be followed for the production of vegetatively propagated planting material (varieties and rootstocks). Certified material is obtained through a fixed number of steps. At each of these steps plants are tested to verify the absence of pests, and they are maintained and multiplied under strict conditions to exclude recontaminations. This certification scheme also includes guidance on testing procedures for virus and virus-like diseases which should be tested for (i.e. fanleaf, grapevine European nepoviruses, leafroll, rugose wood, enation, fleck, vein necrosis, vein mosaic, flavescence dorée, bois noir and other European grapevine yellows). Some guidelines on sanitation procedures are also given. Considering that the EPPO certification scheme was published in 1993, that new diseases have appeared, new diagnostics techniques are available, and that discussions are taking place at the European Union level on certification of grapevine, the EPPO certification scheme for grapevine will soon be revised to reflect all these new developments.

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GRAPEVINE CLONAL AND SANITARY SELECTION: THE POINT OF VIEW OF E.U. SELECTORS

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First application of grapevine clonal selection dated late 19th century on Silvaner in Germany. It was only after the sixties of the 20th century, however, that this methodology became a common tool for vine genetic improvement and since then applied on large extent in the main viticultural Countries of Europe.

Since the beginning, the aim of clonal selection has been to supply the growers with propagation material able to express suitable agronomic and enological characters. Over the years, in parallel with the increasing knowledge on virus diseases and their detrimental effects on vine performances, the sanitary side of the selection has raised importance and, up today, a selected clone must also be free from several virus and virus-like diseases.

E.U. has given since 1968 (EEC recommendation n.° 68/193) common rules for grapevine propagation and selection to the member States, nevertheless each national legislation acknowledged European recommendations according to the social and environmental conditions of domestic viticulture so that differences may still be found in the way to perform clonal and sanitary selection in Europe.

Regardless of different protocols, a tremendous amount of work in the field of clonal selection has been carried out in Europe in the last 30 years. Especially in France, Italy and Germany, public and private selectors obtained the official registration of hundreds of selected clones which are currently propagated by nurseries as 'certified' material to supply domestic markets and export. Selectors also play a fundamental role in clonal material preservation, supervising or directly managing clone repositories, in order to ensure best conservation for licensed clones and to maintain sources of genetic variability for future selections.

Clonal selection important scientific, technical and economic implications on viticulture induced the European selectors to join in a specific association, whose Statute was officially signed in 2001 in France at the Centre de Selection - ENTAV. The European Grapevine Clone Selectors Association (Association Europèenne des Obtenteurs de Clones de Vigne - AEOCV), sited at the Agricultural Institute of S. Michele all'Adige (TN) – Italy, is a no-profit voluntary organization which, up to now, gathers clone selectors of Austria, France, Germany, Greece, Italy, Portugal and Spain. The aim of the AEOCV, according to the article 2 of the its Statute, is to coordinate the activity of the members, to promote researches in the field of clonal and sanitary selection and of the clone preservation, to act for licensed clone protection (patent rights), to improve information and material exchange, to favour the harmonisation among national legislations in the field of grapevine selection and propagation, to promote the maintenance of genetic biodiversity among and within grapevine cultivars.

The selector figure varies depending on the different Countries. In France there is only one clone selector, the Centre de Selection - ANTAV, in Italy there are several officially recognized selectors (mainly public institutions, University or other research Institutes, but also some private ones such as Vivai Cooperativi Rauscedo) pooled in the Italian Grapevine Selectors Association (ACOVIT), public and private institutions carry out clonal selection in Germany (where Geisenheim Research Institute is the most important selector) as well as in Austria, Greece, Portugal and Spain. The first clones of the main indigenous Portuguese cultivars, for instance, have been officially registered in 2003 thanks to a private selector (Viveiros Plansel).

AEOCV wishes to focus on some aspects of clonal selection which are regarded as particularly important:

1) European selectors consider essential:
   - to carry out in parallel both genetic (i.e. agronomic and enological) and sanitary selection;
   - to select clones free from the most harmful virus and virus-like diseases and their causal agents (when known);
   - to make available the clonal material as quickly as possible in the best sanitary condition for nurseries;
   - to detect and preserve as much as possible the variability within-variety to prevent the depletion of genetic diversity.

2) European selectors favour:
   - the pursuit of a point of balance between genetic and sanitary selection aimed at the registration of a large number of clones with different performing characters to provide the wine industry with access to the full genetic range of a given variety;
   - the definition of a common minimal list of virus and virus-like diseases and related causal agents from which the selected clones must be free for certification in E.U.;
   - the liberalization in the use of fast propagation techniques (*in vitro* culture, etc.) for the production of licensed clone 'initial' and 'base' material (scions and rootstocks) to speed up the clone transfer to grapevine nurseries.

AEOCV welcomes the entry of new members in the Association and aims at becoming a privileged forum of discussion and confrontation in the field of grapevine genetic improvement and of clonal selection in particular.

SELECTION AND BIODIVERSITY IN VITICULTURE: THE POINT OF VIEW OF NURSERYMEN

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The CIP (International Coordinating Board of the Professional European Viticultural Nursery Organization), whose members comprise producers of the most important European grapevine-growing countries, has been addressing for some time now problems of major relevance, such as the selection of grapevine material and the necessity to conserve a high biodiversity level of propagative material to be marketed.

The need for improvement was stimulated by associated producers, growers, and winemakers wishing to keep up their production and marketing objectives in order to maintain their business prospects.

For several decades now, European Community regulations on this matter have activated, in member states, a sort of "certification of grapevine-propagation material," privileging the use of material derived from clonal selections.

Clonal selection, which began in the main viticultural countries of Europe in the 60's and 70's, has also encompassed a sanitary improvement of the industry because the incidence of some of the major diseases, with specific reference to virus-induced disorders, was reduced and their negative effects on the crop contained. However, this method of selection has adversely affected the complex of viticultural biotypes, which had evolved over the centuries. These biotypes, if conserved in the existing range, would represent a most valuable source of variation for the grape industry of Europe and the rest of the world.

Surely, clonal selection counts on the positive effects stemming from the improved health conditions and homogeneity of the stocks derived from cloning, but indirectly causes a considerable loss in the biodiversity of extant varieties. If clonal selection does not assure the identification of an adequate number of differentiated clones for each major variety, this would, for the most part, limit the variability present in the vineyards established with them. This line of action is positive in view of uniform productions. However, in a commercial situation this strategy points at obtaining and promoting marketing productions that are typical and differentiated for territorial origin, for the technology used, for basic viticulture and oenology usage, for a more complete use of the traditional (and non) varietal patrimony and a more articulate availability of clones (where possible) to compensate, at least in part, for the loss of biodiversity as a consequence of the vast scale selection methods applied during the last decades. A much ampler cloning strategy than the current one and a more rapid sanitary control, limited to the most harmful diseases may produce a shift of the balance in the direction of conservation of a richer and more articulate biodiversity.

On this specific issue, an active confrontation could be opened between the scientific community and the productive world to define and identify common solutions, simple to implement and, above all, functional to the objectives of the grape industry by and large These lines of action, simple and with contained costs, can coexist so as to guarantee a supply of high quality propagating material, in accordance with marketing requirements which demand a sanitary situation in harmony with the diversification of varieties and sub-varieties (clones), to assure a wide range of transformed products.

These considerations are the expression of the wish to conserve and maintain, but also represent the reasonable necessity that innovative elements be introduced into the productive system in a harmonious and balanced way.

As far as genetically modified organisms (GM.O) are concerned, these must undergo a series of necessary preliminary controls, overcoming (should the conditions permit) political and/or psychological obstacles. These controls will allow a correct and objective evaluation of these "new" plant types to be delivered to nurseries and growers. In those countries where viticulture and oenology have a millennial history, we consider it correct to proceed with caution before modifying the current varietal patrimony, which has served efficiently in the past and which we feel that will continue to be efficient under the economic and mercantile profile of the future. It is, however, necessary to open to GM.O novelties, which might represent, after preventive and thorough checks, an important moment for innovation. The International Board of Nurseries has officially expressed the opinion that GMOs, once gone through the necessary sanitary, environmental, biological, qualitative, etc., controls may be distributed to growers.

It is, however, necessary to discuss the rights, the obligations and the responsibilities of the owners of new varieties and clones. These persons must receive appropriate compensation for their work, with adequate royalties but must not take possession of these products by protecting them with patent-rights. The original genetic patrimony of grapevine varieties is the result of selections made by generations of vinegrowers and is therefore a collective heritage of the entire viticultural sector. Expectations of producers are that the scientific community will develop procedures for the unambiguous identification of clones, so as to avoid suspicions or deception. We retain that the dialogue and confrontation between scientists and producers is extremely useful. This dialogue must be reinforced so that the evolution of the entire system may advance in harmony, optimizing results and making them functional to the needs of production.

Nurserymen have at his disposal a precious commercial database, his own clients, who present him with their relative needs for innovation, evolution, or conservation. Customers evaluate also the effects of the various innovations developed and implemented over time and, in case, may suggest changes in any procedure when they consider it necessary. This information can be most useful for both the scientific world and the Public Administration. We have met European Commission Officers regarding the definition of Directive 2002/11 CE, which regulates the commercialization of grapevine propagation material, and maintain that we have strived towards the definition of guidelines for the viticultural nursery sector which best fit the needs of production.

INTRODUCTION OF A PRIVATE CERTIFICATION SCHEME INTO AN UNREGULATED NATIONAL INDUSTRY: THE NEW ZEALAND EXPERIENCE

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New Zealand has a small and relatively young Wine Industry. There is no National certification scheme for grapevines in New Zealand and consequently there are no mandatory quality controls governing either the clonal identity, the phytosanitary status or any other quality parameters of any grapevine material sold within New Zealand.

The lack of proper audit trails for any grapevine material introduced into New Zealand has resulted in confusion as to the exact identity of many clones. Many clones are now incorrectly named, lost or compromised with virus infections which have probably been introduced by the multiple grafting events preceding the establishment of the commercial vineyards which are used as source blocks.

The lack of any phytosanitary controls has resulted in the distribution of large amounts of virus infected propagation material, particularly material infected with GLRaV-3. This distribution of virus infected material has become most evident only in the last few years during which time the New Zealand wine industry has gone through a period of rapid expansion with extensive new vineyard developments. Some areas of New Zealand are now heavily infected with GLRaV-3 and some wineries are now experiencing significant problems in the quality of the fruit they produce, particularly with respect to red grapes such as Merlot and Pinot noir.

In the absence of any National scheme for the New Zealand Industry, we decided to develop our own certification scheme to address the issues discussed above. The introduction of a private certification scheme is a break from the typically Nationally organised schemes. We developed a scheme based on the HACCP (Hazard Analysis Critical Control Point) system and external auditing is provided through SGS of Switzerland. We decided to focus on a three point system: Verification of true cultivar and clonal identity, high-health phytosanitary status and conformance to optimal physical specifications for production of grafted vines. We have also embarked upon an extensive process of re-selection of the existing New Zealand vine stocks and re-importation from established overseas clonal selection agencies. The process of re-selection is based upon extensive virus testing using ELISA and PCR, verification of proper cultivar and clonal (where possible) identity by overseas ampelographic experts and on viticultural performance. Germplasm blocks of re-selected and re-imported material of high health status and known identity with fully documented audit trails are now maintained in an isolated region away from other vineyards and risk of infection.

This paper discusses some of the issues we have had to consider during the process of developing a private certification scheme and some of the discoveries we have made along the way.

SOUTH AFRICAN VINE IMPROVEMENT ASSOCIATION (VIA) AND THE SOUTH AFRICAN CERTIFICATION SCHEME

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Plant improvement for wine grapes in South Africa officially started in 1963, with an agreement of co-operation between KWV, the Nietvoorbij Institute for Viticulture and Oenology, the Plant Protection Research Institute and the Directorate Plant and Quality Control. The main objective was to select grapevine material free from known harmful virus and virus-like diseases and for distributing and certifying of this material.

In 1986 the Vine Improvement Association (V.I.A.) consisting of two industry members, KWV and the Cape Wine and Spirits Institute (C.W.S.I.), was founded and the executive board, the Vine Improvement Board (V.I.B.) was appointed, the latter on which the state and the nurserymen were represented. The main objective of the V.I.B. is to promote the use of the "best available" plant material by all sectors of the SA wine industry. This is done through the application of the "SA Plant Certification Scheme for Wine Grapes", which officially came into effect in terms of the Plant Improvement Act, 1976 (Act 53 of 1976) in August 1992. The V.I.B. determines minimum certification standards and it serves as the official multiplication organization.

The standard operating procedures for certification are currently being upgraded and re-evaluated and these updated procedures will be presented.

PRESENT STATUS OF GRAPEVINE SANITARY SELECTION IN ARGENTINA

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The grape growing area of Argentina is near 200.000 Has, settled mainly in Mendoza and San Juan provinces. Traditional vineyards remain conformed with selfrooted locally selected materials. At present, a massive contribution of imported grafted plants are used, in the last ten years more than 15 million plants were imported from Europe and other countries. The Instituto Nacional de Tecnología Agropecuaria (INTA) have several Experiment Stations along the viticultural regions, where grapevine were clonaly selected from many years ago, paying attention only to oenologic and agronomic improvement.

