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Comparison of RNA extracts from *in vitro* shoot tip cultures of leafroll-affected and leafroll-free grapevine cultivars

by

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Comparaison des acides ribonucléiques obtenus à partir de cultures *in vitro* de vignes saines ou atteintes de l'enroulement

R é s u m é : Des acides ribonucléiques obtenus à partir de cultures *in vitro* de vignes atteintes de la maladie de l'enroulement ont été comparés à ceux obtenus à partir de cultures de vignes saines. Les cultures *in vitro* de 65 % (11 de 17) des variétés atteintes de l'enroulement contenaient un acide nucléique à simple brin (LMWssRNA), non-signalé jusqu'ici, qui était absent chez les vignes saines. Plusieurs ARN bicaténaires (HMWdsRNA) ont été observés chez les vignes atteintes de l'enroulement. L'intensité et la mobilité (poids moléculaire apparent) de ces HMWdsRNA variaient beaucoup d'une variété de vigne à l'autre, mais elles étaient constantes pour chaque variété. Ces observations s'accordent bien avec l'idée que l'enroulement pourrait être causé par plus d'un agent.

K e y w o r d s : disease, leafroll, virus, RNA, variety of vine, in vitro culture, analysis, etiology.

Introduction

Grapevine leafroll (GLR) is one of the most important diseases of grapevines and it occurs world-wide. The symptoms on red cultivars of *Vitis vinifera* consist of leaf reddening accompanied by a downward rolling of the leaves. On white cultivars, the leaves become slightly chlorotic, rather than reddish. The disease is not usually lethal, but affected vines are less vigorous, have smaller and fewer clusters and their fruit have a lower sugar content, which leads to a delayed harvest (GOHEEN 1970; BOVEY *et al.* 1980).

GLR is graft-transmissible (SCHEU 1936). A number of virus-like particles have been associated with the disease, including potyvirus-like particles (TANNE *et al.* 1977), isometric particles (NAMBA *et al.* 1979a; CASTELLANO *et al.* 1983) and closterovirus-like particles (NAMBA *et al.* 1979b; FAORO *et al.* 1981; CASTELLANO *et al.* 1983; GUGERLI *et al.* 1984; MILNE *et al.* 1984; MOSSOP *et al.* 1985; ZEE *et al.* 1987; ZIMMERMANN *et al.* 1988), but the causative agent of the disease has not yet been conclusively identified. The accepted method for diagnosis of GLR is indexing on suitable woody indicators (GOHEEN 1970), a procedure which takes several years to complete. Heat therapy is conventionally used for the production of disease-free apices (GOHEEN 1970). GLR-free plantlets have also been regenerated from fragmented shoot apices (BARLASS *et al.* 1982), by micrografting *in vitro* (AYUSO 1985), by *in vitro* heat therapy (MUR 1979), and by shoot tip culture, with or without prior heat therapy of the GLR-affected grapevine (MONETTE 1988). Plantlets produced by any of these techniques still need to be indexed on woody indicators, in the absence of a more rapid diagnostic procedure.

The potential value of dsRNA extraction and analysis as a virus-indexing method has been noted by MORRIS *et al.* (1983). MOSSOP *et al.* (1985) have reported the presence of a high molecular weight (HMW) dsRNA in GLR-affected, but not in GLR-free grapevines and recently MONETTE *et al.* (1989) have detected a stem pitting-associated dsRNA

in shoot tip cultures of grapevine cultivars affected with rupestris stem pitting. This communication reports on a comparative analysis of the RNA content of *in vitro* shoot tip cultures from GLR-affected and GLR-free grapevines.

Materials and methods

22 grapevine cultivars were used in this study (Table). All had been indexed on woody indicators (*V. rupestris* St. George, *V. vinifera* Pinot noir and the hybrid LN-33). 12 cultivars were affected with leafroll alone, 2 were affected with leafroll and also contained grapevine fanleaf virus, and 3 were affected with leafroll as well as fleck, stem grooving or corky bark. Another 5 cultivars were GLR disease-free.

Media and procedures for grapevine *in vitro* shoot tip culture were as previously published (MONETTE *et al.* 1989). Procedures for the extraction of nucleic acids, for their analysis by polyacrylamide gel electrophoresis and silver staining and for nuclease digestion have already been described (MONETTE *et al.* 1989). Nuclease digestions were conducted on extracts from shoot tip cultures of Riesling cl. 239.

