Presentation of the Mediterranean network on grapevine closteroviruses (MNGC) and report of activity 1992-97

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Presentation of the Mediterranean Network on Grapevine Closteroviruses (MNGC) and report of activity 1992-97

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SUMMARY - The objectives, the participants and the activities of the Mediterranean research network for the study of the epidemiology of grapevine closteroviruses (MNGC) are briefly illustrated in this presentation. At the end of the review there is a list of the abstracts on subjects dealth with by the MNGC and carried out in part within its framework.

Key words: MNGC, grapevine, closteroviruses, epidemiology

RESUME *- Les objectifs, les participants et les activités du réseau de recherche méditerranéen sur l'étude de épidemiologie des closterovirus de la vigne (MNGC) sont brièvement illustrés dans ce travail. En plus, une liste de résumés est incluse concernant les sujets abordés et les activités menés en partie au sein de ce réseau.*

Mots-clés: *MNGC, vigne, closterovirus, épidemiologie*

1. Objectives of the MNGC

The MNGC was established for fostering cooperative studies on closteroviruses known to infect grapevines, and on leafroll and rugose wood, the diseases they are associated with. Given the extensive ground that such investigations would have covered, it was agreed to start with a somewhat restricted project, centred mainly on two of these viruses, i.e. grapevine virus A (GVA) and grapevine virus B (GVB), which were thought, but not proven, to be involved in the aetiology of rugose wood (RW).

In particular the studies were aimed at:

(i) Ascertaining the distribution of GVA and GVB in selected viticultural areas of the participating countries where RW is present and natural mealybug infestations are known to occur.

(ii) Isolating and characterizing GVA and GVB strains.

(iii) Ascertaining the occurrence of natural transmission of GVA and GVB in mealybug-infested areas.

(iv) Checking vectoring efficiency of mealybugs through transmission trials under controlled conditions and virus detection in the potential vectors.

(v) Establishing whether a cause-effect relationship exists between GVA-Rupestris stem pitting (RSP), and GVB-Corky bark (CB).

(vi) Establishing the role of RSP and CB in the aetiology of RW.

(vii) Obtaining RSP- and CB-free stocks through sanitation and carring out performance tests.

(viii) Conceiving, testing, and implementing integrated control schemes against natural spread of RSP and CB.

2. Participants in the MNGC

The following Institutions participated in the MNGC:

(i) Institut Technique de l'Arboriculture Fruitiere et de la Vigne
Tessala El-Merdja, Boufarik, Algeria

(ii) Plant Protection Institute
Heraklion, Crete, Greece

(iii) Laboratorie de Virologie Végétale INRAT
Ariana Tunis, Tunisia

(iv) Service de Controle des Semences et Plants Ministry of Agriculture
Rabat, Morocco

(v) Istituto Agronomico Mediterraneo
Valenzano Bari, Italy

(vi) Micropropagation Centre, Department of Agriculture and Fisheries
Lija, BZN 04, Malta

(vii) Agricultural Research Institute, Nicosia, Cyprus

(viii) Istituto di Patologia Vegetale
Sassari, Italy

(ix) Dipartimento di Protezione delle Piante
Bari, Italy

(x) Department of Plant Protection
University of Cukurova, Adana, Turkey

3. Meetings held by the MNGC

MNGC met five times as follows:

- December 10-11 1992, MAI-Bari, Italy
- September 6-9 1993, ICVG Conference, Montreux, Switzerland
- May l8-22 1994, Institute of Plant Pathology, University of Sassari, Italy
- July 15 1995, MAI-Bari, Italy
- May 8-12 1996, Centro de Investigaciones y Desarollo Agroalimentario Murcia, Spain.

In two of the above encounters (Sassari, 1994 and Murcia, 1996) MNGC met together with the "European Network for the Establishment of Reference Protocols for Detection and Elimination of Infectious Agents" (EUGN), a group of experts of six European viticultural countries supported by the European Union that investigated aspects of grapevine virology complementary to those addressed by MNGC.

4. Activity

The activity of the MNGC has developed along two main lines whose relevant results are summarised hereafter.

A. Research

The research activity has been very intensive so that several of the goals listed under "Objectives of MNGC" were attained. Furthermore, following the request by some of the participants, the investigations were extended to grapevine leafroll associated closteroviruses (GLRaVs), with special reference to GLRaV-3 and GLRaV-7.