Since 1992 propagation materials are checked in the Mendoza Experiment Station by ELISA technique for a few prevalent viruses (1). At the same time began the work in order to perform a real sanitary selection that guarantee the quality of propagating materials adapted to local conditions and allow to minimize the introduction of foreign plants. The following activities were done:

1. Implantation of a collection of virus free indicator *Vitis* plants in Mendoza (M) and Rama Caída (RC) Experiment Stations (INTA), protected of contaminations.
2. Implementation of the indexing procedures as detailed in the Table 1 (M and RC), the biological indexing was carried out with the ELISA negative plants.
3. Elaboration and approval of the legal support applied to the grapevine certification schedule by the Secretaría de Agricultura, Ganadería, Pesca y Alimentación (2). The regulations on the methodology to be applied is by now under consideration in SENASA (Plant Health Service), being hopefully effective in the near few months.
4. The Instituto Nacional de Vitivinicultura (INV) was designate as Prosecution Agent of official rules. Several courses and training on virus and virus like diseases were given for the qualification of the INV inspectors.
5. Development of the methodology for the multiplication and growing of certified materials.
6. Implantation of the Foundation Collection (RC), where ten plants of each clean clone are protected from vectors and climatic outbreaks.
7. Start up and grant the research on grapevine viruses (M), as a way to support and to keep updated the local Sanitary Selection.

Table 1. Biological indexing

Disease	**Indicator *Vitis***	**Feature**		
Fleck	*V.rupestris*	Winter Ω graft	Field nursery under protection	Symptoms in spring during three years
Fanleaf	*V.rupestris*			
Leafroll	*V.vinifera* cv. Pinot Noire			Symptoms in autumn during three years
Graft incompatibility	Kober 5BB		Plastic house	Symptoms in wood after three years
Rupestris Stem Pitting	V.rupestris			
Kober Stem Grooving	Kober 5BB			
Corky Bark	LN 33	Green graft	Greenhouse	Symptoms in three months
Vein Mosaic	*V.riparia* cv. Gloire de Montpellier			
Vein Necrosis	Richter110			

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SANITARY SELECTION AND DIAGNOSTICS OF GRAPEVINE VIRUS DISEASES IN UKRAINE

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Grapevine clonal selection in Ukraine is conducted by Tairov Research Institute of Viticulture and Wine-Making from the end of 1960's for more than 60 grapevine varieties in different ecological zones. Among 500 clones chosen in the process of long-term trials 50 clones of 37 varieties have been propagated as perspective.

At the same time with clonal selection sanitary selection is realized. Sanitary selection gives a possibility of obtaining healthy grapevine material, free of harmful viruses. Sanitary selection and grapevine virus diseases diagnostics have been begun in 1970's by Dr. B. Milkus (1).

For testing of nuclear stock plants green grafting indexing and ELISA-test have been used (2). Preliminary screening has been completed by dsRNA analysis and molecular hybridization with radioactive labelled dsRNA probes which have been created together with molecular biology Department of Genetics and Breeding Institute, Odessa (3).

During working out of sanitary selection and certification schemes we took into account the levels of spreading and harmfulness of grapevine virus diseases in Ukraine and European Union demands (4). Spreading of grapevine virus diseases has been studied in 5 viticulture regions of Ukraine. Grapevine fanleaf and grapevine leafroll are common diseases for all of them. Rugose wood complex has been revealed in Zakarpaje region and in Crimea. A high level of rugose wood harmfulness in Ukraine has been estimated. Grapevine fanleaf, leafroll (I and III serotypes), fleck, grapevine viruses A and B should be eliminated from propagation material according to the Ukrainian certification scheme. Periodicity and methods of testing depends upon category of planting material. Visual sanitary selection is accomplished twice a year. Plants of nuclear stock should be tested by ELISA every three years for 7 viruses. On base mother blocks randomly laboratory testing by ELISA should be done every five years.

Soils of nuclear stock, base mother blocks and nurseries should be checked up for nematodes *Xiphinema index* and *Xiphinema italiae*.

Base material is propagated in 7 base nurseries of Ukraine. Mother blocks of base virus-free material have been established on more than 200 ha.

Propagation of nuclear stock and base material is performed under strict official control by Ukrainian State Inspection of Pomology and Ampelography and specialized laboratories of Grapevine Clonal Selection Centre.

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SANITARY STATUS OF SELF ROOTED AND GRAFTED DEBINA AND VLACHIKO WINE GRAPE VARIETIES IN EPIRUS GREECE

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Debina and Vlachico are indigenous wine grape varieties of Hepirus, cultivated mainly in Zitsa region. The most important and widespread is Debina (750 hectares), whereas Vlachiko acreage is rather limited. In Zitsa although most of the vines are grafted, in some areas shelf rooted vines still exist. In the present work virus incidence in self rooted and grafted Debina and Vlachico vines was evaluated.

Samples were collected randomly during November 2002 from 21 different fields in Zitsa region. In total 42 samples originated from grafted and 40 from shelf rooted Debina vines were collected. In addition 10 samples from grafted Vlachico vines were also collected. Each sample consisted of three mature canes and phloem grapevine tissue (cortical scrapings) was tested serologically by ELISA by using commercially available diagnostic kits, for the presence of *Grapevine fanleaf virus* (GFLV), *Tomato black ring virus* (TBRV), *Arabis mosaic virus* (ArMV), six different closteroviruses (*Grapevine leafroll-associated virus 1, 2, 3, 5, 6, 7,* GLRaV-1,-2,-3,-5,-6,-7) and two vitiviruses (*Grapevine virus A, B*, GVA, GVB).

In Debina results indicated the presence of GFLV (26%), GVA (43%), GLRaV-1 (12%), GLRaV-5 (36%), and GFKV (5%) in grafted vines, while only three viruses namely GFLV (15%), GVA (20%), and GLRaV-5 (42%) were present in shelf rooted ones (Fig. 1). In grafted Vlachiko vines, only GFLV (10%), GVA (40%), GLRaV-5 (20%), and GFKV (20%) were present.

In shelf rooted plants we did not detect GLRaV-1 and GFKV whereas GVA and GFLV were found in lower incidence compared to the grafted ones. This indicates the contribution of grafting in the introduction and dissimination of GLRaV-1 and GFKV in the local variety Debina, possibly from *Vitis* species from America.

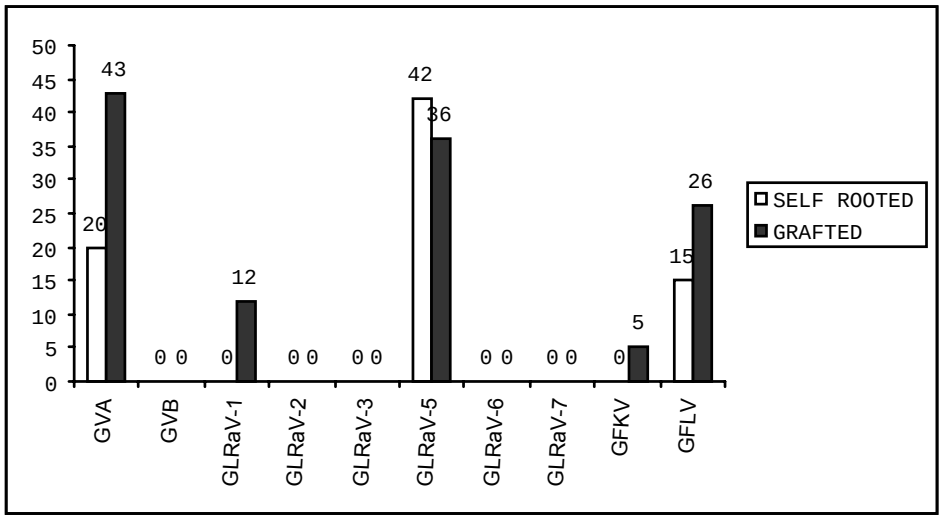

Figure 1. Percentage of virus incidence in self rooted and grafted Debina vines in Zitsa area.

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DEVELOPMENT AND EVALUATION OF A CYPRUS GRAPEVINE GENEBANK

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Introduction

Grapevines are among the most important crops in Cyprus, both from the economic and social point of view. The total area under vines is presently about 19, 000 ha, 90% of which yield wine grapes and 10% table grapes. They cover about 12% of the island's total cultivated area and contribute about 7% of the total value of agricultural production (7). The quality of the grape depends on several influences and one of the most essential production factors is grapevine planting material. For this purpose, the ICVG suggested a uniform EU certification scheme, which aims to ensure varietal identity, purity, and health status (6). The objective of the present study was the development and evaluation of a Cyprus grapevine genebank trough ELISA tests for 14 viruses, in vitro micropropagation and establishment of nuclear pre-basic plants. This investigation was carried out within the framework of a previously reported grapevine selection programme of the Agricultural Research Institute (ARI) of Cyprus (3, 4).

Materials and Methods

The ARI grapevine screenhouse collection at Zygi Experimental Station, including 119 selection lines of 72 genotypes, was used as a basic source for plant material development. A grapevine certification scheme based on Martelli *et al.* (6) and on EEC Directives 68/193 and 00/059 was employed. ELISA analyses for 14 viruses were carried out as described by Voller *et al.* (9) using DAS ELISA and RTA ELISA kits obtained from BIOREBA®. For grapevine clonal micropropagation a modification protocol based on Samson and Gasteran (8) was used. The acclimatisation of grapevine *in vitro* plants were based on a modification of the protocol described by Babrikov *et al.* (1).

Results and Discussion

Results from ELISA tests on grapevine plants grown in the ARI screenhouse collection indicated the presence of GLRaV-1 and GVA at 10.1% and 10.9 %, respectively (Table.1). Infected plants did not express any visible symptoms. Ioannou *et al.*(2, 5) observed high incidence and very fast natural spread of grape leafroll disease in Cyprus vineyards, associated primarily with mealybug-transmitted GLRaV-3. Thanks to the previous selection programme (4), no infections with GLRaV-3, GLRaV-6, GLRaV-2, RpRSV-ch, RpRSV-g, SLRSV and TRSV were found, while other tested viruses showed low incidence percentages, between 0.8% and 1.7%.

Table 1. Incidence of fourteen viruses in ARI screenhouse grapevine collection

Virus	Infected samples (out of 119 tested)	% Infection
Grapevine fanleaf virus (GFLV)	2	1.7
Arabis mosaic virus (ArMV)	1	0.8
Grapevine leafroll-associated virus 1 (GLRaV-1)	12	10.1
Grapevine leafroll-associated virus 2 (GLRaV-2)	-	-
Grapevine leafroll-associated virus 3 (GLRaV-3)	-	-
Grapevine leafroll-associated virus 6 (GLRaV-6)	-	-
Grapevine fleck virus (GFkV)	2	1.7
Grapevine virus A (GVA)	13	10.9
Raspberry ringspot virus-ch strain (RpRSV-ch)	-	-
Raspberry ringspot virus- g strain (RpRSV-g)	-	-
Strawberry latent ringspot virus (SLRSV)	-	-
Tobacco ringspot virus (TRSV)	-	-
Tomato ringspot virus- Chikadee strain (ToRSV-Ch)	2	1.7
Tomato ringspot virus-peach yellow bud mosaic strain (ToRSV-PYBM)	2	1.7

Infected plants were quarantined and additional sanitization work, including tissue culture combined with thermotherapy, was initiated. Healthy plants for the establishment of nuclear pre-basic stock were selected in some cases. Preliminary results of this work are presented in Table 2. Development of nuclear pre-basic plants has been almost completed for 28.5% of the grapevine collection. Molecular analyses, both for variety identification and virus detection are in progress.

Table 2. Development of ARI grapevine genebank

Stock Accessions	No. of genotypes processed	No. of selection lines	No. of successful lines		
			Forced canes	Established in vitro	Established pre-basic nuclear plants
Rootstocks	11	11	8	7	1
Local red wine varieties	4	13	13	9	1
Local white wine varieties	6	14	13	12	6
Local table red seeded varieties	2	21	20	18	1
Worldwide red wine varieties	12	16	16	16	8
Worldwide white wine varieties	12	13	13	12	6
Worldwide table red seeded varieties	5	7	7	7	3
Worldwide table red seedless varieties	4	7	7	7	3
Worldwide table white seeded varieties	10	11	11	9	2
Worldwide table white seedless varieties	6	6	6	6	3
TOTAL (%)	72	119 (100%)	114 (95.8%)	103 (86.6%)	34 (28.5%)

Acknowledgments

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SANITARY IMPROVEMENT OF GRAPEVINE IN GREECE: CREATION OF A GENETIC BANK OF GREEK VARIETIES *IN VITRO*

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Introduction

Use of virus-free propagation material is an important factor to improve quality and quantity of grape production. Many viticultural countries of the world have set up systems for selecting grapevine material free of the most important viruses and for distributing and certifying this material (3, 4, 5, 8). In Greece, regular virological screening of grape varieties for the production of virus-free material started in 1998 in the frame of the project INTERREG II entitled "Improvement of the grapevine germplasm and production of certified grape propagated material in Crete, Ioannina, Lemnos and Samos". Some results of this work were presented at the 13th ICVG Conference in Adelaide (7).

