

## A chemotaxonomic investigation on *Vitis vinifera* L. III: Characterization of *Vitis* biotypes via root apex proteins

by

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**S u m m a r y :** Soluble proteins from root tips were used as biochemical markers to study, taxonomically and systematically, *Vitis* biotypes. Analyses of total proteins in the pH range 3.5-10 and of the enzymes AcP, ADH, EST, PGM and POD indicate that only in the case of proteins and AcP the staining intensity of all samples and the variation of band pattern is taxonomically informative. A comparison between seed storage proteins and root tip proteins and an automatic data acquisition system is included.

**K e y w o r d s :** root apex proteins, isoelectric focusing, enzyme, chemotaxonomy.

### Introduction

The aim of this research was to carry out a systematic study within the *Vitis* species using soluble proteins of primary meristematic root tissue. Compared to pollen and seeds (ARULSEKAR and PARFITT 1986; CARGNELLO *et al.* 1988; GIANAZZA *et al.* 1989; TEDESCO *et al.* 1991, 1990, 1989; SCIENZA *et al.* 1994) roots are available already early in the vine's ontogeny and in large quantities. Moreover woody and semi-woody cuttings of most *Vitis* species root easily and are available throughout the year.

To homologize results presented by SCIENZA *et al.* (1994) we present a comparison of isozymes from seeds and root tips and an automatic data acquisition system.

### Materials and methods

The *Vitis* spp., interspecific genotypes and *V. vinifera* cultivars used in our experiments are listed in the Table. The material was taken from various collections in Italy (Centro Vitivinicolo Provinciale, Brescia, Azienda Agricola Villa Riccagioia, Loc. Riccagioia Voghera, Pavia and the Centre for the increment of vine and fruit growing and cereal culture in the Lombardy region) from 1989 to 1991.

The root tips were obtained from 10-15 woody and semi-woody cuttings which were rooted in a "hot bed" of agriperlite known to enlarge the roots' diameter.

The samples were taken after 30-40 d, using the distal 2-3 mm of the root apex, and kept at -20 °C.

All operations, except the ones which follow, were carried out at 4 °C. 25 mg of the material was ground with 1 ml (1:40 w/v) of 0.2 M glycine and the homogenate was then clarified by centrifugation (15 min at 17400 g; Beckman centrifuge J2-21). The soluble fraction was stored at -80 °C.

10-20 µg of the fraction was used for electrophoresis; the amount was estimated from protein dosage by the stain-

ing capacity, matched with BSA standard solutions (STEGEMANN 1983).

For isoelectric focusing (IEF, Desaga-Desaphor HF and Desaga-Desatronic 3000/200) the following non-linear pH gradients in acrylamide were used: acrylamide = 5 % mixing the ampholyne (LKB Pharmacia) a) pH 3.5-6, 75 %, pH 3.5-9.5, 25 %; b) pH 3.5-9.5, 50 %, pH 2.5-5, 50 %; c) pH 2.5-5, 30 %, pH 4-7, 40 %, pH 3-10, 30 % in a buffer containing: glutamic acid 5 mM, aspartic acid 5 mM, lysine 5 mM, arginine 5 mM in order to stabilize the extremes of the pH gradient and glycine 20 mM and taurine 20 mM to increase the dielectric constant of the gel.

The gel was stained using the silver stain photochemical method of MERRIL *et al.* (1981).

The enzymes to be analysed are: AcP, acid phosphatase (SWALLOW and HARRIS 1972), ADH, alcohol dehydrogenase (SMITH *et al.* 1971), EST, esterase (COATES *et al.* 1975), PGM, phosphoglucomutase (SPENCER *et al.* 1964), POD, peroxidase (TAKETA 1987).

The qualitative presence/absence (1/0) data were elaborated using Jaccard's coefficient (SNEATH and SOKAL 1973) and UPGMA (Unweighted Pair-Group Method using Arithmetical Averages) and the resulting dendrograms were statistically verified using the cophenetical correlation coefficient (SOKAL 1986) (NTSYS package, version 1.3 adapted to microcomputer (ROHLF 1987)).