(a) **Diagnosis:** monoclonal and polyclonal antisera to GVA, GVB and GLRaV-7 were produced providing useful tools for routine identification of these viruses. These antisera, distributed to MNGC participants, allowed the identification of GVA and GVB in several Greek areas (Crete, Northern Greece and North-Eastern Peloponnesus), and of GLRaV-7, an emerging new grape pathogen, in Crete and Turkey. A large

survey was also carried out in Italy, which provided first-hand information on the distribution of GVB in Sardinia and Apulia and GLRaV-7 in the southern regions of the country. Reliable protocols for the molecular detection of vitiviruses and closteroviruses by PCR, dot blot, and tissue blot were also developed and made available to the participants.

(b) **Aetiology:** it was demonstrated that GVA, a former closterovirus now member of a new genus named *Vitivirus,* is closely associated with, and more than likely is, the agent of Kober stem grooving rather than the agent of Rupestris stem pitting as originally postulated. On the other hand several lines of evidence were obtained supporting the notion that GVB is implicated in the aetiology of Corky bark. It seems therefore that at least two of the disorders of the rugose wood complex have been characterised etiologically, which represents a veritable breakthrough in the knowledge of RW and opens to further advances. In addition, grapevine virus D (GVD), a new vitivirus, was described and identified as one of the possible etiological agents of RW

(c) **Epidemiology:** a highly reliable technique for the molecular detection of closteroviruses and vitiviruses by RT-PCR and multiplex PCR in field-collected mealybugs was developed.

The following mealybug collection protocol for PCR analysis was developed and issued to MNGC participants:

1. Mealybug collection from host plants has to be made carefully, to minimise the damage that their detachment from tissues may cause.
2. *Coccids:* remove single insects with a scalpel and place in an appropriate container, easy to handle and seal (1.5 ml eppendorf microfuge tubes are recommended).
3. *Pseudococcids:* avoid collecting very young juveniles and mature adults with the cottony eggsac. Identify middle-aged crawlers, tease the insects gently with a needle or a thin painting brush until they begin to move, collect with the same tool and place in the microfuge tube.
4. *Size of the sample.* Each sample should consist of no less than 5-6 insects. Samples may come from single vines (preferably) or from several vines.
5. *Sample preparation for storage and shipment.* Add to the tube containing mealybugs 1 ml of 70% solution of ethanol in distilled water. Mix gently by inverting the tube to make sure that the insects are wet and there are no air bobbles at the bottom. Seal the tube with parafilm, place in a refrigerator (4°C) as soon as possible, and keep in the cold until shipment. Place the tubes in a hard-walled container that can resist crushing during the travel (e.g. film boxes) to avoid rupture of the thin propylene tubes, and ship by air mail.

6. Tubes should be numbered and, if possible, information should be given on the type of symptoms shown by the host plant (especially leafroll and rugose wood), place and date of collection, severity of the mealybug infestation, and any other detail that may be considered useful.

Mealybug samples mostly collected from vines with RW symptoms were obtained from several Mediterranean countries (Spain, Portugal, Lebanon, Tunisia, Sardinia). These samples proved positive in a very high proportion to the assay for GVA (23 out of 28 samples) and to a lesser extent, for GLRaV-3 (8 out of 28 samples). The reliability and sensitivity of the multiplex PCR application will allow more detailed investigations on the distribution of potentially viruliferous mealybugs and scale insects in the vineyards of the countries participating in the MNGC, giving an essential contribution to the epidemiological studies.

New interesting observations were carried out in Cyprus on the field spread of GLRaV-3 in relation to the presence and activity of the mealybugs *Planococcus ficus* and P. *citri.* Both species were experimentally proven to be GLRaV-3 vectors. *P. ficus* appeared to be more efficient than the other species. The Cypriot studies represent the first example of a systematic investigation of mealybug-mediated field spread of a grapevine closterovirus.

Pseudococcus affinis, the obscure mealybug, was identified in Sardinia as a new experimental vector of GVA and GVB, whilst in Bari, the parameters underlying transmission of GVA by *P. ficus* were determined. In particular it was found that mealybugs can acquire the virus in 15 min and retain it for 48 h. There is no latent period in transmission and the virus is still detectable in the vectors after five successive passages of 1 hour each on healthy plants.