In this work are presented a) the results of the sanitary status of 37 additional varieties coming from three Greek islands (Chephalonia, Rhodos, Santorine) and b) the creation of a genetic bank of Greek traditional wine producing varieties *in vitro*.

Materials and methods

Field surveys for selection of the most productive and healthy looking vines were conducted during the period 1999-2002 in 81 vineyards of Chephalonia, 25 of Rhodos and 32 of Santorine islands. Mature canes were collected from a total of 855, 594 and 113 vines respectively. In total, 37 local wine producing varieties were studied. All samples were analysed for the presence of the following six viruses: *Grapevine fanleaf virus* (GFLV), *Grapevine leafroll-associated viruses 1* and *3* (GLRaV-1 and GLRaV-3), *Grapevine virus A* (GVA), *Grapevine virus B* (GVB) and *Grapevine fleck virus* (GFkV). Cortical scraping extracts were prepared and tested by DAS-ELISA. Polyclonal antisera were used coming from Bioreba AG (Reinmach, Switzerland) and Agritest (Valenzano, Italy).

For the establishment of the genetic bank *in vitro* dormant cuttings of 2-3 nodes of a total 43 Greek local grape varieties (107 genotypes) were planted in perlite for sprouting. One bud microcuttings, 2 cm long, were cultivated in glass tubes containing as nutrient medium modified Murashige and Skoog and were incubated in growth chambers at 23-25o C with 16h photoperiod, 2000-2200 Lux light intensity and 70% relative humidity. In case the plants were to be transplanted *ex vitro*, four weeks after planting were transferred from the tubes to a mixture of peat:perlite (1:4). The acclimatization was performed in Plexiglas boxes (6).

Results

Serological assays (Table 1) showed that from the 37 varieties tested 16 were infected by at least one virus (43,2%). However, virus-free vines were in all cases easy to be found. The most healthy material was found in Santorine where the vines are self-rooted. GLRaV-1 prevailed (6,5%), followed by GLRaV-3 (3,7%), GFLV (2,6%), GFkV (1,7%), and GVA (0,8%).

Table 1:Results of serological detection (ELISA) of six grapevine viruses on 37 local varieties in three Greek islands

Region	Vines	Diseased vines	Viruses					
			GFLV	GLRaV-1	GLRaV-3	GVA	GVB	GFkV
Chephalonia	855	107	4	90	5	7	0	4
Rhodos	594	105	37	11	52	0	-	23
Santorine	113	6	0	0	0	6	-	0
Total	**1562**	**218**	**41**	**101**	**57**	**13**	**-**	**27**

Today, over 5000 grape plants in glass tubes are preserved in four growth rooms. Four hardening rooms are used for the acclimatization of the plants.

Discussion

In the frame of the project INTERREG II (1998-2001) the Plant Protection Institute of Volos was charged to create a grapevine germplasm collection of Greek varieties *in vitro*. The creation of this collection aimed at both the survival of valuable grapevine material of local Greek traditional varieties and the production of high quality, healthy grapevine propagated material. The collection was enriched also with new varieties from other grape producing areas of Greece. At the end of the year 2003 is expected that the local varieties of Zakynthos, Rapsani (Olympus mountain) and Tyrnavos to be added to the collection.

In the cases of the grape producing areas of Crete, Ioannina, Lemnos and Samos the material was clone selected for 3 years by scientists from other Institutes and was tested serologically for the presence of 13 viruses and, in addition, by woody indexing on seven indicator species (7). In the other cases, the selection was made during the summer period and was

based on visual observations. In the last cases, the material was checked serologically (DAS-ELISA) for the presence of six of the most important viruses.

The closterovirus GLRaV-1 appears to be the most widespread in this study and it was detected in 101 out of 1562 vine specimens tested (overall incidence 6,5%). Similar results were recorded during an extensive survey of leafroll disease of grapevine carried out in the main viticultural areas of Greece. GLRaV-1 prevails in South Greece and the islands, while GLRaV-3 has a higher incidence in Northen Greece (2). By contrast, levels of infection determined for the other viruses tested were much lower. In Rhodos, the most widespread virus was GLRaV-3, while in Santorine the only virus detected was GVA. In Santorine the percentage of the infected stocks was low (5%), since the vineyards were established with self-rooted propagated material. The GFLV-vector nematode *Xiphinema index* Thorne and Allen is present in Rhodos and it should be taken into consideration when growers replant their vineyards (1).

Virus-free genotypes are transferred from the genetical bank *in vitro* into a mother block for further propagation. It is very important that Greek wine producers realized that sanitary selection is essential and integral part of clonal selection for optimal vine performance, including wine quality. Sanitary selection must actually proceed genetic clonal selection, in order to allow the evaluation of true genetic differences between clones. Virus and virus-like diseases may alter the phenotypic expression of the genetic characters. Today, the genetic bank comprises 43 local wine producing Greek grape varieties and five rootstocks (110R, 140Ru, 41B, 1103P, SO_4).

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CERTIFICATION OF GRAPEVINE PLANTING MATERIAL AT RESEARCH STATION FOR VITICULTURE STEFANESTI, ROMANIA

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Vitis genus with its numerous species is considered to be one of the most infected crops with viruses (4). The strategy for an ecological viticulture is based on the most advanced biotechnologies for the obtaining of virus and mycoplasma – free planting material. According to the European and International Standards, the production of grapevine certified material to national level requires the application of virus elimination technology.

The activity for sanitary selection and virus elimination in grapevine was carried out at Research Station for Viticulture Stefanesti-Arges since 1988, according to the National Planning for producing planting material, and was based on the certification schemes used in European countries with great production of grapes and wine (3). The program for obtaining virus-free grapevine plants was constantly developed over the last years due to the increasing number of cultivars and clones needed to be available as healthy material. According to the certification scheme, our laboratory tested over 190 cultivars for virus infection using herbaceous test plants and woody indicator plants. ELISA test has been used routinely for the detection of GFLV, ArMV and GLRaVs

In conclusion, the biological value of grapevine planting material obtained by thermotherapy is guarantied and is in accordance with requirements stated in Law 266/2002 and Order 244/ 2002, as follows:

-the grapevine material from nuclear stock and germplasm field is included in “pre-basic” biological category. This material can be used for research activities and for establishing the pre-propagation field. Research Station for Viticulture Stefanesti-Arges was entitled as curator for all the cultivars and clones mentioned in the Official Catalogue;

-the material from the pre-propagation field or mother plantation (scion and rootstock), is included in the “basic” category, and can be delivered to farms as ″certified″ material;

- the grapevine material from “certified” category can be produced by any authorized nurseries by using exclusively mother plants belonging to “basic″ category.

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FIRST DETECTION OF AN AMPELOVIRUS, A MACULAVIRUS AND TWO VITIVIRUSES IN IRANIAN TABLE GRAPES

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The total area allocated to grapevine cultivation in Iran is estimated at around 250,000 hectares with an annual production of 2.5 million tonnes, mainly as table grapes and as dried fruit (1). Since phylloxera is not a pest in Iran, traditional varieties are planted on their own roots as bush vines.

Although the occurrence of grapevine fanleaf virus (GFLV) in Ourmia, North-West Iran has been reported (3), no records of the presence of other viruses are available. However, typical leafroll symptoms have been observed on red varieties in Fars, a major grape-growing province located in the South of the country. Here, we report the detection of three grapevine viruses, each from a different family, in vineyards of Southern Iran.

Vine samples were collected from 20 vineyards in three Iranian provinces of Isfahan (central Iran, courtesy of Dr. Masoud Bahar), Khuzestan (Southwest) and Fars (Southeast). Phloem shavings from green and mature cane were obtained from local red and white table grape varieties in spring or late summer and macerated in a lysis buffer (1:10 w/v) containing 4 M guanidine hydrochloride, 0.2 M sodium acetate, pH 5.0, 25 mM EDTA, 2.5 % polyninyl pyrrolidone (PVP-40), 20% Sarkosyl and 1% freshly added sodium metabisulphite. The extracts in 1 ml screw-cap tubes (5) were sent to Australia for virus assay. Total RNA was extracted using Qiagen RNeasy minicolumns according to the manufacturer's protocol. The RT-PCR assay using specific primers for the following viruses (5) was carried out as described by MacKenzie *et al*. (7):
Closteroviridae: Seven viruses: *Grapevine leafrol- associated virus* (GLRaV) types *1, 2, 3, 4, 5, 9* and Grapevine rootstock stem lesion-associated virus.
Vitivirus: *Grapevine viruses A* and *B* (GVA and GVB).
Foveavirus: *Grapevine Rupestris stem pitting-associated virus* (GRSPaV).
Maculavirus: *Grapevine fleck virus* (GFkV).
Nepovirus: *Grapevine fanleaf virus* (GFLV).

Of a total of 80 samples tested, 40 had either GLRaV-1 (*Ampelovirus*) or GVA (*Vitivirus*), or a mixture of both viruses (Table 1). Mixed infection of GLRaV-1 and GVA has also been observed in Australia, where for 942 samples which tested positive for either GLRaV-1 or GVA, 16% (mostly table grapes) were infected with both viruses (unpublished). However, this rate of co-infection was higher in the Iranian samples (Table 1).

GVB was detected in 7 samples, while GFkV (*Maculavirus*) was detected in 5 samples. No virus was detected in samples collected from Isfahan. Of 50 samples tested from Fars, 22 tested negative to all the viruses assayed. This indicates that it is possible to initiate a sanitary selection program using locally grown grapevine varieties. All samples collected from Khuzestan tested positive for one or more viruses (Table 1).

Table 1. Viruses detected in Iranian vineyards

Province	Vineyards	Samples	GLRaV-1 only	GVA only	GLRaV-1 + GVA	GVB	GFkV
Isfahan	10	10	0	0	0	0	0
Fars	7	50	10	3	7	6	2
Khuzestan	3	20	1	2	17	1	3
Total	**20**	**80**	**11**	**5**	**24**	**7**	**5**

None of the Iranian samples tested positive for GRSPaV, a virus detected in 68% of symptomless vines from Australia (4). Twelve samples of own-rooted local varieties from the Shanxi Province in North China also tested negative to GRSPaV (kindly provided by Runzhi Li, unpublished). It is possible that GRSPaV entered Australia via infected American rootstocks which are used to control Phylloxera both in the United States as well as in Europe.

It has been suggested that GLRaV-1 exists as a mixture of sequence variants in the same vine (6). This was confirmed when we compared the T_m of a 174 bp amplicon from the coat protein gene using the virus specific primer pair of p35LR1h (AAT CCT ATG CGT CAG TAT GC) and p35LR1c (TGG CAT CGT TGC TAA ATT GAG) (2) from different samples. When GLRaV-1 amplicons in the presence of Sybr Green were subjected to slow melting analysis (8), three melting peaks (melting temperatures or T_m) of 84, 86 and 88° C were observed (Table 2, Fig. 1), indicating the presence of a heterologous population of GLRaV-1 in a single sample (Table 2). Most samples contained the predominant variant GLRaV1-86 which had a T_m of 86° C (Fig. 1B and GLRaV-1-86, Table 2). However, GLRaV-1- 84, when present, always appeared as a shoulder (Fig. 1B). Only five of the 20 samples examined contained GLRaV-1-88 with a peak T_m of 88 (Fig. 1A, Table 2 GLRaV-1-88), while 15 others had GLRaV-1-86 as their major variant. Table 2 also shows that detecting GLRaV-1 ampicons by melting curve analysis is more sensitive than its detection by the conventional agarose gel/ethidium bromide staining method. It has been suggested that a single mismatch may be responsible for the shift in the T_m (8), and hence the detection of the mutant. The relationship of these T_m variants with other properties of GLRaV-1 is not known.

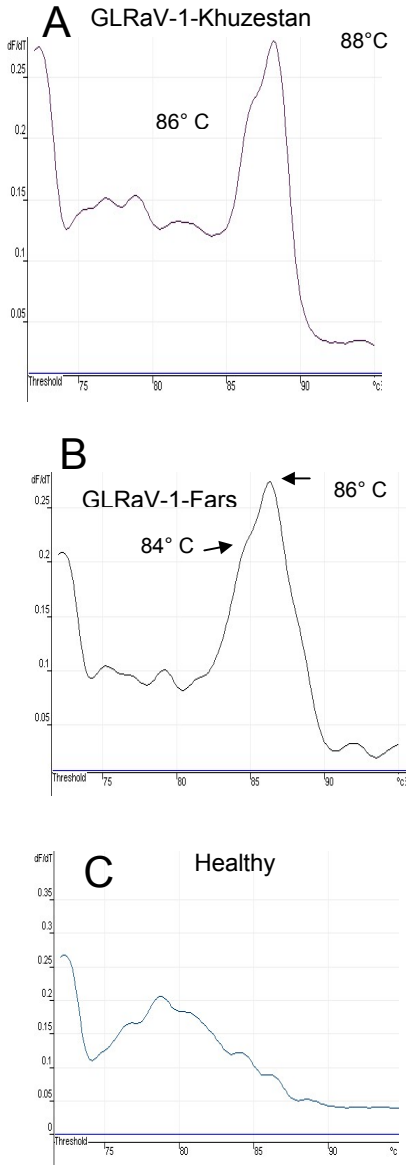

Fig. 1. Typical fluorescence melting peak analysis of GLRaV-1 amplicons in the presence of Sybr Green 1. The T_m for each sample from Iran (A and B) is shown by arrows. Panel C is a healthy Shiraz sample.