Comparison between root apex proteins and seed storage proteins: To compare the data obtained from proteins of root tips with those of seed endosperm, cultivars Brugnera, Maiolina, Erbanno and Orsanella were used. The storage seed proteins (SCIENZA *et al.* 1994) were separated on a non-linear pH 3-10 gradient with 8 M urea (Fig. 1 a), while root tip proteins have been separated in native form with the same pH gradient (Fig. 1 b). A comparison of the AcP isozymes using the above mentioned method is also presented. The same pH gradient marker was positioned to all analysed

Table

*Vitis* spp., interspecific hybrids and *V. vinifera* cvs used in the experiments

<i>V. riparia</i>	125AA
Quebec	157-11
New York 21	
tomentosa	<i>V. Berlandieri</i> x <i>V. rupestris</i>
grand glabre	140 Ruggeri 1
grand glabre 2	140 Ruggeri 2
New York 21 (Manitoba)	775 Paulsen
Martineau	99 Richter
Manitoba M A 1	779 Paulsen
Gloire de Montpellier	1447 Paulsen
<i>V. rupestris</i>	<i>V. riparia</i> x <i>V. rupestris</i>
du Lot	3309 C
St. George	Schwarzmann
metallica	101-14
Martin	
<i>V. Berlandieri</i>	<i>V. vinifera</i> x <i>V. Berlandieri</i>
NN10	333 EM
Wainstadt	41 B
<i>V. cinerea</i>	<i>V. riparia</i> x <i>V. rupestris</i>
New York (male)	x <i>V. cordifolia</i>
Barret 1 58-46	106-8 Mgt
Barret	
<i>V. monticola</i>	<i>V. riparia</i> x <i>V. rupestris</i>
Big cluster	x <i>V. vinifera</i>
Rochester	Gagliardo
68-17	196-17 Cl
<i>V. Labrusca</i>	<i>V. vinifera</i> ssp. <i>sativa</i>
<i>V. solonis Longii</i>	Chardonnay
<i>V. palmata Mexico</i>	Pinot bianco
<i>V. amurensis</i>	Riesling renano
<i>V. Simpsonii</i>	Riesling italico
<i>V. Andersonii</i>	Folle blanche
<i>V. Champinii</i>	Sauvignon
	IM 6.0.13
	Pinot nero R4
	Pinot nero 019379
<i>V. Berlandieri</i> x <i>V. riparia</i>	Pinot meunier
Kober 5BB Cl 46 Fr	Malbec
Kober 5BB	Cabernet franc
420A 1	Cabernet Sauvignon
420A 2	Merlot
Barrisiquou	Abouriou
Rekendorfer 27	Chiavennasca 2NF
SO4	Chiavennasca 2B
Teleki 5C	Brugnera
Teleki 8B	Maiolina
Teleki 8B Cosmo 2	Erbanno
Teleki 8B Cosmo 10	Orsanella
Teleki 5A	
5A Gosek	<i>V. vinifera</i> ssp. <i>silvestris</i>
161-49 C	Svizzera

groups (indicated with M). Next to M, we marked the average pH values for every 0.5 cm of gel. In the schematic presentation of the marker we indicate only the bands declared by the supplier considering the others as artifacts.

**Data acquisition:** The analytical model used for the electrophoretic profiles was performed by recording the electrophoretic image (9.0 x 6.6 cm) from the gel with a videocamera (768 x 564 pixels). The recorded colored image was then converted into a black and white image and analysed with the Eidosoft-Winalgel software. This enabled us to execute a densitometric analysis for every sample lane. Moreover, at the real image (Fig. 2 a) it is possible to place lines in relation to any individual band (Fig. 2 b), in order to evaluate and eventually correct distortions and non-geometrical alignments of the band. For each sample, the software provides an original image, a schematic representation of bands and a density profile (Fig. 2 c). Comparing the schematic lanes of the samples, it is consequently possible to obtain a presence/absence matrix (Fig. 1) which can be utilised for statistical analysis.

