Research Note

Study of the genetic homogeneity of Albariño (*Vitis vinifera* L.) growing in Galicia (Spain) using isozyme and RAPD markers

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Summary: An evaluation of the genetic diversity of cv. Albariño (*Vitis vinifera* L.) was carried out. Centenarian and young plants were selected from vineyards, some of them showing slight ampelographic differences. Using ELISA tests 5 out of 24 plants were found to be infected with grapevine leafroll-associated virus 3 (GLRaV-3). In order to evaluate genetic polymorphism, 10 enzyme systems and 42 RAPD primers were used. The 73 isozyme and 308 RAPD markers were common in the samples tested. The results show the existence of a genetic homogeneity within Albariño cultivated in Galicia. Minor ampelographic differences among samples could be due to external factors rather than to genetic differences.

Key words: genetic diversity, grapevine, isozymes, leafroll virus, RAPD markers.

Introduction: The grapevine cv. Albariño (*Vitis vinifera* L.) is one of the most important cultivars grown in Galicia (Northwest of Spain), where it is cultivated since ancient times (Casares 1843). Due to a lack of official clonal and sanitary selection vinegrowers and nurseries selected their own scions for planting. Thus, at present, there are minor plots with centenarian plants that survived the phylloxera invasion, while in the majority of vineyards young grafted vines are planted the origin of which is not well known.

Growers have reported slight differences in morphology and yield among plants, especially in new vineyards. Differences might have a genetic base but might also be due to environmental factors (soil, climate, cultivation) or to the presence of viruses. In this region, Albariño is grown under different cultivation practices and only the incidence of grapevine leafroll associated virus 3 (GLRaV-3) is important (Cabaleiro and Segura 1997).

Albariño has been described by classical ampelographic methods (Pérez *et al.* 1993), however they can be affected by environmental factors and tissue age (Wolfe 1976) and are not adequate to identify closely related genotypes. Isoenzymes and DNA techniques have been used to differentiate closely related cultivars (Benin *et al.* 1988; Gogorcena *et al.* 1993; Tschammer and Zyprian 1994).

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In this study, we report the use of isoenzyme and RAPD markers to analyse the genetic diversity among 24 plants of Albariño cultivated in different vineyards and to find out whether the reported ampelographic differences have a genetic base.

Materials and methods: Isozymes analysis: One-year-old dormant canes were collected up in late autumn (Table). Cambial scrapings were frozen in liquid nitrogen, ground with a mortar, mixed in extraction buffer (Arulsekar and Parfitt 1986) and ground again. Electrophoresis was carried out in polyacrylamide gels at 4 °C and 20 mA Tris-glycine (pH 8.6) electrode bufer. Gels were 1 mm thick and consisted of 3 layers: resolution (10 % acrylamide), separation (6 %) and sample (4 %); ca. 150 µg of protein were placed into the wells. Ten enzyme systems were studied: acid phosphatase, catechol oxidase, esterases, glucose phosphate isomerase, aspartate aminotransferase, leucine aminopeptidase, malate dehydrogenase, phosphoglucomutase, peroxidases, and superoxide dismutase.

RAPD conditions: DNA extractions were performed according to Torres *et al.* (1993). Amplification was carried out in 25 µl volume with 5 ng DNA template, reaction buffer GoldStar 1X (75 mM Tris-HCl pH 9.0; 20 mM $(NH_4)_2SO_4$; 0.01 % Tween 20), 1.5 mM $MgCl_2$, 100 µM

Table

Albariño plant material. All samples were colleted in the Appellation of Origin Rías Baixas

Code[a]	Vineyard	Morphology[b]
A1	Cambados 1	small bunch and leaf
A2	Sanxenxo	typical morphology
A3	Ribadumia 1	small bunch and leaf
A4	Ribadumia 2	typical morphology
A5	Barro	typical morphology
A6	Meis	typical morphology
A7	Rosal 1	small bunch
A8	Rosal 1	large bunch
A9	Meaño	small bunch and leaf
A10	Meaño	typical morphology
A11	Ribadumia 3	small bunch
A12	Ribadumia 3	large bunch
A13	Ribadumia 4	small grapes
A14	Ribadumia 4	typical morphology
A15	Vilanova 1	typical morphology
A16	Vilanova 2	large bunch
A17	Rosal 2	morphological differences
A18	Rosal 2	morphological differences
A19	Rosal 3	typical morphology
A20	Rosal 4	large bunch
A21	Rosal 5	typical morphology
A22	Arbo 1	large bunch
A23	Arbo 2	typical morphology
A24	Salvaterra	typical morphology

[a] Centenarian (1 to 10) and under 25 years old (11 to 24) samples.
[b] Morphological particularities observed in the vineyard.