Table

PAGE analysis of RNA extracts from *in vitro* shoot tip cultures of GLR-affected and GLR-free grapevines

Analyse électrophorétique sur gels de polyacrylamide des acides ribonucléiques obtenus de cultures *in vitro* de vignes saines ou atteintes de la maladie de l'enroulement

Cultivar	Disease diagnosed on woody indicators[1])	LMWssRNA[2])
Gf. 31-17-115	GLR	+
Schuyler	GLR	+
Pirobelle	GLR	+
Schwarzriesling	GLR	+
Appley Towers	GLR	+
Riesling cl. 239	GLR	+
Müller-Thurgau (1/1 GM)	GLR, fanleaf	+
Auxerrois 22 GM	GLR, fanleaf	+
Pinot noir (Hurlman 2/45)	GLR, fleck	+
Scheurebe	GLR, stem grooving	+
Semillon	GLR, corky bark	+
Kadarka	GLR	−
Limberger	GLR	−
Castel 19637	GLR	−
Jakaranda	GLR	−
Jubileum 75	GLR	−
Vidal 256	GLR	−
Limberger	fleck	−
Vidal 256	none	−
White Riesling	none	−
Pinot Chardonnay	none	−
SO 4	none	−

[1]) Indicators used: *V. rupestris* St. George, *V. vinifera* Pinot noir, LN-33.
[2]) + = Present; − = absent.

Results and discussion

11 of the 17 GLR-affected grapevine cultivars (65 %) contained a low-molecular-weight single-stranded RNA (LMWssRNA) which was absent from GLR-free grapevines (Table). This LMWssRNA was present as a major component in all 11 cultivars (Fig. 1, arrow 1) and may be related to a virus, or a strain of a virus, responsible for GLR. This RNA is primarily a single-stranded molecule, as it was susceptible to RNAse A under both low and high salt conditions (Fig. 2). The observation that it adsorbed to phosphocellulose in TSE containing 15 % ethanol suggests that it may contain some double-stranded regions (DODDS *et al.* 1984). One interesting possibility is that the LMWssRNA reported here may represent a subgenomic component of a virus, for example a closterovirus. Should this be the case, the absence of this LMWssRNA in 6 of the 17 GLR-affected samples might reflect either virus strain differences or a host cultivar influence on virus gene transcription (DODDS *et al.* 1987). Alternatively, this LMWssRNA might be a satellite RNA.

A LMWdsRNA with an apparent molecular weight of 0.24×10^6 Da was recently detected in *in vitro* shoot tip cultures of stem pitting-affected grapevines (MONETTE *et al.* 1989). This double-stranded stem pitting-associated (SP-A) RNA and the LMWssRNA reported here had different migration rates in polyacrylamide gels. The difference was slight but reproducible, with the SP-A RNA migrating farther into the gel than the LMWssRNA from GLR-affected grapevines. A more thorough comparative analysis of these RNAs is in progress in order to evaluate a possible relationship between them.

Numerous HMWdsRNA bands, ranging in apparent size from 1.3×10^6 Da to 15×10^6 Da, were observed in samples from *in vitro* shoot tip cultures of GLR-affected grapevines (Fig. 1). This region of the gel may contain a band similar to the leafroll-associated 8×10^6 Da band detected by MOSSOP *et al.* (1985) in RNA extracts from phloem tissue, but this band could not be clearly identified in our gels. The HMWdsRNAs detected in shoot tip cultures of GLR-affected cultivars varied both in intensity and mobility (apparent molecular weight) from cultivar to cultivar, but were reproducible for each cultivar. Certain banding patterns were quite distinctive. For example, GLR-affected Limberger (Fig. 1 A, lane 4) showed a pattern of HMW bands which were absent from either GLR-free Limberger (Fig. 1 A, lane 3) or the other GLR-affected cultivars. The pattern obtained with Semillon (Fig. 1 A, lane 10) was also different from those observed in the other GLR-affected cultivars. Semillon, however, was corky bark- as well as GLR-affected. Consequently, the banding pattern obtained with this cultivar might in part be related to corky bark disease. 4 GLR-affected cultivars, Gf. 31-17-115, Pinot noir, Riesling cl. 239 and Appley Towers, appeared to share a common band (Fig. 1, arrow 2) migrating slightly farther than the 0.6×10^6 Da marker fragment. The multiplicity of clear banding patterns obtained with RNA extracts from GLR-affected grapevine shoot tip cultures is consistent with the view that grapevine leafroll disease may consist of several diseases with very similar symptoms. The apparently conflicting reports concerning the association of several distinct virus-like particles with grapevine leafroll disease may be easily reconciled if GLR does prove to have more than one causal agent.