A most interesting result was the development of an artificial GVA acquisition system, whereby the virus is picked up by the mealybugs through a membrane from a medium containing purified particles suspended in a sucrose-tryptone-yeast extract solution. It is expected that this technique will speed up the investigations on vitivirus-mealybug relationships, providing information essential for defining the transmission strategy.

(d) **Sanitation:** treatments based on the combined effect of heat therapy and *in vitro* culture were carried out for the production of rugose wood- and leafroll-free plants. A number of rooted explants were obtained, which are currently under evaluation for ascertaining their freedom from GVA, GVB, GLRaV-3, and GLRaV-7.

B. Intranetwork relationships

One of the goals of the MNGC was to establish links between participants so as to maximise the exploitation of the results obtained by the different parties. This goal was achieved to a satisfactory extent. Diagnostic tools produced at Bari (antisera in particular) were distributed to and used by other participants. In turn, various participants collected and supplied

samples for analysis. ln all cases there was an intensive flow of information between the co-operating parties.

The connection with the EU Network (EUGN) was highly beneficial and the two meetings held jointly were an undoubtful success. Members of the MNGC have taken part in some of the activities of the EUGN for they have received antisera for comparative tests and are contributing grapevine accessions to the "*European Grape Reference Virus Collection*" established in France. On the other hand, members of the EUGN have participated in the epidemiological studies, contributing samples and information on the occurrence and prevalence of grapevine mealybugs (psudococcids and scales) in their areas, and carrying out transmission trials.

4. Resolution approved at the 5th Meeting of MNGC (Murcia, 1996)

The MNGC has entered the fifth year of activity which, according to the foreseen duration of the Network, might represent its whole lifespan. The participating parties, therefore, in the course of the last Meeting (Murcia, 1996) have reviewed the achievements of four year's activity and discussed possible future developments.

The consensus was that the MNGC has given a substantial impulse to research on grapevine virology in many of the participating countries and has helped creating or reinforcing ties between laboratories of the Northern and Southern banks of the Mediterranean. After an initial period of settlement, the collaboration between parties has gained momentum and is now proceeding at a satisfactory pace. The technology developed for the assessment of the potential virulificity of mealybug populations is seen as a powerful tool for detailed investigations on the epidemiology of viruses transmitted by these vectors, which rank among those of major economic relevance for the Mediterranean viticultural industry.

Therefore, the MNGC is unanimous in recommending that the Network should continue its activity. However, it is reckoned that a re-orientation of the research work would be desirable, as well as the possible opening to other participants. Addressing the problem of an harmonised implementation of national certification schemes, could, for example, represent one of the future lines of activity. The involvement of other Mediterranean countries that are now developing clean stock programmes (e.g., Albania and Lebanon) is also seen with favour.

Developing close contacts with a EU Network has represented a very happy experience and has confirmed the value of the North-South exchanges in technology and information. In the joint Meeting at Murcia, both Networks have expressed the willingness to continue the relationship and to foster a more profound integration of the activities in the common areas of expertise.

5. Papers on subjects dealth with by the MNGC and carried out in part within its framework

1993

Archives of Virology 130, 109-120.

Properties of a filamentous virus isolated from grapevines affected by corky bark

D. Boscia, V. Savino, A. Minafra, S. Namba, V. Elicio, M.A. Castellano, D. Gonsalves, G.P. Martelli

SUMMARY – A virus with highly flexuous filamentous particles *c.* 800 nm long, showing distinct transverse striation was isolated with high frequency (60%) by inoculation of *Nicotiana occidentalis* with sap from grapevine accensions indexing positive for corky bark. The virus, for which the name grapevine virus B (GVB) is proposed, has a ssRNA genome with mol. wt of *c.* 2.5x10^6 daltons (*c.* 7600 nt) and coat protein subunits with mol. wt of *c.* 23,000 daltons. GVB has a very restricted herbaceous host range and was experimentally transmitted by the mealybug *Pseudococcus ficus.* The physicochemical and ultrastructural properties of GVB resemble those of closteroviruses. However it is serologically unrelated to other grapevine closteroviruses including grapevine virus A, with which it shares some biological and physicochemical properties.