Table 2. Melting temperature (T_m) (♣C) of GLRaV-1 amplicons in the presence of Sybr Green G

Country	Sample	Vineyard	**GLRaV-1-84**	GLRaV-1-86	GLRaV-1-88	PCR band
Iran	AA6-F1	Sheidan 2	84.2[1]	86.2	**88.3**	1[2]
	AA7-F1	Sheidan 3	np[3]	86.6	**88.2**	2
	AA9-F2	Imamzadeh 1	84.6	**86.3**	np	5
	AA18-F3	Saadatshahr 1	np	86.5	**88.2**	1
	AA19-F3	Saadatshahr 3	84.4	**86.6**	88.2	2
	AANov02-06	Mezaigan 8	84.4	**86.4**	88.2	4
	AANov02-07	Mezaigan 14	np	**86.3**	88.2	5
	AANov02-21	Urmia-2	np	**86.2**	88.2	5
	AANov02-22	Urmia-1	np	**86.2**	88.2	3
	KhuRam03	Ramhormoz 1	84.4	**86.4**	np	1
	KhuRam04	Ramhormoz 1	84.5	**86.3**	np	4
	KhuRam13	Ramhormoz 2	np	**86.6**	88.2	1
	KhuRam18	Ramhormoz 2	84.6	86.2	**88.2**	5
	KhuRam19	Ramhormoz 2	np	**86.6**	88.2	3
New Zealand	685-20 NZ	ChardMendoza	84.4	**86.4**	88.2	2
Australia	692-7A	Yenda	84.4	**86.2**	88.2	3
	615-2	Coonawarra	84.4	**86.4**	88.3	1
	567-F	Jacobs	np	86.2	**88.2**	1
	CS-13	Abbey	84.4	**86.4**	88.2	3
	Grenache	Waite	84.4	**86.2**	np	1

[1] Thin digits show that the T_m peak appeared as a minor or as a shoulder peak; bold digits imply a major T_m peaks. [2] 1: no PCR band, 5: a strong PCR band.
[3] np: no T_m peak was observed.

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THE PRODUCTION OF GRAPEVINE CERTIFIED PLANTING MATERIAL IN THE UKRAINE

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In Ukraine, the researches of clonal selection realized at Tairov Research Institute of Viticulture and Enology (Odessa) and at Institute of Viticulture and Enology "Magaratch" (Yalta, Crimea). According to the "Technology of Production the Certified Planting Matrisl of Fruit-trees, berry cultures and grapevine" (Moscow, 1989) (1) the grapevine clones should be free from GFLV, GLRaV 1, GLRaV 3, GFkV, Rugose wood complex and from crown gall disease.

For the detection and the identification of viruses we used ELISA-test and grafting method. The indicator varieties are the next: Cabernet Franc, *Vitis rupestris* St.George and Kober 5BB). For ELISA-test we used the sets produced by Agritest (Italy). During the last two years we tested the clones not only produced at the Ukraine but also introduced to the Ukraine from France and Moldova.

As a result of our research it was established that on the South of the Ukraine the most distributed viruses are: GFLV (13%), GLRaV 3 (13%) and GFkV(80%). In Crimea the most prevalent viruses are: GLRaV 1 (50%), GFLV (3%), GLRaV 3 (3%) and GFkV (6%). GLRaV 1 and GLRaV 3 (100%), GFLV (80%) and GFkV (12%) infected the grapevine plants introduced from Moldova. Clones of Cabernet Sauvignon, Chardonnay, Pinot noir, Pinot menje cultivars for France were free from virus infection. However, the Cabernet Sauvignon from France planted at 2000 appeared to be infected by crown gall disease (24%).

For testing the grapevine plants on the presence of *Agrobacterium vitis* we applied the Lechoczky method (2) and Roy and Sasser semi-selective media (3). Colonies with characteristic morphology we replanted on potato dextrose agar snd checked the bacteria culture by the ELISA-test and polyclonal antiserum that was prepared to some *A.vitis* strains. The pathogenicity test was provided by using test-plants of tomatoes, disks of carrots and green grapevine cuttings. For further dividing the pathogenic from non-pathogenic strains we used PCR method (ipt primers)(1). For the PCR we used 2 days culture bacteria growing on potato agar media. We also studied the bleeding sap from infected plants. The results have shown that 24, 1% of visually asymptomatic Cabernet Sauvignon plants are infected by *A.vitis* (Fig.1). When studying the bleeding sap from infected plants by PCR we did not find *A.vitis*. This result corresponds with Szegedi's and Botka's data (4).

In connection with distribution of crown gall disease to the Ukrainian vineyards, the program of grapevine clones certification should be extended and includes not only the clones free from grapevine viruses but also free from crown gall disease (5).

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LATENT INFECTIONS BY DIFFERENT VIRUSES RECENTLY DETECTED IN GRAPEVINE DURING SANITARY SELECTION IN LOMBARDIA (NORTHERN ITALY)

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Clonal and sanitary selection of the grapevine in Lombardia (Northern Italy) was initiated more than 40 years ago (1). At the beginning it was mainly based on visual selection; then it was continuously improved through the introduction of biological and serological tests (2). Now molecular tests are also used frequently (e.g., for Rupestris stem pitting).

In recent years several candidate clones, that did not show any symptom during two growing seasons, were tested by DAS-ELISA for the presence of the following viruses: *Grapevine fanleaf virus* (GFLV), *Arabis mosaic virus* (ArMV), *Grapevine leafroll-associated viruses 1* and *3* (GLRaV-1, -3), *Grapevine fleck virus* (GFkV), *Grapevine virus A* (GVA) and *Grapevine virus B* (GVB).

Out of 126 candidate clones, selected in four viticultural districts (Oltrepò pavese, Colli mantovani, Valtellina and Valtenesi), 65 gave negative results while 61 resulted to be infected with one or more of the tested viruses. The results obtained are summarized in Table 1 and deserve some considerations.

1. ArMV and GVB were never found in any of the tested vines.
2. All the other viruses produced latent infections.
3. Particularly frequent were latent infections caused by GLRaV-1 and by GFkV in cv Chiavennasca, where the two viruses often resulted to be present in mixed infections. Therefore Chiavennasca seems to be a tolerant variety for those viruses.
4. GVA was only found in Valtellina and in Colli mantovani, always in double infection with GLRaV-1. This could be caused by a double transmission made by the same vector, as already reported (3).
5. Quite rare were latent infections caused by GFLV and GLRaV-3. This probably means that the tested varieties are sensitive to those viruses and visual selection could discard most of the infected clones.

Table 1. Virus infections detected by ELISA in candidate clones selected in vineyards of Lombardia.

Cultivar	Tested samples No.	Negative samples No.	POSITIVE FOR						
			ArMV	GFLV	GLRaV-1	GLRaV-3	GFkV	GVA	GVB
Oltrepò pavese									
Barbera	12	8	0	1	0	0	4	0	0
Croatina	21	17	0	0	0	2	2	0	0
Merlot	3	3	0	0	0	0	0	0	0
	36	28	0	1	0	2	6	0	0
Colli mantovani									
Lambrusco Viadanese	18	6	0	0	9	2	2	9	0
Valtellina									
Chiavennasca	33	10	0	1	19	2	10	6	0
Fortana	1	0	0	0	1	0	1	1	0
Pignola	2	2	0	0	0	0	0	0	0
Rossola	5	1	0	0	3	0	2	1	0
	41	13	0	1	23	2	13	8	0
Valtenesi									
Cabernet franc	13	9	0	3	0	0	1	0	0
Groppello	9	3	0	4	0	2	1	0	0
Nebbiolo	1	0	0	1	0	0	0	0	0
Trebbiano di Lugana	8	6	0	1	0	1	0	0	0
	31	18	0	9	0	3	2	0	0
TOTAL	**126**	**65**	**0**	**11**	**32**	**9**	**23**	**17**	**0**

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SURVEY OF FILAMENTOUS VIRUSES IN PORTUGUESE VINEYARDS

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Viral diseases remain one of the main threats to quality and yield of grapevine production in Portugal. In addition to yield loss, high sensitivity to viral infection could lead to the elimination of interesting clones. Diagnosis of viral diseases has been hindered by the lack of suitable ELISA reagents for a significant number of viruses affecting grapevine. The use of methodologies targeting the viral genome, such as RT-PCR, may circumvent this problem. Previous surveys on the sanitary status of budwood material used for grapevine propagation and vineyards in Portugal revealed a significant presence of several Closteroviruses, Vitiviruses and *Rupestris stem pitting-associated virus* (2,3,4). In this work we report the results of an extended survey of vineyards for diverse regions in Portugal. The methods and primers used were previously described (4). The primers LR127 and LR45 are degenerate primers targeting the HSP70 and were designed in such way to be able to detect *Grapevine leafroll-associated viruses 1*, *2* or *7* and *4* or *5*, respectively. *Grapevine leafroll-associated virus 3* was specifically detected with primers LR3 (4). Primers RSP48 and 49 were used to detect RSPaV (provided by Dr. A. Rowani). The primers GVA1:5´ AACACTCTCTTCGGGTACAT 3´ and GVA2:5´ TATATCTCAACAGCCTGCTC 3´, were used to detect GVA and they amplify the coat protein region of *Grapevine virus A*.

Approximately 400 samples from 12 wine regions (DOC) were tested. The results obtained are presented on Table 1. As expected, based on previous results from our lab (2,3), the prevalence of RSPaV was high in all regions. In contrast with a previous study focused on nursery material (1), the prevalence of leafroll associated viruses found in this work, in the vineyards, is significantly higher. GVA incidence is low (8%).

Table 1. Filamentous virus detection in 12 wine regions in Portugal

	Grapevine leafroll associated viruses						RSPaV		GVA	
	1,2 or 7		4 or 5		3					
Region	Nº Samples	%	Nº Samples	%	Nº Samples	%	Nº Samples	%	Nº Samples	%
Vinhos Verdes (Minho)	0/50	0	2/50	4	8/50	16	15/45	33.3	nt*	nt
Douro/Vinho do Porto	2/71	2.8	11/72	15.3	36/81	44.4	70/81	86.4	nt	nt
Bairrada	10/30	33.3	9/32	28.1	13/32	40.6	19/32	59.4	2/32	6.25
Dão	3/51	5.9	5/51	9.8	7/51	13.7	26/51	51	nt	nt
Oeste	17/28	60.7	13/30	43.3	15/29	21.7	20/30	66.7	5/29	17.2
Carcavelos	0/5	0	0/5	0	1/5	20.0	2/4	50	1/4	25.0
Colares	1/12	8.3	3/14	21.4	8/14	57.1	2/13	15.4	0/10	0
Ribatejo	5/20	25.0	2/18	11.1	13/20	65.0	14/20	70	5/20	25.0
Terras do Sado	3/19	15.8	4/20	20.0	2/20	10.0	5/6	83.3	1/19	5.3
Pegões	1/6	16.7	1/6	16.7	1/6	16.7	2/3	66.7	0/6	0
Alentejo	11/48	22.9	9/48	18.8	3/51	5.8	26/56	46.4	0/50	0
Algarve	5/34	14.7	5/33	15.2	13/34	38.2	23/37	62.2	3/34	8.8
Total	58/374	15.5	64/379	16.9	120/393	30.5	224/378	59.3	17/204	8.3

(*) nt - not tested

These samples corresponded to 46 Portuguese varieties. The RSPaV was present in 36 with an incidence of 68.3%. The leafroll associated virus (GLRaV1, 2 and 7; GLRaV4, 5 and GLRaV3) were present in 11, 21 and 28 varieties, with an incidence of 23.5, 28.6 and 44.4%, respectively. Besides the reduced number of samples analysed for GVA, this virus is present in six varieties, with an incidence of 34.3%. The prevalence of these viruses incidence in important Portuguese grape varieties is presented on Table 2.

Table 2. Incidence of some filamentous viruses on six Portuguese grape varieties.