The use of *in vitro* shoot tip cultures as samples for RNA extraction and analysis clearly has potential for the elucidation of grapevine leafroll disease etiology and the subsequent development of appropriate diagnostic tests. This approach may be a valuable supplement to current serological and electron microscopical studies (TZENG 1984; ROSCIGLIONE and GUGERLI 1986; ENGELBRECHT and KASDORF 1987; ZEE *et al.* 1987; ZIMMERMANN *et al.* 1988).

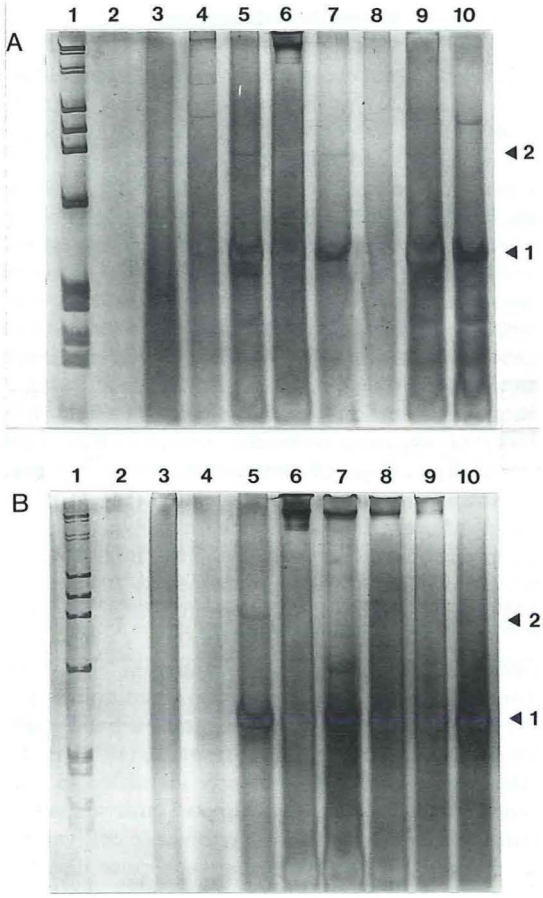

Fig. 1: PAGE analysis of RNA extracts from *in vitro* shoot tip cultures of GLR-free and GLR-affected grapevines. — Lanes in (A) show DRIgest III (Pharmacia) markers (lane 1), empty lane (2), GLR-free (fleck-affected) Limberger (3), GLR-affected Limberger (4), GLR- and fleck-affected Pinot noir (5), GLR- and stem grooving-affected Scheurebe (6), GLR-affected Appley Towers (7), GLR-affected Jubileum 75 (8), GLR-affected Riesling cl. 239 (9) and GLR- and corky bark-affected Semillon (10). — Lanes in (B) show DRIgest markers (1), empty lane (2), GLR-free Vidal 256 (3), GLR-affected Vidal 256 (4), GLR-affected Gf. 31-17-115 (5), GLR-affected Schuyler (6), GLR-affected Pirobelle (7), GLR-affected Schwarzriesling (8), GLR- and fanleaf-affected Müller-Thurgau (9) and GLR- and fanleaf-affected Auxerrois 22 GM (10). — Arrow 1 points to the LMWssRNA. Arrow 2 points to a band common to Gf. 31-17-115, Pinot Noir, Riesling cl. 239 and Appley Towers.