The schematic figure (related to Fig. 1) marked with C and V, refers to the presence of common bands of all samples and the variable bands, respectively. Comparing the pH marker lanes of different groups it is possible to identify the position of each band, to pair the common ones (C) and to recognize its own elements (V). This elaboration, as well as the previous, is obtained by the arbitrary position of the lines relating to the individual bands.

## Results

A comparison between soluble protein bands obtained from root apices of a number of *Vitis* species is shown in Fig. 3 a. The individual bands, not occurring in each biotype, were utilised to draw the dendrogram presented in Fig. 3 b. In spite of some intraspecific variability, this dendrogram shows that the biotypes of a particular species are grouped together. On the basis of IEF profiles (pH gradient c) for the AcP isoenzymatic activities the different biotypes are grouped without any apparent order.

Differences between the components of total protein of the interspecific hybrids were of low significance. Neither a model, nor a correlation with the morphological parameters were detected. A comparison of isoenzymatic activity of AcP (pH gradient c) profiles is shown in Fig. 4; it enabled us to identify three groups, each characterised by common parents.

The profiles of the extracts of a number of *V. vinifera* cultivars and that of a *V. silvestris* biotype are shown in Fig. 5. Of the two groups identified, one includes all the vines with dark berries as well as the *V. silvestris* biotype; the other group comprises all remaining cultivars with light-coloured berries. On the contrary, the isoenzymatic AcP test (pH gradient c) carried out with the same samples suggests that *V. silvestris* is distinct from all other *V. vinifera* cultivars.