	Grapevine leafroll associated viruses			
	1,2,or 7	4 or 5	3	RSPaV
Varieties	%	%	%	%
Aragonez	0	7.1	53.3	86.7
Arinto	12.0	15.4	22.2	81.8
Baga	41.7	33.3	33.3	41.7
Malvasia-Fina	10.0	11.1	54.5	63.6
Tália	30.0	20.0	77.8	80.0
Touriga-Nacional	15.4	10.0	15.4	92.3

Acknowledgements

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SANITARY STATUS OF WINE GRAPE VARIETIES (*VITIS VINIFERA* L.) IN NORTHERN GREECE ORIGINATING FROM CLONAL SELECTION AND COLLECTION FOR PRESERVATION

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During a project[1] concerning the preservation and clonal selection of wine grapevine varieties of Macedonia, Thrace and Epirus, 107 biotypes were collected. Eighty-two biotypes from 41 varieties, originated from plant collection for preservation that was based on the ampelographic descriptions and the absence of virus-like symptoms. Grapevine plants were marked just before harvest and mature canes were collected in winter for virus indexing. Twenty-five biotypes from 7 varieties (Moschomavro, Xinomavro, Athiri, Assyrtiko, Negoska, Agiorgitiko and Limnio), originated from clonal selection that started in old vineyards from areas, which are representative of cultivation for each variety. Clonal selection was performed by following a combined protocol based on the respective ones of France (1), Italy (2) and the Laboratory of Viticulture of the Agricultural University of Athens. Biotype selection was based on the typical characteristics of each variety with special emphasis on grape characteristics. Ampelographic descriptions, ampelometric measurements of leaves and agronomic characters were observed and recorded for period of three years. Plant material was collected and kept *in planta* in pots and *in vitro*.

Phloem grapevine tissue (cortical scrapings) was tested serologically by ELISA by using commercially available diagnostic kits, for the presence of *Grapevine fanleaf virus* (GFLV), *Tomato black ring virus* (TBRV), *Arabis mosaic virus* (ArMV), six different closteroviruses (*Grapevine leafroll-associated virus 1,2,3,5,6,7*, GLRaV -1,-2,-3,-5,-6,-7) and two vitiviruses (*Grapevine virus A, B*, GVA, GVB). The same samples were also tested by RT-PCR (using two primer pairs), for the presence of *Rupestris stem pitting associated virus-1*, (RSPaV-1) (3).

Total results from all 107 biotypes, indicated high incidence of GFkV (42%), GVA (21%), GLRaV-1 (12%), GLRaV-2 (22%), GLRaV-3 (21%), and GFLV (13%), whereas incidence of GVB (2%), GLRaV-5 (3%), GLRaV-6 (1%) and GLRaV-7 (6%) was lower. Finally, RT-PCR revealed high infection levels of RSPaV-1 (79%) whereas ArMV and ToBRV were not detected (Table 1).

Results indicate that plants originating from clonal selection had a lower incidence of GFLV, GVA, GLRaV-1, -2 and -7 compared to plants originating from plant collection for preservation (Fig. 1) probably due to the strict criteria of the clonal selection (observation during a three years period). Incidence of two latent viruses (GFKV and RSPaV-1) and GLRaV-3 was very high and it was independent of the way the biotypes were selected. Consequently, clonal selection reduces the risk of infection by viruses causing characteristic virus-like symptoms. However, even the clonal selection protocol is inadequate to reduce the risk of infection by latent viruses such as GFKV and RSPaV-1.

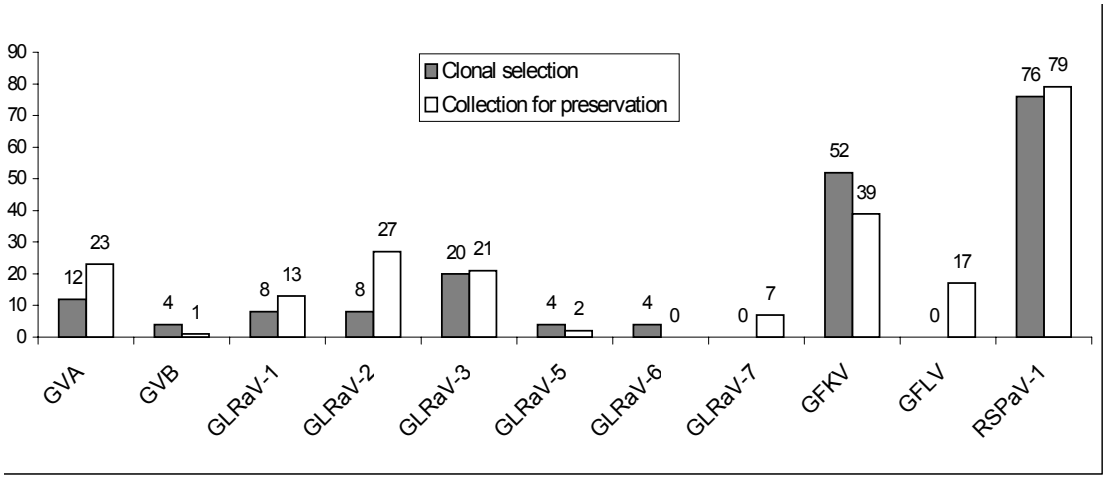

Figure 1. Percentage of virus incidence in plants originating either from clonal selection or collection for preservation

[1]the project was financed by the Wine Roads of Macedonia (Leader II).

Table1. Incidence of viruses in 107 plants originating from clonal selection and collection for preservation

Variety	GVA	GVB	GLRaV-1	GLRaV-2	GLRaV-3	GLRaV-5	GLRaV-6	GLRaV-7	GFkV	GFLV	RSPaV-1
Moschomavro	2/6		1/6	2/6	1/6	1/6	1/6		4/6		5/6
Xinomavro	1/9	1/9							3/9		7/9
Athiri			1/1		1/1						0/1
Assyrtiko					1/1				1/1		1/1
Negoska					2/5				5/5		4/5
Agiorgitiko			1/1								1/1
Limnio											1/2
Zoumiatiko					1/1				1/1		1/1
Trinka			1/1						1/1		1/1
Koiniaro	3/4								1/4		4/4
Karapapas*											1/1
Sefka	3/7	1/7	5/7	5/7	4/7				6/7		6/7
Asprouda				1/3	1/3						2/3
Mavroudi				1/3	1/3				2/3		3/3
Pamidi				2/4	1/4				1/4	1/4	3/4
Roditis	3/6			1/6	2/6			2/6	3/6		5/6
Fartsalo											1/2
Karnahalas	1/1		1/1	1/1						1/1	1/1
Tsougianides				1/1				1/1			1/1
Voulgaroudia	1/1		1/1							1/1	1/1
Muskat blanc of Soufli				1/1							1/1
Bogiamas				1/1							1/1
Keratsouda				1/1	1/1				1/1		1/1
Aleponoura				1/1					1/1		1/1
Vergiotiko									2/2		1/2
Sklithro				1/2					2/2	1/2	2/2
Nevro			1/2								2/2
Korithi n									1/1	1/1	1/1
Nigrikiotiko	1/3								3/3	1/3	3/3
Korithi b					1/1				1/1	1/1	1/1
Nerodebina										1/1	0/1
Muskat noir	1/2			1/2	1/2				1/2		2/2
Kokinouska				2/2					2/2		2/2
Cinsaut	1/2			1/2	1/2	1/2		1/2	1/2		2/2
Italiko									1/1		2/1
Stavroto	1/1										2/1
Debina	1/10		1/10		1/10	1/10		1/10	1/10	1/10	7/10
Koukouli	1/1									1/1	2/1
Malagousia	1/1									1/1	0/1
Preknadi									1/1		0/1
Batiki											1/1
Fokiano									1/1		0/1
Tsapournakos*								1/1			0/1
Vapsa Velventou	1/5			1/5	2/5					2/5	3/5
Vlachiko											0/1
Pachipeko Piknoasa			1/1							1/1	1/1
TOTAL	**22/107**	**2/107**	**13/107**	**24/107**	**22/107**	**3/107**	**1/107**	**6/107**	**45/107**	**14/107**	**84/107**
	21%	**2%**	**12%**	**22%**	**21%**	**3%**	**1%**	**6%**	**42%**	**13%**	**79%**

*Biotypes not officially registered

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VIRUS TESTING AND THE CANADIAN PLANT PROTECTION EXPORT CERTIFICATION PROGRAM FOR GRAPEVINE NURSERY STOCK (PPECP)

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Grapevine viruses and virus-like diseases often have a significant economic impact on grapevine growth, yield, and fruit quality. They may adversely affect winter hardiness, cause graft incompatibility, and increase plant mortality. Production costs are often higher. Full range virus testing combined with a comprehensive grapevine certification program are needed to reduce or eliminate these detrimental effects.

Canadian Plant Protection Export Certification Program for Grapevine Nursery Stock (PPECP) (1)

The Canadian Plant Protection Export Certification Program for Grapevine Nursery Stock (PPECP) is the national, Canadian grapevine certification program. The PPECP is a voluntary certification program administered by the Canadian Food Inspection Agency (CFIA) (2), Canada's National Plant Protection Organization (NPPO). Established prior to 1980, the PPECP is based on the propagation of grapevines derived from mother plants tested in Canada at the Nuclear level (i.e. Nuclear Stock) under conditions that prevent disease contamination. These grapevines, when propagated, normally descend through successive certification levels from Nuclear to Elite, to Foundation, and to Certified Planting Stock. Grapevine plants and propagative material produced under the program are of the highest phytosanitary quality. This material meets the import requirements of the United States and other countries such as New Zealand and Australia. The plants are distributed widely to Canadian nurseries and viticulturists.

Varieties and rootstocks must be fully tested for viruses and virus-like diseases at the Centre for Plant Health (CPH) in Sidney, British Columbia, Canada to be eligible for the PPECP. A mother plant from a candidate variety is chosen for full range testing. Testing is done by ELISA, PCR (2), and inoculation on herbaceous and woody bioassays (table 1). Each candidate plant is indexed on herbaceous and woody bioassays and tested by at least one lab-based test for as many viruses as possible.

Following testing the mother plants are either kept at the Nuclear level in isolated, screened houses or field plots at the CPH or, distributed to the proprietary owner where they are eligible for planting at the Elite level. While in the Nuclear repository they are regularly tested for naturally transmitted viruses. Testing on Nuclear plants is continuously being upgraded by new, more sensitive techniques. Testing for newly identified viruses or virus-like diseases are done as tests become available. Propagative material is distributed from the mother plants to nurseries registered in the PPECP.

The PPECP is a comprehensive phytosanitary certification program administered across the country by Canada's NPPO. Growers intending to participate in the PPECP must apply for approval at least three (3) months prior to planting. Both the production practices and planting material must meet specific requirements. Prior to approval, all planting sites must be sampled for the presence of virus-vectoring nematodes. Sites are re-sampled on a regular schedule for nematodes. The detection of these nematodes in Elite and Foundation sites results in more regular nepovirus testing. Testing for nepoviruses known to occur in Canada is done upon nematode detection and every five years thereafter.

Elite and Foundation blocks cannot be planted on land on which non-certified *Vitis* spp. have been grown within the last 10 years, or non-certified fruit trees or other non-tested rosaceous plants within the last two years. All sites must be separated from non-certified grapevine plantings by prescribed buffer zones. Suitable precautions must be taken to prevent the introduction of virus-vectoring nematodes. Accurate records of plantings, purchases and sales of plant material produced under the PPECP must be kept.

Plants are inspected by the CFIA at least once during each growing season, and at other times as deemed necessary. Material suspected of being infected may be tested. The PPECP identifies the conditions under which certification of a variety, planting site or grower may be suspended or cancelled, and re-established.

Virus Testing Activities at the Canadian Centre for Plant Health in Sidney, BC, Canada (4)

The Centre for Plant Health (CPH) in Sidney, British Columbia is the CFIA`s national post-entry quarantine and virus testing facility for grapevines, fruit trees, small fruit, and crops other than potatoes. It is the only Canadian facility accredited to quarantine and test foreign non-certified grapevines. The CPH tests varieties on behalf of Canadian and foreign consignees. Established in 1966, the Centre has received 2656 foreign varieties and rootstocks for quarantine and testing for viruses and virus-like diseases. In addition, 1680 domestic grapevine varieties and rootstocks have been submitted from Canadian breeders, growers and nurseries. Custom testing for selected viruses is also done.

The CPH is responsible for testing all imported non-certified grapevines and tree fruit plants. Canadian import requirements permit commercial importation only from a few approved foreign certification programs. Other plants may be imported in small quantities through the CPH. Plants or their propagative material are released to the importers after full range testing. Plants in which viruses or virus-like diseases are detected are either subjected to virus elimination by hot air thermotherapy or a replacement plant is obtained and tested. New plants are tested after thermotherapy. During testing, the imported mother plants are maintained as potted plants in isolated, insect resistant screened houses.

The CPH is responsible for testing samples (audits) taken from approved commercial grapevine and tree fruit importations. This audit testing allows the CFIA to monitor the status and compliance of foreign certification programs.

The CPH maintains a collection of over 100 virus-tested grapevine varieties and rootstocks in a secure repository that prevents infection by naturally transmitted viruses. Potted mother plants are maintained in screened houses. Additional plants may be placed in isolated field blocks. Propagative material from these plants are eligible for export and used to establish certified blocks under the PPECP. Repository material is distributed around the world. Proprietary conditions are upheld.