Rivista di Patologia Vegetale, S. V, 3, 15-22

A cloned probe to grapevine virus B

P. Saldarelli, A. Minafra, R. Garau, G.P. Martelli

SUMMARY – A 1070 bp cDNA fragment was synthesized by random priming on the genomic RNA of grapevine closterovirus B (GVB), and cloned in *Escherichia coli* DH 5a. The probe proved to be virus-specific for it hybridized with homologous RNA but not with total nucleic acid extracts from healthy grapevines and herbaceous hosts. It recognized with strong signals sequences of six GVB isolates of different geographic areas, but not of a virus isolate from USA nor of grapevine closterovirus A (GVA). A Sardinian isolate of GVB gave weak hybridization reactions possibly because of lower sequence homology. The probe was successful for GVB detection in sap from infected *Nicotiana occidentalis*, and recognized with a much lower efficiency GVB sequences in total nucleic acid extracts from infected grapevines.

Rivista di Patologia Vegetale, S. V, 3, 83-89

Studies on grapevine virus B isolates from corky bark-affected vines in Sardinia

R. Garau, V.A. Prota, D. Boscia, R. Piredda, U. Prota

SUMMARY – In the course of indexing trials carried out in Sardinia two distinct groups of *Vitis* donors were identified, which induced corky bark reactions in LN 33 differing in the intensity and consistency of symptoms expression. Grapevine virus B (GVB) was isolated in over 60% of the cases by mechanical inoculation to *Nicotiana occidentalis*, from donors inducing severe reactions in LN 33, but not from donors eliciting weak and erratic reactions in the same host. Based on the reaction of herbaceous hosts, biological variants of GVB were identified. Dodder (*Cuscuta campestris*) acquired GVB from grapevine and *N. occidentalis* but was unable to transmit it to other hosts.

List of extended abstracts presented at the 11th Meeting ICVG, Montreux, Switzerland, 6-9 September 1993 by MNGC members

Boscia D., N. Abou Ghanem, P. Saldarelli, A. Minafra, M.A. Castellano, R. Garau, V. Savino, G.P. Martelli. A comparison of grapevine virus B isolates. 25-26

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Garau R., V.A. Prota , D. Boscia, R. Piredda, U. Prota. Grapevine virus B in Sardinia. 47-48

Martelli G.P., D. Boscia, E. Choueiri, M. Digiaro, M.A. Castellano, V. Savino. Rugose wood of grapevine in Yemen. 51

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Garau R., V.A. Prota , R. Piredda, U. Prota. A stunting factor in *Vitis vinifera* transmitted by grafting to Kober 5BB. 74-75

Ioannou N. Occurrence and natural spread of grapevine leafroll-associated closterovirus in Cyprus. 111-112

Ozaslan M., S. Baloglu, M.E. Guldur, M.A. Yilmaz. Virus diseases of grapevine in Southeastern Anatolian region of Turkey. 122

Saldarelli P., H. Guglielmi Montano, G.P. Martelli. Detection of three grapevine closterolike viruses by non radioactive molecular probes 136

Minafra A., A. Hadidi, P. Saldarelli. Sensitive immunocapture and multiplex reverse transcription polymerase chain reaction for the detection of grapevine leafroll associated virus III and grapevine virus B. 137-138

Minafra A., A. Hadidi. Detection of grapevine virus A in single mealybugs by immunocapture - reverse transcription - polymerase chain reaction. 139

1994

Vitis 33, 157-160

Non-radioactive molecular probes for the detection of three filamentous viruses of the grapevine

P. Saldarelli, H. Guglielmi Montano, G.P. Martelli

SUMMARY – Digoxigenin-labelled riboprobes (DIG-RNA) were developed for the detection in infected tissue extracts of grapevine trichovirus A (GVA), grapevine trichovirus B (GVB) and grapevine leafroll-associated closterovirus III (GLRaV III). The probes were virus-specific and could be used for the identification of the respective viruses in sap expressed from infected *Nicotiana* species (GVA and GVB) and in total nucleic acid extracts from infected grapevines (GVA, GVB and GLRaV III). The efficiency of detection was the same as (GLRaV III), or slightly less than (GVA), with ELISA. No difference was found in detection efficiency between DIG-RNA and cDNA radioactive probes.