Analyse électrophorétique sur gels de polyacrylamide d'ARN obtenus à partir de cultures *in vitro* de vignes saines ou atteintes de l'enroulement. — Les pistes dans (A) contiennent: (1) des acides nucléiques à poids définis (DRIgest III), (2) une piste vide, (3) Limberger atteinte du «fleck» mais non atteinte de l'enroulement, (4) Limberger atteinte de l'enroulement, (5) Pinot noir atteinte de l'enroulement et du «fleck», (6) Scheurebe atteinte de l'enroulement et du «stem grooving», (7) Appley Towers atteinte de l'enroulement, (8) Jubileum 75 atteinte de l'enroulement, (9) Riesling cl. 239 atteinte de l'enroulement et (10) Semillon atteinte de l'enroulement et du «corky bark». — Les pistes dans (B) contiennent: (1) des acides nucléiques à poids définis, (2) une piste vide, (3) Vidal 256 saine, (4) Vidal 256 atteinte de l'enroulement, (5) Gf. 31-17-115 atteinte de l'enroulement, (6) Schuyler atteinte de l'enroulement, (7) Pirobelle atteinte de l'enroulement, (8) Schwarzriesling atteinte de l'enroulement, (9) Müller-Thurgau atteinte de l'enroulement et du «fanleaf», et (10) Auxerrois 22 GM atteinte de l'enroulement et du «fanleaf». — La flèche numéro 1 indique la location du LMWssRNA.

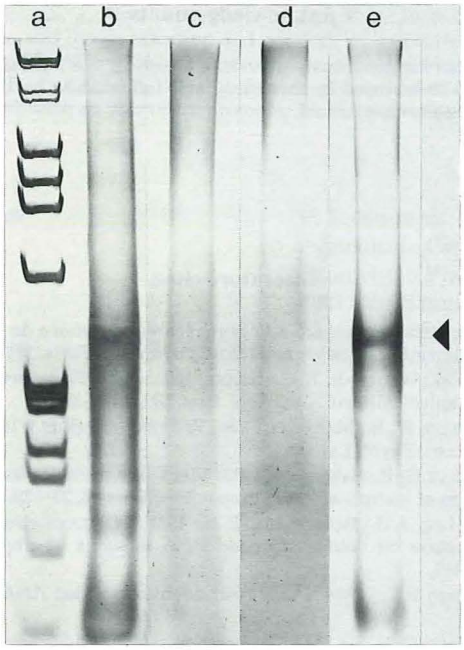

Fig. 2: Polyacrylamide gel electrophoresis of RNA extracted from GLR-affected Riesling cl. 239. Lane (a) contains size markers and lane (b) RNA not subjected to nuclease treatment. Lanes (c), (d) and (e) were exposed to RNase A in low salt, RNase A in high salt and DNase 1, respectively, at 31 °C for 2 h prior to silver staining. The arrow points to the LMWssRNA.

Electrophorèse d'ARN obtenus à partir de cultures *in vitro* de la variété Riesling cl. 239. Le gel (a) contient des acides nucléiques à poids définis. Le gel (b) contient les ARN extraits du Riesling cl. 239 et n'a été soumis à aucune digestion enzymatique. Les gels (c), (d) et (e) contiennent les mêmes ARN que le gel (b), mais ils ont été placés à 31 °C pour 2 h avec, respectivement, du RNase A sous une faible concentration de sels, du RNase A sous une forte concentration de sels et du DNase 1, avant d'être colorés à l'argent. La flèche indique la location du LMWssRNA.

Summary

The RNA content of *in vitro* shoot tip cultures from grapevine leafroll (GLR) disease-affected grapevines was analyzed and compared to that of similar cultures from GLR-free grapevines. A previously unreported low-molecular-weight single-stranded RNA (LMWssRNA) was detected in *in vitro* shoot tip cultures of 65 % (11 out of 17) of GLR-affected cultivars. This LMWssRNA was absent from disease-free cultivars and may be associated with a virus or a strain of a virus responsible for GLR. Numerous high-molecular-weight (HMW) dsRNA bands were also detected in GLR-affected grapevine cultivars. The intensities and mobilities (apparent molecular weights) of these dsRNA bands varied considerably from one GLR-affected cultivar to the next, but were reproducible for each cultivar. The detection of multiple distinctive RNA banding patterns is consistent with the possibility that more than one agent can cause grapevine leafroll disease.

La flèche numéro 2 indique la location d'un ARN en commun à quatre variétés atteintes de l'enroulement, Gf. 31-17-115, Pinot noir, Riesling cl. 239 et Appley Towers.

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