In addition to these activities, the CPH is involved in research and technology development. Research is carried out on virus isolation, identification and purification (5). New diagnostic tests are developed and implemented to improve virus detection (3). Tests developed in other laboratories are evaluated and validated for possible implementation (6). The CPH maintains a collection of grapevines infected with viruses and virus-like diseases from around the world. These are used as virus controls for tests and in the evaluation of new detection techniques.

The tests currently used at the CPH are listed in table 1. The CPH is ISO accredited under standard 17025 for the ELISA tests, and the herbaceous and woody bioassays. New tests are continuously being added.

Table 1. Diagnostic tests routinely used at the Centre for Plant Health for full range testing of grapevines.

ELISA*:	**PCR*:**
- *Arabis mosaic virus*	- *Grapevine fanleaf virus*
- *Grapevine fanleaf virus*	- *Arabis mosaic virus*
- *Grapevine leafroll-associated virus 1*	- *Tomato ringspot virus*
- *Grapevine leafroll-associated virus 3*	- *Tomato blackring virus*
- *Raspberry ringspot virus*	- *Grapevine leafroll-associated viruses 1, 2, 3, 4, 5*
- *Strawberry latent ringspot virus*	- *Grapevine viruses A, B, D*
- *Tomato ringspot virus*	- *Grapevine rupestris stem pitting-associated virus*
	- *Grapevine fleck virus*
Woody Bioassay Indicators:	- phytoplasmas
- *Vitis riparia* x *berlandieri* 5BB	
- *V.* hybrid LN33	**Herbaceous bioassay indicators:**
- *V. vinifera* Pinot Noir	- *Chenopodium quinoa*, 'New York'
- *V. rupestris* du lot St. George	- *C. quinoa*, 'Summerland'
- *V. riparia* Gloire de Montpellier	- *C. amaranticolor*.

***** Antibodies and PCR primers to other viruses are available to confirm other diagnostic test results.

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GRAPEVINE INSIDIOUS VIRUSES IN SPANISH VITICULTURE

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The grapevine plant material in Spain with respect to breeding nurseries has a contrasted quality, also from the sanitary and genetic point of view. Authorized nurseries have to fulfil current legislation: Technical Regulation of Control and Certification of Grapevine Breeding Plants, that began to be applied from the 1st of July of 1986, later on modified on 24th of June of 1991 and reformed on 21th of February of the present year, in agreement with the European Directive 2002/11/CE that corrects the already obsolete 68/193/CEE one as well.

At the present time we can say that rootstocks and a great part of the wine and table cultivars, have enough favourable conditions to consider this plant material as certified. The greater efforts made by the different Autonomous Communities have been dedicated to the sanitary clonal selection of local cultivars, starting by those of greater importance and those where it exists a sufficient amount of variability to perform clonal selection, so we can speak of varietal quality.

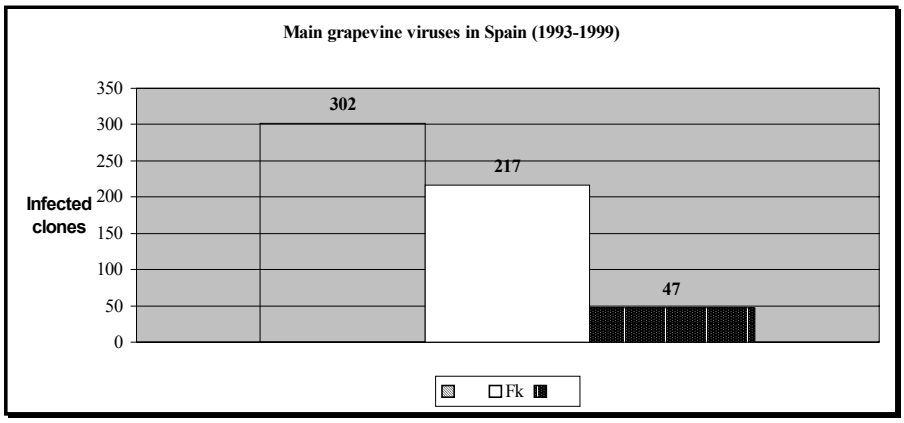
This survey allowed the selection of 1378 clones, which were subsequently tested (ELISA and indexing) for GFLV, GFkV and GLRaV from which 812 clones resulted negative. In the figures it is illustrated the summary of relative incidence of each different virus for the remaining positives clones.

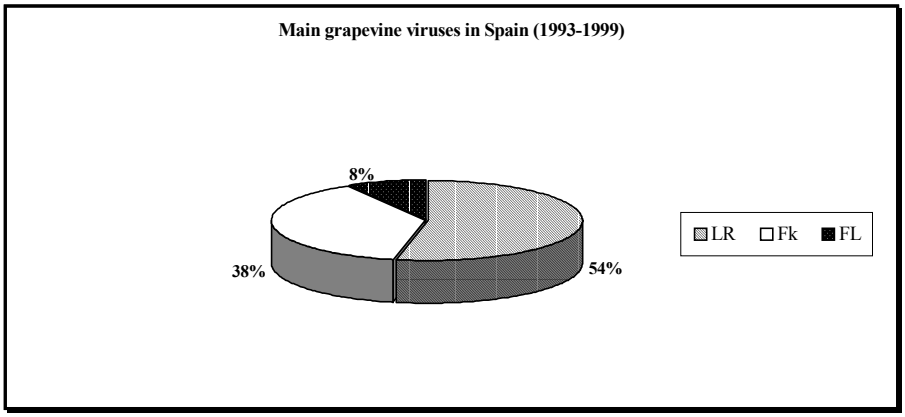
For GLRaV, controls were made considering viruses as a whole, without detaching the different strains, since until recently it has not been possible to discern properly among them. In the case of Spain, determinations have been made for T1, T2 and T3; in the case of T4 there have not appeared any positive samples whereas for T6 there was only a positive case. With respect to T5 and T7 we do not have available data.

Conclusion:

In agreement with the already mentioned legal norms, we can consider that in Spain we have *Vitis* plant material, which is both healthy and genetically adequate.

As an added consideration we have to mention that we do not understand why GLRaV2 is not included in the legislation with an obligatory determination, since our studies have revealed its presence in a relative high percentage of the samples.





SANITARY STATUS OF 7 VARIETIES OF WINE GRAPEVINE IN SOME REGIONS OF CENTRAL ITALY

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In the period 1997-2002, grapevines were selected by their sanitary status from the most important vine-growing areas of Tuscany and another two nearby regions: Bolsena (VT) and Montefalco (PG) situated in Latium and Umbria, respectively. The surveys were not only based on the need to check the health conditions of selected vines against the standard provided for official registration, but also on the need to collect information on the distribution of virus diseases and their potential associations.

The sanitary survey was carried out on 700 individual vines from 7 varieties: Aleatico, Mammolo, Moscadello di Montalcino, Prugnolo gentile, Sagrantino, Sangiovese and Vermentino. The vineyards examined were altogether 92 divided as follows: Aleatico 18, Mammolo 5, Moscadello 6, Prugnolo 7, Sagrantino 22, Sangiovese 30 and Vermentino 4. All the plants were analysed for the presence of the following viruses: Arabis mosaic virus (ArMV), Grapevine fanleaf virus (GFLV), Grapevine fleck virus (GFkV), Grapevine virus A (GVA), Grapevine virus B (GVB) and Grapevine leafroll-associated viruses 1, 2, 3 and 7 (GLRaV 1, 2, 3 and 7). Samples of 20 leaves, randomly collected from each plant selected, were tested by ELISA.

The serological assays (Tab. 1) showed that 508 (72.6%) vines were infected with at least one of the following viruses: GFLV, GFkV, GLRaV 1, GLRaV 3 and GVA, whereas ArMV, GLRaV 2, GLRaV 7 and GVB were not detected.

The frequency of the viruses detected in single or mixed infections may be summarised by the following scale: GVA>GLRaV 1>GLRaV 3>GFkV>GFLV.

The infection rates of the first 4 viruses ranged from 24.9% (GVA) to 20.2% (GFkV). GFLV was detected, instead, on 81 plants (infection rate 9.9%).

GLRaV 1 and GVA were detected mainly in the Sagrantino variety with infection rates of 41.5% and 41.1%, respectively. GLRaV 3 was detected in 169 plants (infection rate 20.7%) and was most widespread (43.7%) in the Aleatico variety.

GFkV was the most frequent virus in the varieties Moscadello (48.3%), Mammolo (44.0%), Sangiovese (35.9%) and Prugnolo (35.3%). GFLV, that was more frequent in Sangiovese (21.2%), was almost absent (0.7%) in the Sagrantino variety.

The results, summarised in Table 1, showed that the health conditions of 5 varieties (Aleatico, Moscadello, Sagrantino, Sangiovese and Vermentino) are extremely worrying. Particularly compromised appear the phyto-virological conditions of Sagrantino (100.0% of infected plants), Aleatico (86.3%) and Moscadello (81.8%). The opposite was true of the varieties Mammolo and Prugnolo, both located in the area of Montepulciano (SI), with about 50.0% of the selected grapevine giving a negative response for all viruses tested.

Table 2 shows the distribution of single or mixed infections in the 508 ELISA-positive plants. 271 (53.3%) vines were infected with a single virus, even if high mixed infection percentages were found in the Moscadello (88.9%), Sagrantino (77.1%) and Vermentino (60.0%) varieties.

All mixed infections showed 21 virus combinations. The association GVA+GLRaV1, apart from the combination observed, proved to be the most widespread one (62.9%). The highest infection rate of this association was found in the Sagrantino variety (91.9%).

The results of the present survey show:

- a high frequency of GVA, GLRaV 1, GLRaV 3 and GFkV in the grapevine germoplasm examined;
- deteriorated sanitary conditions, particularly in the Sagrantino, Aleatico and Moscadello varieties;
- a high incidence of GVA and GLRaV 1 in the Sagrantino variety. The results obtained suggest a long-time presence of these two viruses in this variety.

Table 1. Sanitary status of 700 grapevines of 7 *Vitis vinifera* varieties from central Italy. Frequency of single or mixed infections detected by ELISA. In each row the percentage values refer to overall infections considered equal to 100.

	Plants		**Frequency of detected virus infections (%)**				
Varieties	**tested**	**positives**	**GFLV**	**GFkV**	**GLRaV 1**	**GLRaV 3**	**GVA**
Aleatico	124	107	10.8	8.9	16.2	43.7	20.4
Mammolo	39	17	8.0	44.0	12.0	24.0	12.0
Moscadello	22	18	10.3	48.3	13.8	17.2	10.3
Prugnolo	59	30	14.7	35.3	8.8	26.5	14.7
Sagrantino	144	144	0.7	8.0	41.5	8.7	41.1
Sangiovese	277	167	21.2	35.9	13.4	16.4	13.0
Fermentino	35	25	4.4	15.6	26.7	28.9	24.4
TOTAL	**700**	**508**	**9.9**	**20.2**	**24.3**	**20.7**	**24.9**

Table 2 Distribution of single and mixed infections on 508 grapevine plants after ELISA tests. Percentages are in brackets.

	Infected	Infections		Plants with mixed infection/
Varieties	**plants**	**single**	**mixed**	**overall infected plants**
Aleatico	107	5	14	45/107 (42.1%)
Mammolo	17	4	4	6/17 (35.3%)
Moscadello	18	2	6	7/18 (38.9%)
Prugnolo	30	5	4	4/30 (13.3%)
Sagrantino	144	4	10	111/144 (77.1%)
Sangiovese	167	5	13	49/167 (29.3%)
Vermentino	25	4	8	15/25 (60.0%)
TOTAL	**508**	**5**	**21**	**237/508 (46.7%)**

CLONAL AND SANITARY SELECTION OF THE GRAPEVINE IN THE MARCHE, CENTRAL-EASTERN ITALY

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Clonal and sanitary selection of grapevine, a fundamental activity for improving the quality and quantity of the produce, was carried out in the Marche (Central-Eastern Italy), on both major and minor locally grown varieties all grafted on American rootstocks. For cv. Verdicchio, the main white-berried wine grape variety, the sanitary status of 15 vineyards established from 1900 to 1993 was surveyed. Assays for the presence of *Grapevine virus A* (GVA), *Grapevine virus B* (GVB) (looked for only in major varieties), *Grapevine fanleaf virus* (GFLV), *Grapevine fleck virus* (GFkV), and *Grapevine leafroll-associated viruses 1, 2, 3, 7* (GLRaV-1, GLRaV-2, GLRaV-3, GLRaV-7) were performed by ELISA on cortical scrapings from mature canes collected in winter.

Overall, the main grapevine cultivars proved to be infected by GVA and GLRaV-1 with an incidence of 38% each, followed by GFkV (24.1% infection) and GLRaV-3 (13.1% infection) (Tab. 1). GFLV and GLRaV-2 were found in 11.3% and 3.5% of the tested vines, respectively. The presence of GVB, detected in only three plants, was negligible, while no infections by GLRaV-7 were found. All 61 plants of cv. Lacrima were infected, 59 of which by GVA, while lower infection levels (around 30%) were observed in cvs Pecorino and Trebbiano toscano.

