Research Note

Effects of various types of virus on Vitis vinifera L. cv. Albariño cultivated in vitro

ELена GONZALEZ, ТЕРЕЗА DIAZ и MARIA VICTORIA MOSQUERA

Summary: The shoot tips of grapevines (Vitis vinifera L. cv. Albariño), either healthy or infected with the grapevine leafroll disease type III (GLD-III), grapevine fleck disease + grapevine leafroll disease type I (GFD+GLD-I), and grapevine stem pitting (GSP) were cultivated in vitro. Multiplication and rooting, were best in healthy shoots and decreased according to the type of virus.

Key words: Vitis, in vitro culture, virus diseases.

Introduction: Several studies have been carried out to evaluate the physiology of virus-infected grapevines cultivated in vitro. According to Walter (1988) the rooting capacity and the vegetative multiplication in vitro depends on the healthy condition of the plant. Barba et al. (1989) found that shoots of Merlot affected by leafroll and infected by closterovirus type III cultivated in vitro displayed stems of a much smaller size than healthy plants. Abracheva et al. (1994) observed that in Rupestris du Lot the grapevine fanleaf virus (GFV) and stem pitting (GSP) provoked a decrease of growth.

The objective of this study was to investigate the effect of three grapevine viruses on the multiplication rate and rooting ability of vine shoots cultivated in vitro.

Material and methods: Shoots of 4 clones of Vitis vinifera L. cv. Albariño, one of them healthy and three virus-infected, were used with the following variants: grapevine leafroll disease type III (GLD-III), grapevine fleck disease + grapevine leafroll disease type I (GFD+GLD-I), and grapevine stem pitting (GSP). For in vitro cultivation apical explants (2-3 cm) were isolated from a greenhouse derived starting material of infected and healthy plants.

The initial explants (apical shoots, 2-4 mm) were isolated under sterile conditions using a stereo-scopic microscope. Explants were placed in glass tubes, one explant per tube, containing 25 ml of liquid culture medium consisting of Murashige and Skoog (1962) mineral salts, vitamins as described by Ferro (1989), and 3 % sucrose. Growth regulators were used: 1 mg/l of BA (6-benzyladenine) and 0.5 mg/l of NAA (1-naphthalen-acetic acid). The pH was adjusted to 5.6 before autoclaving. Explants growing in the liquid medium were then placed in tubes with Watman No1 filter paper bridges.

After 30 d, healthy and virus-infected plants, were placed in a solid multiplication medium with 1 mg/l of BA and 0.5 mg/l of zeatine. The medium were solidified with 0.7 % of Difco-Bacto Agar. Shoots larger than 1 cm in length, developed from this culture, were sectioned and subcultured onto the same multiplication medium. This procedure was repeated 4 times. The time for each culture was 30 d. At the end of 4 multiplications the following variables were determined: percentage of explants showing proliferation, number of shoots per explant and maximum shoot length.

Rooting was studied with shoots produced after 5 subcultures. Microshoots (2-3 cm) were cultured in liquid multiplication medium but only with 1 mg/l of NAA for 7 d, then transferred to the same medium without growth regulators. After 30 d in culture, the following variables were determined: percentage of rooted shoots, number of roots per shoot and maximum root length.

In all cases cultures were kept grown in a growth cabinet at a 16 h light regime, 25/20 °C and an illumination of 25 µmol photons. m⁻².s⁻¹. The experiments were repeated 3 times. For each culture 24 explants were used. The percentages were transformed into arcsin % before statistical analysis (Sokal and Rohlf 1979). The differences were tested by analysis of variances by Duncan’s multiple range test (Ruiz-Mayá 1977).

Results and discussion: The proliferation percentage, number of shoots per explant and shoot length of infected plants were reduced compared to healthy shoots; only shoots infected with GSP showed results similar to those of the control (Tab. 1). The results are in accordance with the data obtained with citrus plants by Grego et al. (1998), who demonstrated that the infection of citrus with virus and virus-like pathogenes can have a marked effect on the development of explants cultured in vitro, however this effect depends on the type of pathogene.

Table 1

<table>
<thead>
<tr>
<th>Type of virus</th>
<th>Percentage of proliferation</th>
<th>Shoots per explant</th>
<th>Maximum shoot length (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy</td>
<td>87.5 a</td>
<td>3.1 a</td>
<td>4.9 a</td>
</tr>
<tr>
<td>GLD-III</td>
<td>53.9 b</td>
<td>1.6 b</td>
<td>3.3 a</td>
</tr>
<tr>
<td>GFD+GLD-I</td>
<td>61.4 b</td>
<td>2.3 a</td>
<td>4.2 a</td>
</tr>
<tr>
<td>GSP</td>
<td>87.1 a</td>
<td>2.4 a</td>
<td>5.0 a</td>
</tr>
</tbody>
</table>

Values within column followed by the same letter are not significantly different at the 5% level (Duncan’s multiple range test).

Abracheva et al. (1994) observed that shoot length of Rupestris du Lot infected with GFV and GSP was significantly lower than that of healthy vines. In our experiments the shoot length with GSP was almost identical to that of healthy shoots and, although the remaining shoots were shorter, the differences were not significant.

University of Santiago de Compostela, Facultad de Farmacia, Fisiologia Vegetal, E-15706 Santiago de Compostela, Spain. Fax: (981) 594912.
The rooting percentage was very high, especially in the healthy shoots and those infected with GLD-III (Tab. 2). Although no significant differences were found, the number of roots per explant demonstrates a behaviour similar to the rooting percentage. Maximum root length was significantly higher in healthy shoots, which is confirmed by the observations of Abracheva et al. (1994) with the vine Rupestris du Lot infected with GFV and GSP.

Table 2

Effect of different types of virus on rooting

<table>
<thead>
<tr>
<th>Type of virus</th>
<th>Percent of rooted shoots</th>
<th>Roots per shoot</th>
<th>Maximum root length (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy</td>
<td>100 a</td>
<td>6.6 a</td>
<td>6.0 a</td>
</tr>
<tr>
<td>GLD-III</td>
<td>100 a</td>
<td>7.6 a</td>
<td>3.4 b</td>
</tr>
<tr>
<td>GFD+GLD-I</td>
<td>87 b</td>
<td>4.6 a</td>
<td>2.1 b</td>
</tr>
<tr>
<td>GSP</td>
<td>80 b</td>
<td>5.0 a</td>
<td>2.5 b</td>
</tr>
</tbody>
</table>

Values within column followed by the same letter are not significantly different at the 5% level (Duncan's multiple range test).

Our results confirm that differences in the capacity of rooting and vegetative multiplication in vitro of grapevines are due to the health condition of the plants (Walter 1988) and to the type of virus affecting the shoot (Greño et al. 1998).

This research has been supported by Consellería de Educación e Ordenación Universitaria de la Xunta de Galicia (Xuga 81505488).


