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Research Note

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

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S u m m a r y : 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.

K e y w o r d s : 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. ABRACHEWA *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 N°1 filter paper bridges.

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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 (RUÍZ-MAYA 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 GREÑO *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.

T a b l e 1

Effect of different types of virus on the multiplication of shoots

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

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.

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

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

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).

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ABRACHEVA, P.; ROZENEVA, L.; TODOROVA, M.; 1994: L'influence de grapevine fanleaf virus et de stem pitting sur la cultivation de la vigne *in vitro*. Vitis **33**, 181-182.

BARBA, M.; CUPIDI, A.; FAGGLIOLI, G.; 1989: *In vitro* culture of grapevine infected by closterovirus type III. J. Phytopathol. **126**, 225-230.

FERRO, E.; 1989: Aplicación de las técnicas del cultivo *in vitro* de ápices caulinares en el saneamiento de clones selec-cionados de vid, cv. Albariño. Tesis Doctoral, Santiago de Compostela, Spain.

GREÑO, V.; NAVARRO, L.; DURAN-VILA, N.; 1988: Influences of virus and virus-like agents on the development of citrus buds cultured *in vitro*. Plant Cell Tiss. Org. Cult. **15**, 113-124.

MURASHIGE, T.; SKOOG, F.; 1962: A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol. Plant. **15**, 437-479.

RUIZ-MAYA, L.; 1977: Métodos estadísticos de investigación (Introducción al análisis de la varianza). Presidencia del Gobierno. Instituto Nacional de Estadística, Madrid.

SOKAL, R. R.; ROHLF, F. J.; 1979: Biometría. Principios y métodos estadísticos en la investigación biológica. H. Blume, Madrid.

WALTER, B.; 1988: Some examples of the physiological reaction of the vine in the presence of viruses. Bull. O.I.V. **61**, 383-390.