Vitis 33, 161-163

On the possible relationship between Kober stem grooving and grapevine virus A

R. Garau, V.A. Prota, R. Piredda, D. Boscia, U. Prota

SUMMARY – Investigations were carried out to establish possible correlation of two diseases of the rugose wood complex, i.e. Rupestris stem pitting (RSP) and Kober stem grooving (KSG) with grapevine virus A (GVA), and grapevine leafroll-associated virus I (GLRaV I) and III (GLRaV III). To this purpose 84 clonal accessions of different wine grape cultivars were analyzed by ELISA and by indexing onto the indicators *Vitis rupestris*, Kober 5BB and LN 33. The results obtained clearly indicated that none of the viruses taken into consideration is apparently involved in the etiology of RSP. Conversely, a remarkably close association of GVA with KSG was discovered.

Phytopathologia mediterranea 33, 187-193

On the correlation between grapevine virus A and rugose wood

M. Digiaro, M. Popovic Bedzrob, A.M. D'Onghia, D. Boscia, V. Savino

SUMMARY – Surveys were carried out in different vineyards of Apulia to assess the incidence of infection by the following grapevine viruses: virus A (GVA), leafroll-associated I (GLRaV I), leafroll-associated III (GLRaV III), fanleaf (GFLV) and fleck (GFkV). These studies were aimed especially at establishing possible correlation of rugose wood with closterolike viruses. Of 1828 vines checked individually, 88.3% were infected by at least one of the five viruses. GLRaV III (67.3%), GVA (55.7%) and GFkV (59.3%) were the most widespread. GLRaV I (11.8%) and GFLV (7.1%), which prevailed in older vineyards, were detected less frequently. The consistent presence of closterolike viruses in vineyards established with certified material was interpreted as a consequence of their possible spread by mealybug vectors. GVA appeared to be the key factor for the development of wood abnormalities and to interact with GLRaV I and GLRaV III in the enhancement of the incidence of rugose wood.

Rivista di Patologia Vegetale, S. V, 4, 11-24.

A comparative study of grapevine virus B isolates

D. Boscia, N. Abou-Ghanem, P. Saldarelli, A. Minafra,
M.A. Castellano, R. Garau, V. Savino, G.P. Martelli

SUMMARY – Nine grapevine virus B (GVB) isolated from Italy (Apulia and Sardinia), USA and Canada were studied comparatively with one another and with an isolate of grapevine virus A (GVA). The Apulian isolate GVB-BA was one of a batch of 12 that were recovered from an equal number of grapevine accessions representing the totality of those indexing positive for corky bark found so far in the region. The host range of all GVB isolates was restricted to a few *Nicotiana* species, two of which (*N. occidentalis* and *N. cavicola)* reacted differentially so as to identify two groups of viral variants which induced, respectively, an outstanding chlorotic clearing of the veins or necrotic local lesions followed by yellowing and extensive necrosis of the upper leaves. All isolates behaved in the same way during purification, had particles with the same morphology and coat protein subunits with Mr of 23,000 daltons, except for a Sardinia isolate (GVB-SS-D) whose coat protein co-migrated in electrophoresis with that of GVA (Mr 22,500 daltons). Differences in cytopathology among strains were minor. The genomic RNA of all isolates hybridized with a probe to GVB-Se, the type strain, but that of the two necrotic isolates GVB-SS-D and GVB-LRNOV with a lower affinity. Serologically, all isolates were ricognized in western blots, ELISA and immunoelectron microscopy by different GVB antisera. However, the low titre of the antisera available did not allow conclusive serological characterization of the isolates presently studied.

Journal of Virological Methods 47, 175-188

Sensitive detection of grapevine virus A, B and leafroll-associated III from viruliferous mealybugs and infected tissue by cDNA amplification.

