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Occurrence of grapevine virus A (GVA) and other closteroviruses in Tunisian grapevines affected by leafroll disease

by

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Vorkommen von Grapevine-Virus A (GVA) und anderen Closteroviren in blattrollkranken tunesischen Reben

Zusammenfassung: Reben, die aus den Hauptweinbaugebieten Tunesiens stammten, wurden auf die Anwesenheit von Closteroviren hin überprüft. Während in keiner der symptomfreien Reben Viruspartikel entdeckt wurden, enthielten alle Reben mit Blattrollsymptomen — außer zweien — Closteroviruspartikel, die durch IEM (immune electron microscopy) in konzentrierten Blattextrakten oder unmittelbar in Rohsaft durch ISEM (immunosorbent electron microscopy) identifiziert wurden. Alle vier derzeit bekannten Closteroviren (GCIV-1, GCIV-2, GCIV-3 und GVA) waren, meistens im Gemisch, in Reben mit Blattrollsymptomen vorhanden. GCIV-3 und GVA wurden in 77 bzw. 50 % der geprüften Reben entdeckt. Ein tunesisches Isolat von GVA, das durch *Planococcus citri* auf krautige Testpflanzen übertragen wurde, unterschied sich in biologischer Hinsicht, aber nicht in den charakteristischen physikalisch-chemischen und serologischen Eigenschaften von zwei italienischen Isolaten desselben Virus.

Key words: virosis, leaf roll, closterovirus, GCIV-1, GCIV-2, GCIV-3, GVA, analysis, serology, testplant, vector, transmission, Tunisia, Italy.

Introduction

Leafroll of grapevine (*Vitis vinifera* L.) occurs in all viticultural areas of Tunisia (MARTELLI 1986, 1989). The etiology of this disease, long suspected to be viral, has recently been determined and closteroviruses have been identified as possible causal agents. Several such viruses differing from one another in particle length, serological characteristics and transmissibility by pseudococcid mealybugs are known to occur in leafroll-affected vines (for review see BOVEY and MARTELLI 1986; MARTELLI 1988).

Preliminary unpublished observations had shown that unidentified closteroviruses were also present in Tunisian vines with leafroll symptoms. The present paper gives an account of further studies in which the nature of closteroviruses associated with diseased vines was investigated in more detail.

Materials and methods

Source material

The grapevine accessions under investigation originated from different Tunisian viticultural districts (Bizerte, Tunis, Cap Bon, Gabes and other southern oases). Acces-

sions of the TN series (see Table 1) were supplied by F. ASKRI, whereas those of the T series were collected by G. P. MARTELLI or Dr. C. CHERIF during field surveys. All accessions were self-rooted in plastic containers prior to transplanting in a collection plot near Bari. The vines were inspected for expression twice a year for the last 3 years. Out of 67 accessions 45 remained symptomless and 22 showed leafroll symptoms of varying intensity.

Table 1

Closteroviruses present in Tunisian grapevines with leafroll symptoms

Das Vorkommen von Closteroviren in tunesischen Reben mit Symptomen der Blattrollkrankheit

Grapevine accession	Viruses			
	GCIV-1	GCIV-2	GCIV-3	GVA
TN.1	–	–	–	–
TN.6	–	+	+	–
TN.13	–	–	+	+
TN.30	–	–	+	+
TN.32	–	+	+	+
TN.36	–	–	–	+
TN.38	–	–	–	+
TN.40	–	–	+	+
TN.41	+	–	+	–
TN.42	+	+	+	+
TN.44	–	–	+	+
TN.48	–	–	+	+
T/6	–	+	+	–
T/8	–	+	+	–
T/9	+	–	+	–
T/10	–	–	+	–
T/13	–	+	+	–
T/20	–	–	–	–
T/21	–	–	+	–
T/22	–	–	+	+
T/23	–	–	–	+
T/41	+	–	+	–

+ = Presence, – = absence of closterovirus particles.

Extraction and identification of viruses

Virus particles were extracted essentially as described by GUGERLI *et al.* (1984) from 10 g samples of basal grapevine leaf tissues mostly collected in autumn and stored at – 20 °C.

For virus identification, partially purified extracts were placed on microscope grids for particle decoration (MILNE and LUISONI 1977) and floated on drops of 10-fold diluted antisera to grapevine closterovirus type 1 (GCIV-1) (supplied by Dr. P. GUGERLI), grapevine closterovirus type 2 (GCIV-2) (produced at Bari), grapevine closterovirus type 3

(GCIV-3) (supplied by Dr. D. GONSALVES) and grapevine virus A (GVA) (produced at Bari). Immunosorbent electron microscopy (ISEM), made according to ROBERTS and HARRISON (1979), was also used for detecting and identifying GVA in leaf veins or petioles.