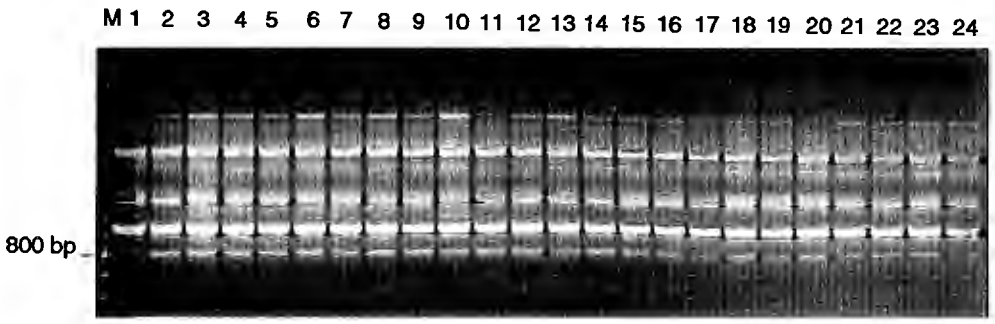

Figure: RAPD patterns obtained with primer BC536 (GCCCCTCGTC). Numbers indicated at the top correspond to the plants of Albariño as in the Table. M indicates DNA molecular weight marker (100 bp ladder). Number on the left side represents molecular weight (bp).

dNTPs, 0.2 µM primer British Columbia (BC) and 0.2 units of GoldStar DNA polymerase (Eurogentec, Belgium). The thermocycler (Perkin Elmer 480) was programmed for one initial step of 2 min at 94 °C followed by 40 cycles of 1 min at 94 °C, 1 min at 36 °C and 2 min at 72 °C.

Virus detection: Bark scrapings (see above) were tested for GLRaV-3 virus by DAS-ELISA (double antibody sandwich enzyme-linked immunosorbent assay). Virus extraction and diagnosis were carried out as described by CABALEIRO and SEGURA (1997). Antibodies and their conjugates were obtained from Bioreba AG (Switzerland). Samples of virus-free grapevine tissues were used as a negative control.

Results and Discussion: Forty-two primers producing unambiguous amplifications and polymorphism between the two varieties, Albariño and Garnacha, were selected from screening of 100 BC RAPD primers (Set 6). The discriminative power of most of the primers used was previously tested on 16 cultivars (VIDAL *et al.* in press).

A total of 73 isozyme and 305 RAPD bands were studied. The plants of Albariño showed the same banding patterns for all 10 enzyme systems and the 42 selected primers (Figure). Esterases showed the highest number of bands (16) and the leucine aminopeptidases the lowest (3). An average of 7.2 amplified fragments per primer was obtained, ranging from 16 (BC526) to only two (BC577). Previous studies demonstrated that closely related grape cultivars can generally be differentiated by their isozyme and RAPD patterns (BENIN *et al.* 1988; TSCHAMMER and ZYPRIAN 1994). Moreover, if no differences are detected between samples after an intensive RAPD analysis, they probably represent the same cultivar (GOGORCENA *et al.* 1993). Therefore, our molecular study indicates that probably all selected samples belong to the same cultivar.

Plants infected with GLRaV-3 virus (samples 8, 17, 18, 21 and 24) shared profile with healthy plants. The presence of the GLRaV-3 virus in Albariño (A17 and A18) induced changes of ampelographic patterns, although some of the infected samples (A21 and A24) showed neither virus symptoms nor ampelographic differences. The infected sample A8 (named "large bunch"), without virus symptoms and with different morphology as compared to the virus-free sample A7 (named "small bunch"), was found in the same vineyard. WALTER (1988) reported that virus infections can alter the physiology and morphology of grape. The banding patterns of centenarian plants (samples 1 to 10), was not different from those of young plants (samples 11 to 24).

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