Interestingly, the sanitary status of cv. Verdicchio varied very much according to the age of the vineyards (Tab. 2). The oldest plantings, established around 1900 with own-rooted plants, were free from viral infections, except for two vines with GLRaV-1 and one with GLRaV-3. A vineyard planted in 1958 showed a relatively low incidence of viral infections (30%), while higher levels were recorded in vineyards established in the 1960s, and incidence was almost always over 65% in 1970s and 1980s plantings. An improvement apparently occurred later as the last vineyard surveyed which was planted with certified material, did not contain any of the viruses taken into consideration. In cv. Verdicchio, GVA, GFkV, and GLRaV-3 had the highest incidence (23-27%), being present in 12 of 15 stands. GLRaV-1 and GFLV were detected in 15.4% and 11.5% of the vines, respectively, while a lower incidence of GLRaV-2 (4.9%) and GLRaV-7 (4.0%) was observed. GLRaV-3 was the most frequently encountered virus, while GVA and GLRaV-1 had a lower incidence as compared with the average infection rates of the main cultivars. GFLV, GFkV, and GLRaV-2 had more or less the same incidence. In contrast with all other cultivars, infections by GLRaV-7 (4.0%) were also found.

Local grapevine varieties or clones showed a certain variability of the sanitary status. For instance, cvs Aleatico, Balsamina 2 and 4, Brugentile, Cimiciola car., Cotrognone, Malvasia bianca, and Morgentino were infected by three viruses, several other cultivars and/or clones by one or two viruses, whereas 12 cultivars and/or clones were free from all tested viruses (Tab. 3). Some of the varieties and/or clones showing good pomological and enological characteristics (e.g. Ciliegiolo 68, Gaglioppo 75, and Vernaccia moscatella) can therefore enter already a certification program.

Table 1. Incidence of viral infection in main grapevine cultivars grown in the Marche

Cultivar	Samples (n.)	Infected plants (%)									
		GVA	GFLV	GFkV	GLRaV-1	GLRaV-2	GLRaV-3	GLRaV-7	GVB	Total infected	Total not infected
Lacrima nera	61	96.7	3.3	4.9	83.6	1.6	14.8	0.0	1.6	100.0	0.0
Montepulciano	63	47.6	4.8	26.9	44.4	1.6	6.3	0.0	1.6	74.6	25.4
Passerina	14	21.4	0.0	50.0	0.0	0.0	35.7	0.0	0.0	71.4	28.6
Pecorino	21	14.3	0.0	4.8	23.8	4.8	0.0	0.0	0.0	28.6	71.4
Sangiovese	38	16.1	41.9	29.0	51.6	16.1	29.0	0.0	3.2	81.6	18.4
Trebbiano toscano	6	33.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	33.3	66.7
Vernaccia nera	23	35.3	29.4	52.9	64.7	0.0	5.9	0.0	0.0	73.9	26.1
Total	226										
Average		38.0	11.3	24.1	38.3	3.5	13.1	0.0	0.9	66.6	33.4

Major varieties showed, on the average, 66.6% of infected plants, 18.4% of which by more than two viruses. Therefore, just 33.4% of the vines were apparently free from viruses as shown by ELISA. In accordance with the results of previous surveys (1, 3), the present investigation confirms that the sanitary status of the Marche's viticultural industry is far from being satisfactory, although visual observations can be misleading for most of the vines did not show clear disease symptoms in the field. In fact, the presence of latent infections or attenuated virus strains is one of the main obstacles to sanitary selection of grapevines (2). The increased frequency of viral infections registered from the early plantings of cv.

Verdicchio to the more recent ones, is more than likely due to the uncontrolled use of infected rootstocks in a recent past. The fact that one of latest vineyards established with certified material was in an apparently optimal sanitary condition. further confirms the importance of certification for improving the quality of propagating material of both scions and rootstocks.

Table 2. Incidence of viral infections in cv Verdicchio vineyards established in the Marche from 1900 to 1993.

Municipality and province	Samples (n.)	Year of Planting	Infected plants (%)								
			GVA	GFLV	GFkV	GLRaV-1	GLRaV-2	GLRaV-3	GLRaV-7	Total infected	Total not infected
Apiro (MC)*	25	1900	0.0	0.0	0.0	8.0	0.0	4.0	0.0	12.0	88.0
Cupramontana (AN)	10	1958	0.0	0.0	20.0	0.0	0.0	10.0	10.0	30.0	70.0
Fermo (AP)	10	1963	30.0	0.0	10.0	20.0	0.0	20.0	10.0	60.0	40.0
Apiro (MC)	20	1967	20.0	5.0	30.0	25.0	15.0	25.0	0.0	60.0	40.0
Matelica (MC)	32	1971	53.6	21.4	25.0	64.3	42.9	25.0	7.1	87.5	12.5
Serra S. Quirico (AN)	20	1974	80.0	15.0	45.0	20.0	0.0	30.0	0.0	95.0	5.0
Staffolo (AN)	20	1975	25.0	10.0	25.0	15.0	0.0	10.0	5.0	50.0	50.0
Cupramontana (AN)	20	1979	40.0	50.0	60.0	0.0	0.0	65.0	0.0	80.0	20.0
Apiro (MC)	20	1980	40.0	30.0	40.0	0.0	0.0	30.0	0.0	70.0	30.0
Pratelli –Rosora (AN)	20	1980	15.0	15.0	25.0	0.0	0.0	20.0	5.0	65.0	35.0
Staffolo (AN)	25	1985	16.0	16.0	12.0	8.0	0.0	8.0	8.0	44.0	56.0
Cupramontana (AN)	20	1987	15.0	5.0	45.0	15.0	5.0	40.0	0.0	75.0	25.0
Cupramontana (AN)	20	1989	50.0	5.0	45.0	30.0	5.0	30.0	5.0	90.0	10.0
Cupramontana (AN)	20	1993	20.0	0.0	25.0	25.0	5.0	30.0	10.0	60.0	40.0
Agugliano (AN)	10	1993	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Total	292	Average	27.0	11.5	27.1	15.4	4.9	23.1	4.0	58.6	41.4

*Vineyard with own-rooted plants established around 1900

Table 3. Viral infections in 38 local grapevine cultivars and/or clones grown in the Marche

Cultivar	GVA	GFLV	GFkV	GLRaV-1	GLRaV-2	GLRaV-3	GLRaV-7
Cacciò	+	-	-	-	-	-	-
Mostosa	-	+	-	-	-	-	-
Ciliegiolo F7P2	-	-	+	-	-	-	-
Empibotte	-	-	-	+	-	-	-
Ciliegiolo Remia, Cimiciola 78/1, Montonico, Ribona	-	-	-	-	-	+	-
Cimicino, Moscato	+	-	-	+	-	-	-
Balsamina 1, Ciliegiolo car., Ciliegiolo F7P1, Fava, Pignoletto, Uva cane, Uva d'oro, Uva Regno	-	-	+	-	-	+	-
Brugentile	+	-	-	+	-	+	-
Balsamina 4	+	-	+	+	-	-	-
Aleatico, Cimiciola car., Malvasia bianca	+	-	+	-	-	+	-
Cotrognone	+	-	-	+	-	+	-
Morgentino	-	+	+	-	-	+	-
Balsamina 2	-	-	+	+	-	+	-
Chiapparù, Ciliegiolo 68, Cocacciara, Famoso, Gamay, Gaglioppo 75, Maceratino, Montonico 55/r, Occhio nero, Trebbiano rosso, Vaccù, Vernaccia moscatella	-	-	-	-	-	-	-

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A PRELIMINARY SURVEY FOR GRAPEVINE VIRUSES IN EGYPT

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With a surface of about 62,000 Ha, the grapevine ranks second among the fruit crops of Egypt, being preceded only by citrus. Vineyards are concentrated along the river Nile valley and in new reclaimed desert lands. Table grape varieties are by far the most widely grown, with the two local varieties Banaty Abiad and Romy Ahmer prevailing by and large. A significant introduction of new grapevine varieties from abroad, mainly seedless varieties (cvs Flame, Superior, King's Ruby, Fantasy, etc.), has taken place in these last years, giving a strong impulse to the renewal of Egyptian varietal platform. Due to the absence of phylloxera, Egyptian vines are almost exclusively own-rooted. Nevertheless, because of soil salinity and nematode problems, a number of rootstocks were recently introduced from abroad.

The sanitary status of Egyptian viticulture is little known as few published reports are available, recording the occurrence of leafroll, rugose wood and fanleaf, primarily in vines of foreign varieties (1, 2). Given the paucity of information on the presence and the incidence of virus infections in Egypt, an investigation was initiated, the preliminary results of which are reported herein.

Field inspections and collection of samples were conducted in December 2002. Mature canes were collected at random from 780 individual vines (664 *Vitis vinifera* varieties and 116 rootstocks) in vineyards of seven different areas (Daqahlia, Minofia, Giza, Gharbia, El-Minya, Fayoum and El-Nobaria). All samples were brought to Bari to be analysed for the presence of the following viruses: GFLV, GFkV, GVA, GVB, GLRaV-1, GLRaV-2 and GLRaV-3. Tests were made on cortical scrapings extracts by DAS-ELISA (GFLV, GLRaV-1, GLRaV-2 and GLRaV-3), DASI-ELISA (GFkV and GVB), and protein A-DAS ELISA (GVA) (3). Polyclonal antisera and monoclonal antibodies raised at the University of Bari were used as reagents.

The leaf extracts of forced cuttings were mechanically inoculated to a standard series of herbaceous hosts from about 300 samples of native varieties chosen at random.

Due to the period of the survey the only symptoms observed and identified with reasonable confidence in the field were those typical of leafroll (i.e. rolling and reddening of the leaves, in particular on red berried varieties). Other symptoms, resembling those induced by fanleaf (i.e. fasciations and bifurcations of the canes) were also observed, in particular on cvs. Banaty Abiad and Romy Ahmer.

Heavy infestations of unidentified pseudococcid mealybugs were observed in most of the surveyed vineyards.

No virus was recovered by sap transmission tests, notwithstanding the high number (c. 300) of samples tested. Serological assays were more informative (Tab. 1). A total of 78% of the ELISA tested *V. vinifera* vines (519 out of 664) were infected by one (29%) or more (49%) viruses. GVA was the most widespread virus (67.9%), followed by GLRaV-3 (55.9%). All the other viruses tested were only scarcely represented, i.e. GLRaV-1 (1.8%), GLRaV-2 (1.4%), GVB (0.6%) and GFkV (0.2%), or completely absent (GFLV). Infection level in native grapevine varieties was c. 86% and c. 60% in imported varieties. The two main local cultivars Banaty Abiad and Romy Ahmer, had infection levels of 77.6% and 88.8%, respectively. In other important native varieties, either not a single vine was free from the viruses tested for as in cv. Bez El-Anza or, as in the case of cvs Fayoumy and Ghariby, average infection levels exceeded 95%, with peaks of 100% in some areas. Totally infected were also many minor native cultivars (Edkawy, Eswid El-Wady, Romy Abiad, Ta'afi, Farg El-Tair and Siwi Abiad) of which, however, a low number of samples was analyzed.

Among the most promising seedless varieties introduced, cvs. Flame, Superior, and Crimson had an average infection level of about 50% (Fig. 1)

Vineyards of El-Fayoum were heavily infected since 96% of the vines tested contained at least one virus, while in West of El- Nobaria infection rates were in the range of 50%. This higher infection level in El-Fayoum may be due to the cultivation in this area of a limited number of very old local varieties (Fayoumy, Ghariby, and Bez El-Anza), to the presence of very old vineyards, and the high incidence of mealybug infestations.

Markedly better (11.5% of infection) was the sanitary condition of rootstocks. GVA and GLRaV-3 (5.5% of infection for both) were the only viruses encountered, except for occasional infections by GVB and GLRaV-1 (Tab. 1).

Some vines of cv. Ahmer Romy that showed leafroll symptoms in the field were ELISA-negative, which is taken as an indication that other leafroll-associated viruses may occur Egyptian vineyards, for whose identification more extensive assays are needed.

As reported above, GFLV was not detected in any of the samples tested, including those collected from vines with cane deformations. It is therefore likely that these abnormalities were not of viral origin.

Given the very high infection level in several local varieties, the implementation of a sanitation programme seem highly desirable to improve the sanitary status of Egyptian viticulture.

The limited number of virus species detected in Egypt, notwithstanding the recent heavy introduction of grapevine varieties from abroad, represents a favourable trait of Egyptian viticulture which must be conserved by introducing stricter rules for the importation of plant propagating material from abroad.