A. Minafra, A. Hadidi

SUMMARY - DNA primers specific for grapevine virus A, grapevine virus B, or grapevine leafroll associated virus III were constructed based on the nucleotide sequence of a segment of each viral genome. DNA primers were utilized for cDNA synthesis and polymerase chain reaction (PCR) amplification of a 430 bp fragment from extracts of GVA-infected grapevine tissue or viruliferous mealybugs and 450 bp and 340 bp DNA fragments from extracts of GVB and GLRaV III-infected grapevine tissue, respectively. The amplified DNA fragment of each virus was identified by Southern hybridization analysis with a cRNA probe of cloned viral genome. Reverse transcription-PCR, immunocapture-PCR and/or multiplex PCR assays were developed for the detection of GVA, GVB and GLRaV III in extracts of infected grapevine leaves, dormant cuttings and in viruliferous mealybugs. Viral specific DNA was absent from amplified extracts of uninfected grapevine tissue or nonviruliferous mealybugs. IC-RT-PCR was easier to perform than RT-PCR for the detection of GVA from viruliferous mealybugs. M-RT-PCR was easier and faster than IC-RT-PCR for the detection of GLRaV III from infected grapevine tissue and it allows the sensitive detection of GVB, for which a high titer antiserum is not yet available.

Phytopathologia mediterranea 33, 146-151

Occurrence of filamentous viruses and rugose wood of grapevine in Yemen

G.P. Martelli, D. Boscia, E. Choueiri, M. Digiaro, M.A. Castellano, V. Savino

SUMMARY – Rugose wood symptoms were observed in a single variety (cv. Asimi) of the 18 or so native grapevine cultivars grown in Yemen. All diseased vines were infected by a mechanically transmissible isolate of grapevine virus A (GVA), but some contained also grapevine leafroll-associated virus I (GLRaV I) or III (GLRaV III). ELISA and IEM tests, did not reveal the presence of grapevine fanleaf or grapevine fleck virus. The Yemeni isolate of GVA was able to infect herbaceous hosts other than *Nicotiana* species, but showed physico-chemical and ultrastructural properties similar to those of Italian isolates of the same virus with which it is being compared. The present finding confirm the alleged relationship of GVA with rugose wood, and provide support to the notion that closterolike viruses of the grapevine had a long-lasting association with *Vitis vinifera* L., with which they may have co-evolved and spread from its centres of origin.

1995

Vitis 34, 67-68.

Pseudococcus affinis Mask, new vector of grapevine trichoviruses A and B

R. Garau, V.A. Prota, D. Boscia, M. Fiori, U. Prota

SUMMARY – Grapevine trichovirus A (GVA) and grapevine trichovirus B (GVB) were successfully transferred with bulk transmission trials under controlled conditions, from infected grapevines to herbaceous hosts by *Pseudococcus affinis* Mask., a pseudococcid mealybug that may attack grapevines. *P. affinis* is the fourth mealybug species capable of vectoring GVA and GVB, confirming that transmission by mealybugs of grapevine trichoviruses may not be species-specific.

Vitis 34, 171-175.

Nomenclature of grapevine leafroll-associated putative closteroviruses

D. Boscia, C. Greif, P. Gugerli, G.P. Martelli, B. Walter, D. Gonsalves

SUMMARY - Comparative immunoenzymatic (ELISA), immunoelectron microscopic (IEM) and immunoblotting tests were carried out with antisera produced in different laboratories and commercial diagnostic kits on closterolike viruses reported in the literature under the name of grapevine corky bark-associated virus (GCBaV) and grapevine leafroll-associated viruses IIa and IIb (GLRaV IIa and GLRaV IIb). The results of these studies have established that GCBaV is the same as GLRaV IIb and that both viruses are apparently identical to an isolate of GLRaV-2 identified in France, whose designation as the authentic GLRaV-2 is proposed. GLRaV IIa is serologically distinct from all known clostero-like viruses of the grapevine and, therefore, the provisional name of grapevine leafroll-associated virus 6 (GLRaV-6) is suggested for it.

1996

Vitis 35, 53-58.

