Isozyme pattern comparison between tissue-cultured grapevines and mother plants [1])

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Summary: Isozyme analysis is one of the means suitable to characterize clonally propagated cultivars.

Isoelectric focusing was used to reveal differences in isozyme patterns between tissue-cultured plants and mother plants, for the cultivars Barbera, Queen of the Vineyards, Dolcetto and Delight. In cultivar Barbera both 2n and 4n plants were considered.

Leaf samples were collected from shoots grown on cuttings under controlled environmental conditions and from plants obtained by tissue culture. The buds used for tissue culture were taken from the same shooted cuttings.

Leaf extracts were analyzed by isoelectric focusing considering the following isozymes: AcPH (acid phosphatase), GPI (glucose phosphate isomerase) and PGM (phosphoglucomutase).

The banding patterns of GPI and PGM showed differences among the cultivars, while for AcPH there seemed to be no differences among them in the pH range considered. There were no differences between isozyme patterns of the Barbera 2n and Barbera 4n.

The main difference between *in vitro* plants and mother plants was the amount of isozyme evaluated by densitometric measurements. In all the cultivars, the amount of isozymes for AcPH was higher in mother plants than in *in vitro* ones, while for PGM and GPI it was the opposite.

This can be due to the different environmental conditions affecting cellular metabolism.

Key words: enzyme, protein, analysis, leaf, variety of vine, clone, tissue culture, ampelography.

Introduction

Isozyme analysis of grapevine has been carried out with leaves, berries and canes using either starch gel or polyacrylamide gel electrophoresis (Wolfe 1976; Dal Belin Peruffo *et al.* 1981; Arulsekar and Parfitt 1986; Benin *et al.* 1986; Subden *et al.* 1987; Bachmann and Blaich 1988; Benin *et al.* 1988; Paludetti and Calo 1988). These authors pointed out that isozyme analysis is one of the means suitable to characterize cultivars and clones.

In this investigation isoelectric focusing was used to ascertain possible differences in isozyme patterns between tissue-cultured plants and mother plants for the cultivars Barbera, Queen of the Vineyards, Dolcetto and Delight.

The grapevines used came from a vineyard of irradiated plants (γ-rays) and presented interesting characters: Dolcetto was early ripening; Delight had berries larger than usual; Queen of the Vineyards was parthenocarpic; Barbera was tetraploid. In cv. Barbera a diploid plant was also considered.

Materials and methods

Samples of young, expanding leaves were collected from the 1st up to the 3rd node of shoots grown from cuttings and of tissue-cultured plants. The buds used for tissue culture were taken from the same shooted cuttings.

The cuttings were kept in the Knoop nutritive solution at 22 °C with a 16-h photoperiod.

The tissue-cultured plants were grown on MS (Murashige and Skoog) medium added with 5 μM BAP (6-benzylaminopurine); to stimulate rooting 1 mg/l IBA (3-indolebutyric acid) was

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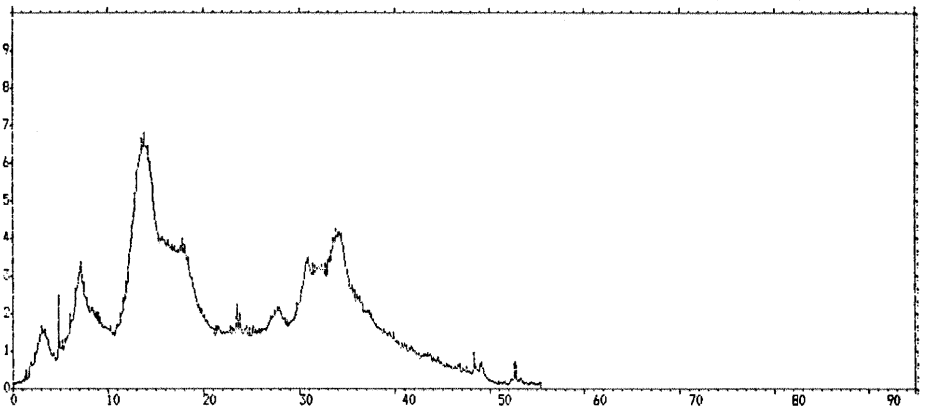
A



PEAK AREAS of
16/6PGM04 (Scale = 8, AbsR.= 0.5)

Scaling factor : 1.000
Background : horizontal line
Peak areas : fitted

Peak	Position	Area	Area %	Peak	Position	Area	Area %
1	3.1	162.3	2.3	7	31.2	716.5	10.3
2	7.7	812.6	11.7	8	34.0	512.5	7.4
3	13.6	1379.3	19.9	9	37.3	897.7	12.9
4	17.2	1086.1	15.6	10	43.1	165.6	2.4
5	23.3	874.7	12.6	11	47.4	112.4	1.6
6	27.8	228.1	3.3				

Total Area 6947.8 = 100.0 %

Fig. 1: Cultivar Delight; densitometric measurement of PGM banding pattern. A) *in vitro* cultured plants; B) mother plants. (Continued overleaf.)

added. The environmental conditions were kept at 24 °C during 16 h light and 16 °C during 8 h dark.