Table 1. Incidence of seven different viruses in Egyptian grapevine varieties and rootstocks

Virus	*V. vinifera* cvs.						Rootstocks	
	Native		Imported		Total			
	Inf. samples	%	Inf. samples	%	Inf. samples	%	Inf. samples	%
GVA	357	76.5	94	47.7	451	67.9	6	5.2
GVB	3	0.6	1	0.5	4	0.6	1	0.9
GLRaV-1	12	2.6	0	0	12	1.8	0	0
GLRaV-2	7	1.5	2	1.0	9	1.4	0	0
GLRaV-3	291	62.3	80	40.6	371	55.9	6	5.2
GFLV	0	0	0	0	0	0	0	0
GFkV	0	0	1	0.5	1	0.2	0	0
Total	**401**	**85.9**	**118**	**59.9**	**519**	**78.2**	**10**	**8.6**

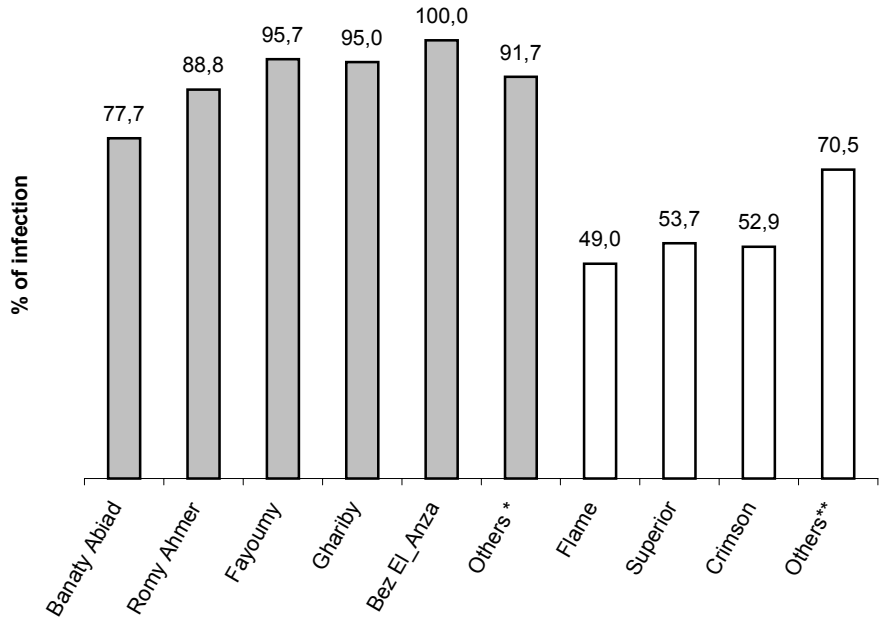

Figure 1. Extent of virus infections in different native (□) and imported (□) grapevine varieties in Egypt
***** Farg El-Tair, Siwi Abiad, Marsa Matroh Eswid, Ta'afi, Rich Baba, Siwi Eswid, Khalily, Abiad El- Wady, Romy Abiad, Eswid El-Wady and Edkawy varieties.
******: Early Superior, King's Ruby, Rubiera, Perlette, Exotic, Black Monnukka, Muscat of Hamburg, Soltanin Noir, ARG1, Red Globe, Queen, Fantasy, Viola, Italia, Fiesta and Thompson 2A.

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CONTRIBUTION TO THE STUDY OF GRAPEVINE VIRUS DISEASES IN SERBIA

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Viticulture is an important industry in Serbia and grapevines are grown on ca. 90.000 hectares (4). Virus-like symptoms have been observed on grapevines growing in different areas in Serbia. The most frequent are: (a) on leaves: fan-leaf distortion, vein mosaic and vein banding, yellow mosaic, chlorotic spots, yellowing, leaf rolling and reddening etc., (b) on canes: short internodes, double nodes, curved (zig-zag) canes etc., (c) on berries: dropping off, small berries, discoloration, irregular ripening, etc., (d) on the vines: stunting, decline, early dying, etc., (e) the vines showing the above mentioned symptoms produced small yield. In previous investigations the following viruses were identified on grapevine in Serbia: *Grapevine fanleaf virus* (GFLV), *Arabis mosaic virus* (ArMV), *Tomato ringspot virus* (3), and *Grapevine Bulgarian latent virus* (2). In the present work a further contribution to the knowledge of virus diseases of grapevine in Serbia was attempted.

Samples were collected during November 2002, in the region Zupa Aleksandrovac from 11 vines with virus-like symptoms and 19 symptomless. Each sample consisted of three mature canes one or two years old. They were stored at 0-4^{o}C until testing and cortical scrapings were used for virus detection. Virus diagnosis was based on serology (DAS ELISA, TAS ELISA and Biotin-Streptavidin ELISA), by using polyclonal and monoclonal antibodies for the detection of the following viruses: GFLV, *Tomato black ring virus* (TBRV), ArMV, *Grapevine leafroll-associated virus 1,2,3,5,6,7*, (GLRaV-1,-2,-3,-5,-6,-7), *Grapevine fleck virus* (GFkV), *Grapevine virus A*, and *B*, (GVA, GVB). Obtained results are presented in Table 1.

Table 1. Grapevine symptoms and viruses detected

Symptoms	Variety	Viruses detected detected
I - reddening and leaf rolling	Gamay tenturier	GFkV, GVA, GLRaV-1, GLRaV-3
	Gamay tenturier	GFkV, GVA, GLRaV-3
	Plovdina	GFkV
II- yellowing and leaf rolling	Italian Riesling	GFkV
	Italian Riesling	
	Smederevka	GFkV, GLRaV-1, GLRaV-3
	Riesling	GFkV, GVA, GLRaV-3
III-fanleaf-like leaf distortions, chlorotic yellowing, vein banding, smaller bunches, shot berries	Prokupac	GFkV
IV- vein mosaic	Zimsko belo	GFkV, GLRaV-1, GLRaV-3
V – chlorotic spots	Rkaciteli	
	Traminer	GFkV
VI- symptomless vines	Italian Riesling	GLRaV-3
	Pinot Noir, Chardonnay	GFkV
	Pinot Noir, Merlot, Plovdina	
	Berl.x Riparia SO4,	
	Berl.x Riparia Kober, Berl.xRiparia Teleki 5C Riesling red, Pinot blac, Rkaciteli, Sauvignon white, Italian Riesling, Prokupac, Jagodinka, Berl.xRiparia SO4 (2 plants), St.George	

Results indicated that 13 out of 30 samples were infected with GFkV, GLRaV-1, GLRaV-3 and GVA. Eight vines were singly infected, seven with GFkV and one with GLRaV-3, whereas mixed infections with three viruses (GFkV, GVA and GLRaV-3) were found in three cases. The most frequent viruses found were GFkV (detected in 12 vines), and GLRaV-3 (detected in 6 vines), while GVA was detected in three samples. From the limited number of samples tested, our results showed as it was suspected on the basis of observed symptoms that grapevine virus incidence in Serbia is high. From the samples collected from grapevine plants showing virus-like symptoms, the majority (10 out of 11 samples) were found to be virus infected, whereas in symptomless plants GFkV, a known latent virus, was detected in two cases and GLRaV-3 was found in one case.

Leaf reddening in case of coloured varieties and yellowing in white varieties, accompanied with downward leaf rolling, has been showed to be associated with the presence of GLRaV-1 and GLRaV –3. GFkV and GVA are also reported for the first time in Serbia. GFLV, previously found to be present in Serbia (3) was not detected during this study, although its presence was suspected in one sample. The possible explanations for this are: a) the low number of samples tested and b) the ELISA assays were performed in February with samples collected in November (GFLV is more reliably detected in leaves early in spring) and this might not give reliable results (1).

GLRaVs (GLRaV-1 and GLRaV –3), GFkV and GVA were not previously known to be present on grapevine in Serbia and they are reported for the first time. On the base of observed symptoms in field surveys we would suspect the presence of some more viruses on grapevine in Serbia. However, an extensive and throughout the country survey is urgently needed in order to collect useful information on the aetiology of the virus-like symptoms observed in grapevine plants in Serbia.

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OCCURRENCE OF GRAPEVINE VIRUSES IN THE CZECH REPUBLIC

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To start the certification of grapevine in the Czech Republic, survey on healthy status of clones of grapevine varieties and rootstocks was started. The presence of sixteen viruses was evaluated - seven nepoviruses, *Grapevine fanleaf virus* (GFLV), *Arabis mosaic virus* (ArMV), *Raspberry ringspot virus* (RpRSV), *Strawberry latent ringspot virus* (SLRSV), *Tomato black ring virus* (TBRV), *Tomato ring spot virus* (TomRSV), *Tobacco ring spot virus* (TRSV), two vitiviruses, *Grapevine virus A* (GVA) and *Grapevine virus B* (GVB), three closteroviruses, *Grapevine leafroll-associated virus 2* (GLRaV-2), *Grapevine leafroll-associated virus 6* (GLRaV-6), *Grapevine leafroll-associated virus 7* (GLRaV-7), three ampeloviruses, *Grapevine leafroll-associated virus 1* (GLRaV-1), *Grapevine leafroll-associated virus 3* (GLRaV-3), *Grapevine leafroll-associated virus 5* (GLRaV-5), and one maculavirus, *Grapevine fleck virus* (GFkV).

From grapevine clones, 126 clones of 40 varieties and 7 rootstocks, maintained at eight viticulture breeding stations, were selected for certification. From prebasic propagation material of each clone, several vines were selected and tested. More than 500 individual vines were examined for presence of most important viruses (ArMV, GFLV, GLRaV-1, GLRaV-3, GVA, GVB, GFkV), some of them also for presence of other viruses (SLRSV, TBRV, RpRSV, TRSV, TomRSV, GLRaV-2, GLRaV-5, GLRaV-6, GLRaV-7). For testing, dormant canes were sampled from these vines during winter (1). Viruses were detected using DAS-ELISA. Antisera were purchased from different producers: Agritest, Italy (antisera against GFLV, ArMV, GVA, GVB, GFkV, GLRaV-1, GLRaV-2, GLRaV-3, GLRaV-7), Bioreba, Switzerland (GLRaV-6, TRSV), Bio-Rad, France (SLRSV, GLRaV-5, TomRSV) and Loewe, Germany (TBRV, RpRSV) and they were used in DAS - ELISA method according to instructions of manufacturer. Presence of individual viruses is summarized in Table 1.

Table 1: Summary of testing of grapevine prebasic propagation material for individual viruses

Virus tested	Number of positive vines	Number of tested vines	% of positive vines
ArMV	96	585	16.4
GFLV	39	585	6.7
TBRV	3	103	2.9
SLRSV	19	162	11.7
RpRSV	3	97	3.1
TRSV	0	42	0
TomRSV	0	51	0
GLRaV-1	76	396	19.2
GLRaV-2	1	66	1.5
GLRaV-3	44	391	11.3
GLRaV-5	9	97	9.3
GLRaV-6	0	61	0
GLRaV-7	8	102	7.8
GVA	32	522	6.1
GVB	25	517	4.8
GfKV	55	552	10.0

From 126 clones tested, in 23 clones negative vines were not found. In the rest 103 clones, at least one negative vine was found. Such vines were promoted as candidate plants into screenhouse for grapevine certification located in Faculty of Horticulture Lednice and they will be further tested by other methods (woody indicators, herbaceous indicators, polymerase chain reaction).

GLRaV-1, ArMV, SLRSV, GLRaV-3 and GFkV were found to be widely spread in Czech propagation material of grapevine. These viruses occurred in more then 10% of examined vines and are considered as economically important for grapevine production in the Czech Republic. Smaller number of vines was found to be infected with GFLV, GLRaV-5, GVA and GVB. Other viruses were found in negligible number (RpRSV, TBRV and GLRaV-2).

Quarantine nepoviruses TRSV and TomRSV were never found in Czech grapevines. Similarly, GLRaV-6 was not found during our experiments.

Harmfulness of grapevine viruses and their effect on growth and fertility of grapevine in our conditions is still to be determined. Sanitation of infected Czech grapevine clones is needed in near future.

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PROBLEMS IN THE PRODUCTION OF CERTIFIED GRAPEVINE PLANTING MATERIAL IN BULGARIA

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The problem of grapevine virus diseases like fanleaf and leafroll, their origin and impact on the host has been addressed in Bulgaria since the beginning of the past century . Schemes for obtaining certified planting material that, besides the major virus diseases, included also the agents of crown gall (*Agrobacterium vitis*) and phomopsis cane and leaf spot (*Phomopsis viticola*), were developed considerably later. Under the climatic conditions of Bulgaria and because of the lack of efficient means of control, A. vitis represents the worst problem to face and the most difficult to solve. At present, the main issues to address for the production of certified grapevine planting material in Bulgaria concern: (i) determination of the range of diseases object of sanitary control; (ii) development of rapid and reliable methods for diagnosis; (iii) development of protocols for the establishment and maintenance of mother vine plots for the production of basic and certified material; (iv) establishment and implementation of an efficient system for the distribution of the sanitarily improved stocks to producers (nurserymen and growers). During the last years a great quantity of allegedly certified grapevine planting material has been imported to Bulgaria. However, the presence of viral disease symptoms in some of the vineyards planted with this material, casts serious doubts on the authenticity of the sanitary condition declared by the importation documents.