Studies on "corky rugose wood" of grapevine and on the diagnosis of grapevine virus B

M. Bonavia, M. Digiaro, D. Boscia, G. Bottalico, V. Savino, G.P. Martelli

SUMMARY – Vines affected by corky rugose wood (CRW), a field syndrome characterized by pronounced cork production by the scion of several grapevine varieties just above the graft union, contain a number of filamentous and isometric phloem-limited viruses, such as grapevine leafroll-associated virus 1, 2 and 3 (GLRaV-1, GLRaV-2, GLRaV-3), grapevine virus A and B (GVA and GVB), and grapevine fleck virus (GFkV). However, the same viruses, with the exception of GVB, are widely represented also in vines with rugose wood without excessive corkyness. Although GVB was found in all vines indexing positive in LN 33 for corky bark disease, its occurrence in CRW-affected vines was not consistent enough to suggest that it may have a determining role in the induction of this syndrome. Monoclonal antibodies to GVB raised previously were characterized and their possible use for reliable detection of GVB in field-grown vines investigated in detail. A triple antibody sandwich ELISA protocol that under our experimental conditions afforded consistent and repeatable results, was based on the use of cortical scraping extracts from mature canes collected in autumn, antibodies from a polyclonal antiserum for plate coating (trapping) and a monoclonal antibody for antigen detection.

Vitis 35, 91-93

Some properties of a hitherto undescribed filamentous virus of the grapevine

E. Choueiri, D. Boscia, M. Digiaro, M.A. Castellano, G.P. Martelli

SUMMARY - An apparently new, non mechanically-transmissible clostero-like virus, for which the name "grapevine leafroll-associated virus 7" (GLRaV-7) is proposed, was found in Albanian grapevine accessions. Virus particles were filamentous, had conspicuous cross banding and a length of 1500-1700 nm. Virions had coat protein subunits with an estimated Mr of ca. 37 kDa and a ssRNA genome with size of ca. 19.5 kb as deduced from the estimate of dsRNA (ca. 19.5 kbp) extracted from grapevine tissues. A virus-specific antiserum was raised, which decorated virions at a dilution of 1:1000. This antiserum did not recognize particles of any of the six grapevine leafroll-associated clostero-like viruses (GLRaV-1 to -6) known to date, nor of grapevine trichovirus A (GVA) and B (GVB). Grapevine indicators graft-inoculated with material from accessions containing GLRaV-7 reacted with mild leafroll-like symptoms. In a survey in which 2226 vines from 30 different countries were examined by ELISA, GLRaV-7 was found in 141 plants from Albania, Greece, Hungary, Egypt, and Italy.

Vignevini 4, 15-17

Risanamento e propagazione *in vitro* di varietà di vite autoctone della Tunisia
***(In vitro* cleaning and regeneration of conformable Tunisian autochthon grapevine varieties)**

F. ben Abdallah, D. Hmouni, H. Zemni, N. Chabbouh, F. Askri, A. Ghorbel

SUMMARY - Different meristematic size have been used to regenerate virus free vitro-plants of the Tunisian autochthon varieties. For studying the best growth of explants and preserving genetic stability of the cultivars, many culture media with various concentrations of hormones have been tested. We have demonstrated, therefore, that the suppression of $CaCl_2$ from the rooting medium improved considerably the quantity and the quality of roots. The survival of *ex vitro* plants was increased, too. *In vitro* behaviour of plantlets showed differences in comparison with regenerative varieties.
Serological tests were performed to control the sanitary state of regenerated plants. The regeneration of healthy Tunisian grapevine cultivars associated to the preservation of their genetic stability were considered in order to establish a collection of local varieties, for subsequent transfer to the field.

1997

Vitis 36, 39-41

Grapevine virus A and grapevine virus D are serologically distantly related

E. Choueiri, N. Abou-Ghanem, D. Boscia

SUMMARY – Grapevine trichovirus A (GVA), B (GVB) and D (GVD) are not serologically related as ascertained by ELISA and IEM test using polyclonal antisera. A study to investigate in detail their serological relationships was carried out with a larger number of reagents, including monoclonal antibodies (MAbs), and serological techniques (ELISA, IEM, tissue blot, Western blot). The results show that (i) polyclonal antisera to GVA, GVB and GVD cross-reacted in Western blot with all antigens; (ii) one out of 4 Mabs to GVA (Mab PA3.B9) reacted in ELISA, Western blot and tissue blot with the homologous virus and GVD but not with GVB. It is concluded that GVA, GVB and GVD are serologically distantly related and that the single antigenic determinant common to GVA and GVD is likely to be a cryptotope