Leaf extracts were obtained according to BENIN *et al.* (1986, 1988) with 1 ml of the buffer (pH 8.5) proposed by SCHAEFER (1971) using 0.1 or 0.2 g of leaves.

The homogenized samples were centrifuged at 4 °C for 15 min at 10,000 rpm; 20 μl of each sample were put on a polyacrylamide gel plate (LKB Ampholine-PAGplate) for isoelectric focusing.

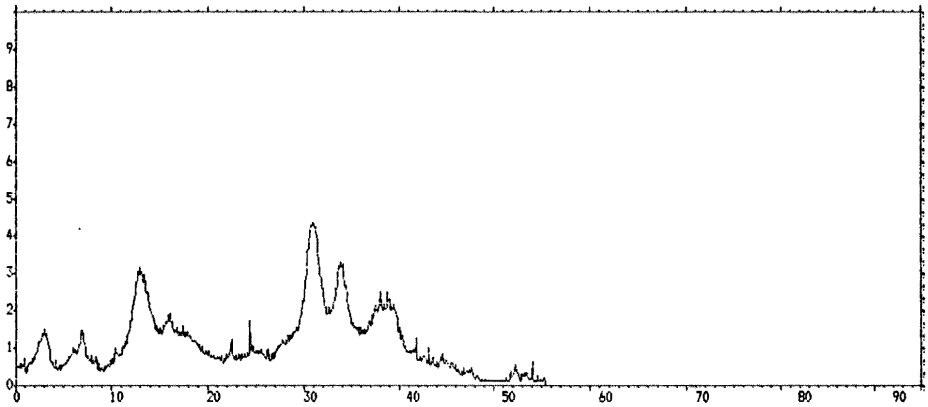
PAGplate pH range was 4.0-6.5 for AcPH and PGM with running conditions of 2,000 V, 40 mA, 50 W at 4.3 °C for about 2 h. The pH range was 3.5-9.5 for GPI and the running conditions were 1,500 V, 50 mA, 30 W at 5 °C for about 2 h. The isoelectric points were estimated with LKB markers.

Staining solution for AcPH was prepared according to ARULSEKAR and PARFITT (1986); for PGM and GPI a staining solution modified from SHAW and PRASAD (1970) was used.

The densitometric measurements were made with an LKB 2202 ULTROSCAN laser densitometer.

B

INTENSITY PROFILE of
16/6PGM05 (meas., Scale = 8, AbsR.= 0.5)



PEAK AREAS of
16/6PGM05 (Scale = 8, AbsR.= 0.5)

Scaling factor : 1.000
Background : horizontal line
Peak areas : fitted

Peak	Position	Area	Area %	Peak	Position	Area	Area %
1	2.9	163.7	4.0	7	31.0	487.2	12.0
2	6.7	212.8	5.2	8	33.8	432.6	10.6
3	12.9	426.5	10.5	9	38.2	776.8	19.1
4	16.5	630.1	15.5	10	43.9	203.8	5.0
5	23.7	317.7	7.8	11	45.6	2.7	0.1
6	29.2	411.6	10.1				

Total Area 4065.5 = 100.0 %

Fig. 1 (continued)

Results and discussion

The isozymes studied catalyze reactions in the glucide cycle leading to the formation of sugars and starch that can be accumulated in the leaves.

The leaf amount used for the extraction (0.1 g/ml) of PGM and GPI gave scarcely evident bands for the mother plants. For this reason, the extraction was performed doubling the leaf amount of the mother plants (0.2 g/ml), thus obtaining more evident bands and revealing the different isozyme content of the plants. This difference was confirmed by densitometric measurements (Fig. 1).

The banding patterns of PGM showed no differences between *in vitro* cultured and mother plants and between Barbera 2n and 4n (Fig. 2) and the differences among the cultivars that are depicted in Fig. 3. The isoelectric range of PGM was 4.85-6.45.

Considering GPI, the cultivars showed similar anodal and cathodal bands; in the isoelectric range 5.65-5.92 the cultivar Barbera showed two bands more than the cultivars Delight and Queen of the Vineyards, while the cultivar Dolcetto had only one of them. No differences between *in vitro* cultured and mother plants or between Barbera 2n and 4n were observed. The isoelectric range of GPI was 5.65-7.3.