Virus characterization

A Tunisian isolate of GVA obtained, as specified below, by mealybug transmission to herbaceous hosts was characterized in comparison with two Italian isolates of the same virus from Sicily (GVA-PA) (CASTROVILLI and GALLITELLI 1985) and Apulia (GVA-151).

Virus isolates were purified as described by CASTROVILLI and GALLITELLI (1985) from systemically infected *Nicotiana benthamiana* DOMIN. plants.

RNA was obtained by treating purified virus with phenol-SDS (CASTROVILLI and GALLITELLI 1985) and electrophoresed in 1.2 % agarose slab gels in a 1 : 1 solution of 50 % formamide and 0.5 M Loening's buffer pH 7.6 after denaturation at 55 °C for 10 min in 50 % formamide. Reference markers were: *Escherichia coli* ribosomal RNAs (2.9 Kb and 1.5 Kb, respectively) and arabis mosaic nepovirus genomic RNA 1 and 2 (6.8 Kb and 3.9 Kb, respectively). The gels were stained with ethidium bromide.

Coat protein subunits were obtained by boiling virus preparations for 5 min in presence of 1.5 % SDS and 3.5 % 2-mercapto-ethanol in 35 mM Tris-HCl buffer pH 7. Protein samples were electrophoresed in 12.5 % polyacrylamide slab gels using LAEMMLI's (1970) discontinuous buffer system and stained with Coomassie brilliant blue. Reference markers (mol. wt in parentheses) were: α-lactoalbumin (14,400), soybean trypsin inhibitor (20,100), carbonic anhydrase (30,000), ovalbumin (43,000), bovine serum albumin (68,000) and phosphorylase B (94,000).

An antiserum to the Tunisian GVA isolate was produced in a rabbit which had been given virus purified by caesium sulphate gradients in one intramuscular injection in Freund's incomplete adjuvant followed by two intravenous injections at weekly intervals. The antiserum was collected 2 weeks after the last injection. Its titre was determined by immune electron microscopy through particle decoration.

Attempts were made to infect herbaceous hosts (*N. clevelandii* and *N. benthamiana*) with crude leaf extracts or partially purified preparations of Tunisian GVA, and grapevine seedlings with purified preparations of GVA-PA.

Mealybug transmission of Tunisian GVA

Trials for transmitting GVA with mealybugs were made using the 11 grapevine accessions that had been found to be infected by this virus (see Table 1). Colonies of *Planococcus citri* RISSO to be used for transmission were reared on virus-free potato sprouts. Donor vines were 2-month-old rooted cuttings caged under fine-mesh cloth in a glasshouse at 24 °C. Groups of no less than 20 *P. citri* crawlers were transferred to each donor vine and allowed to feed for 3 weeks. All surviving mealybugs were transferred to *N. clevelandii* plants for 10 d, then killed by insecticide treatment. Disinfested plants were moved to another fine-mesh screen cage and observed for appearance of symptoms. Controls consisted of *N. clevelandii* seedlings infested with non viruliferous mealybug crawlers.

Results

Presence and distribution of closteroviruses

Both methods (ISEM and micropurification) used for detection and identification of closteroviruses in grapevine leaves were satisfactory. ISEM in particular worked well with GVA.

No virus particles were ever found in the 45 asymptomatic grapevine accessions, whereas, as shown in Table 1, all vines with leafroll symptoms but 2 (accessions TN.1 and T/20) contained one or more closteroviruses. GCIV-3 ranked first, as it was detected in 77 % of the samples (Table 2), followed by GVA (50 %), GCIV-2 (27 %) and GCIV-1 (18 %). In most cases (17 out of 22) closteroviruses occurred in mixture of two or three. In a single instance all four viruses were found in the same accession (TN.42).

Table 2

Relative incidence of closteroviruses in 22 Tunisian grapevines with leafroll symptoms

Rel. Häufigkeit von Closteroviren in 22 tunesischen Reben mit Symptomen der Blattrollkrankheit

Virus	Records			
	Number	%	Single infection	Mixed infection
GVA	11	50	3	8
GCIV-1	4	18	0	4
GCIV-2	6	27	0	6
GCIV-3	17	77	2	15

Mechanical inoculations

In no case transmission of Tunisian closteroviruses to herbaceous hosts was obtained by mechanical inoculation of crude sap or partially purified leaf extracts. Equally unsuccessful were all attempts to infect grapevine seedlings with a purified preparation of GVA-PA.