Journal of Virological Methods 66, 103-108

A spot-PCR technique for the detection of phloem-limited grapevine viruses

P. La Notte, A. Minafra, P. Saldarelli

SUMMARY – Specific amplification of genomic fragments of grapevine trichovirus A (GVA), grapevine trichovirus B (GVB) and grapevine leafroll-associated closterovirus 3 (GLRaV-3) was obtained by reverse transcription-PCR on total nucleic acid solubilized from small pieces of charged nylon membrane, on which a drop of crude infected grapevine sap was spotted (spot-PCR). A thermal treatment (95°C for 10') of spotted sap in a buffered solution improved the release of viral template. Consistent amplification was obtained with three viruses from fragments of the same respective blots up to 1 month after spotting, while a detection threshold limit comparable with standard PCR techniques was found for this method. Duplex PCR (i.e. amplification of different viruses from a mixed-infected grapevine source) was also found to be effective, since GVA and GLRaV-3 were amplified by a mixture of specific primers in the same reaction. This rapid and easy sampling technique, using leaf petioles to express crude sap, may have a wide field application for screening grapevine viruses.

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Properties of grapevine virus D, a novel putative trichovirus

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SUMMARY – An apparently new sap-transmissible virus was isolated in Apulia (Southern Italy) from a grapevine with corky rugose wood symptoms. Virus particles were filamentous with distinct cross-banding and a size of 825 x 12 nm. Coat protein subunits had an estimated M_r of *ca* 20.5 kDa and the genome was an ssRNA *ca* 7600 nt in size. A virus-specific antiserum was raised, which decorated virions to a dilution of 1/50 but did not recognize particles of grapevine trichovirus A (GVA), B (GVB), and C (GVC). Antisera to GVA, GVB, GVC, Heracleum latent trichovirus (HLV), grapevine berry inner necrosis virus (GBINV) and to each of the seven grapevine leafroll-associated closteroviruses (GLRaV-1 to -7) known to date, did not decorate virus particles. The cytopathology of infected *Nicotiana occidentalis* plants largely conformed to that elicited by GVA and GVB. A cloned cDNA probe 420 bp in size hybridized with purified viral RNA and total nucleic acid extracts from virus-infected *N. occidentalis*, but not comparable preparations of GVA and GVB. Sequencing of a 963 nt long 3' terminal fragment of the viral genome revealed the presence of two open reading frames (ORFs). ORF 1 encoded a polypeptide with a M_r of 17.6 kDa, which was identified as the coat protein (CP) and possessed 75% and 64% identical residues with GVA and GVB CPs, respectively. ORF 2 coded for a polypeptide with a M_r of 10.4 kDa, resembling the putative nucleic acid-binding proteins identified in the same position in GVA and GVB genomes. The above data strongly support the hypothesis that this newly recovered grapevine virus belongs to the *Trichovirus* genus, representing a new species for which the name grapevine virus D (GVD) is proposed. An investigation for the presence of GVD, carried out with RT-PCR on 307 vines of diverse varieties and geographical origins, showed that the virus occurred in *ca.* 4% of 218 accessions with rugose wood symptoms, but in none of 89 disease-free vines.

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Acquisition and transmission of grapevine virus A by the mealybug *Pseudococcus longispinus*

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SUMMARY – Transmission of grapevine virus A (GVA) by the pseudococcid mealybug *Pseudococcus longispinus* was studied. Phases of the transmission process (acquisition access time, retention, and inoculation access time) were checked by bioassays using *Nicotiana clevelandii* and RT-PCR. Virus was acquired either from infected *N. clevelandii* or from a purified preparation through a stretched parafilm membrane. Evidence was obtained that *P. longispinus* instars transmit GVA in a semi-persistent manner: they acquired GVA in as little as 15 min when feeding on *N. clevelandii* or 12 h when feeding on purified virus preparations through a membrane; they retained the virus for up to 48 h when fasting, but no longer than 15 h when allowed to feed on herbaceous hosts following serial transfers; they were able to transmit GVA to healthy plants with no latent period, after a 30 min feeding (the shortest inoculation access time tested). A preliminary survey of populations of different Mediterranean countries showed that 77% contained GVA and 33% contained grapevine leafroll-associated virus 3 (GLRaV-3). Many samples, including a population of the coccid mealybugs *Ceroplastes rusci* from Tunisia, contained both viruses.

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