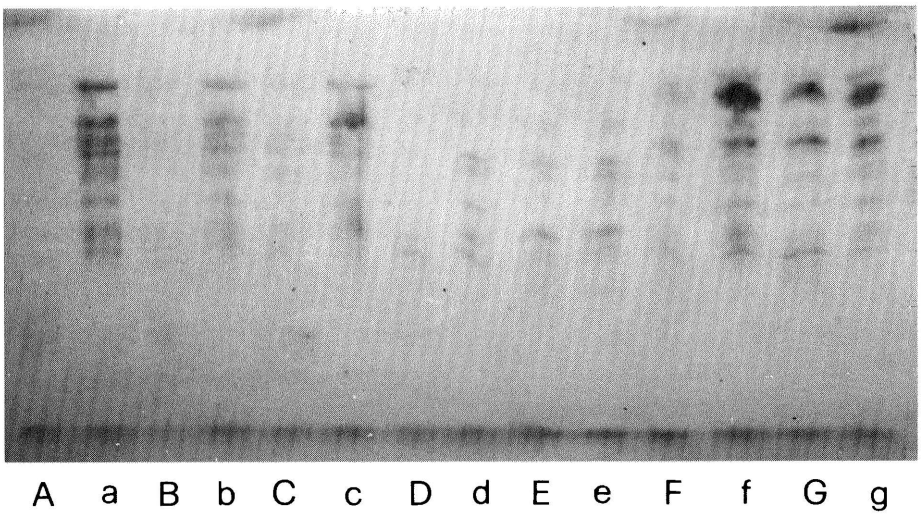


Fig. 2: banding patterns of PGM. A-a and B-b: Barbera 4n; C-c: Barbera 2n; D-d: Queen of the Vineyards; E-e: Dolcetto; F-f and G-g: Delight. Capital letters for mother plants; small letters for *in vitro* cultured plants.

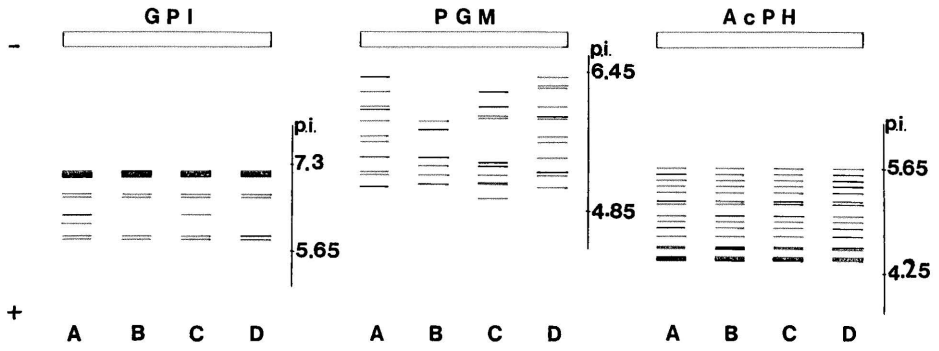


Fig. 3: Banding patterns of both *in vitro* cultured and mother plants. A) Barbera 2n and 4n; B) Queen of the Vineyards; C) Dolcetto; D) Delight.

The AcPH banding patterns showed many and quite close bands which made the comparison among the patterns difficult. Yet, the banding patterns seemed to be the same for all the cultivars considered (Fig. 4) and no differences were noticed between *in vitro* cultured and mother plants, apart from the isozyme content which was lower for the *in vitro* plants. The isoelectric range of AcPH was 4.25-5.65.

The banding patterns of PGM, GPI and AcPH are depicted in Fig. 3.

Therefore, for the three isozymes, the differences between tissue-cultured plants and mother plants were only in their amount. This can be due to the different environmental conditions affecting cellular metabolism.

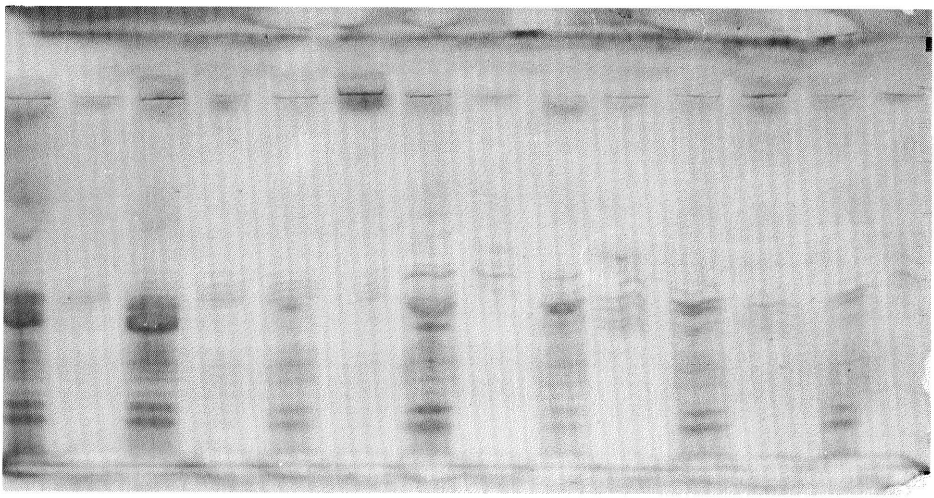


Fig. 4: Banding patterns of AcPH. For explanation of letters see legend to Fig. 2.

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