Whereas isolates GVA-PA and TN.40 were unable to infect herbaceous hosts when inoculated in purified form, comparable preparations of the Apulian isolate GVA-151 could successfully be transmitted to *N. clevelandii*.

Both Italian GVA isolates were readily transmitted by sap inoculation from *N. clevelandii* to *N. benthamiana* and vice versa. However, the Tunisian GVA isolate, which was originally inoculated by mealybugs to *N. clevelandii*, could not be transferred by mechanical means to the same species, but only to *N. benthamiana*. Virus cultures were therefore maintained in this latter species.

Regardless of the differential behaviour in mechanical transmissibility, all three GVA isolates induced comparable symptomatological reactions in herbaceous hosts. The symptoms (vein clearing, reduced growth, mild deformation and crinkling of the leaves) were the same as described in the literature (CONTI *et al.* 1980; ROSCIGLIONE *et al.* 1983; CASTROVILLI and GALLITELLI 1985).

Mealybug transmission of Tunisian GVA

Of the 11 Tunisian grapevine accessions containing GVA (Table 1) tested for virus transmission by *P. citri* instars, only 1 (TN.40) gave positive results. *N. clevelandii* plants came down with typical GVA symptoms short of 3 weeks after exposure to mealybugs crawlers which had fed on infected donor vines. In all other instances no symptoms were shown by *N. clevelandii* seedlings nor could virus be detected by ISEM or recovered by mechanical inoculation from them.

Properties of Tunisian GVA

During purification the isolate TN.40 behaved pretty much the same as the Italian isolates of the same virus (CASTROVILLI and GALLITELLI 1985). In caesium sulphate den-

sity gradient centrifugation, most TN.40 preparations yielded two bands, the upper of which was made up of fragmented particles and host material, whereas the lower denser band contained apparently intact virus particles and was infective.

In agarose gel electrophoresis the nucleic acid migrated as a single species with an apparent size of 7.8 Kb (mol. wt of ca. 2.6 × 10 daltons). The rate of migration was the same as that of RNA extracted from Italian GVA isolates.

Coat protein subunits also migrated at the same rate of the Italian samples and as a single species with an estimated mol. wt of ca. 25,000 daltons.

Serology

The antiserum to Tunisian GVA did not give visible reactions with healthy plant sap in immunodiffusion tests. It clearly decorated homologous and heterologous particles (GVA-PA and GVA-151) to a dilution of 1 : 128.

Discussion

The results of the present investigation confirm that there is a close association between closteroviruses and leafroll disease of grapevines. All four grapevine closteroviruses currently known as separate entities were found in symptomatic Tunisian accessions, usually in mixed infections, but with a clear-cut prevalence of GCIV-3.

GVA was also widely represented occurring in half of the examined samples, an unusually high incidence as compared with records from southern Italy, which rarely exceed 10 % (unpublished information).

The single Tunisian GVA isolate transmitted to herbaceous hosts had physico-chemical characteristics comparable to those of Italian isolates of the same virus studied so far (CONTI *et al.* 1980; BOCCARDO and D'AQUILIO 1981; CASTROVILLI and GALLITELLI 1985) and was serologically very close or indistinguishable from them. However, differences were observed in the biological behaviour of Italian and Tunisian GVA isolates with reference to transmissibility to herbaceous hosts by manual inoculation and mealybug vectors, which warrant more detailed comparative investigations.

Summary

Grapevine accessions coming from the major viticultural areas of Tunisia were surveyed for the presence of closteroviruses. Whereas virus particles were not detected in any of the asymptomatic vines, all accessions showing leafroll symptoms but 2 contained closterovirus particles which were identified by immune electron microscopy in concentrated leaf extracts or directly in crude sap by immunosorbent electron microscopy. All four grapevine closteroviruses currently known (GCIV-1, GCIV-2, GCIV-3 and GVA) were present in symptomatic vines, most frequently in mixtures. GCIV-3 and GVA were detected in 77 % and 50 % of the accessions, respectively. A Tunisian isolate of GVA transmitted by *Planococcus citri* to herbaceous hosts differed biologically but not in physico-chemical and serological characteristics from two Italian isolates of the same virus.

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