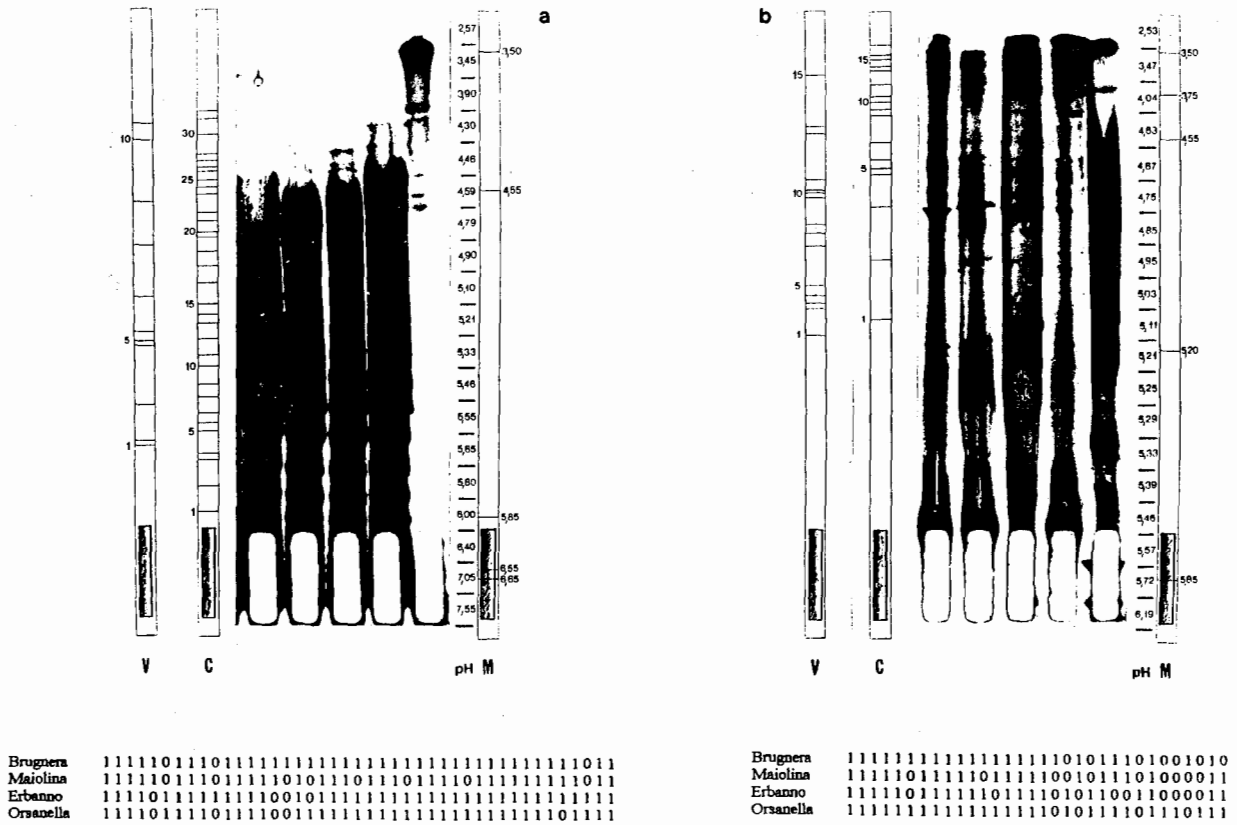


Fig. 1: The IEF of a non-linear pH gradient with 8 M urea of storage seed proteins (a) and without urea of proteins from root tips (b). Column C bands are present in all 4 varieties, bands in column V were not always detected; M=marker.

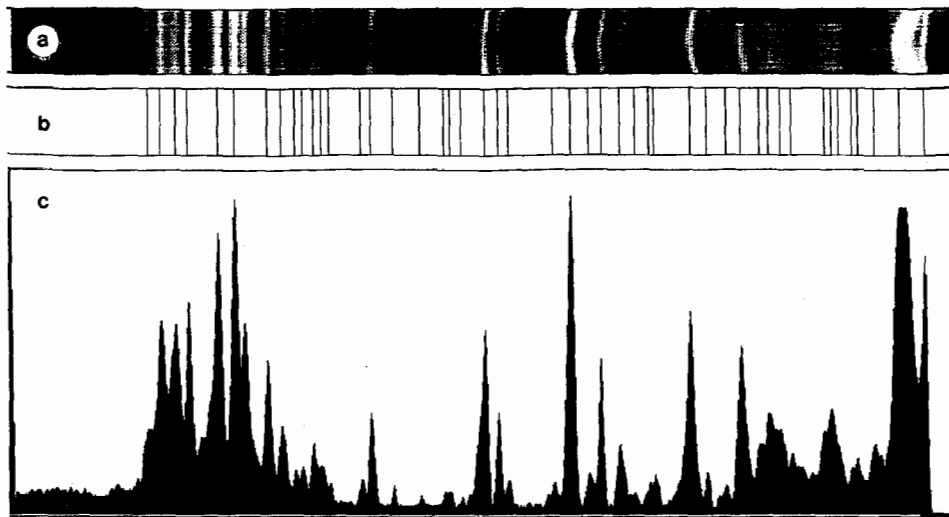


Fig. 2: Densitometric analysis (c) of the real image (a) and the line markers corresponding to the individual bands (b).

**Conclusions**

The use of soluble proteins from root apical meristems for taxonomic studies is advantageous if compared with alternative materials, including those previously used (pollen and seeds) because of its availability throughout the year. Both, woody and non-woody cuttings of *Vitis vinifera* easily root independently of the stage of maturity. The protein content of meristematic tissue, as analysed by our

method, seems to be independent from the ontogenesis. The object of this preliminary study was to verify the suitability of the method to identify the taxonomic hierarchies at lower levels, i.e. species or varieties.

A species can therefore be identified by use of soluble proteins separated via IEF, i.e., the variability among biotypes belonging to a single species (Fig. 3) does not hinder its identification. The obtained groupings are sufficiently coherent with the data in literature: increasing den-

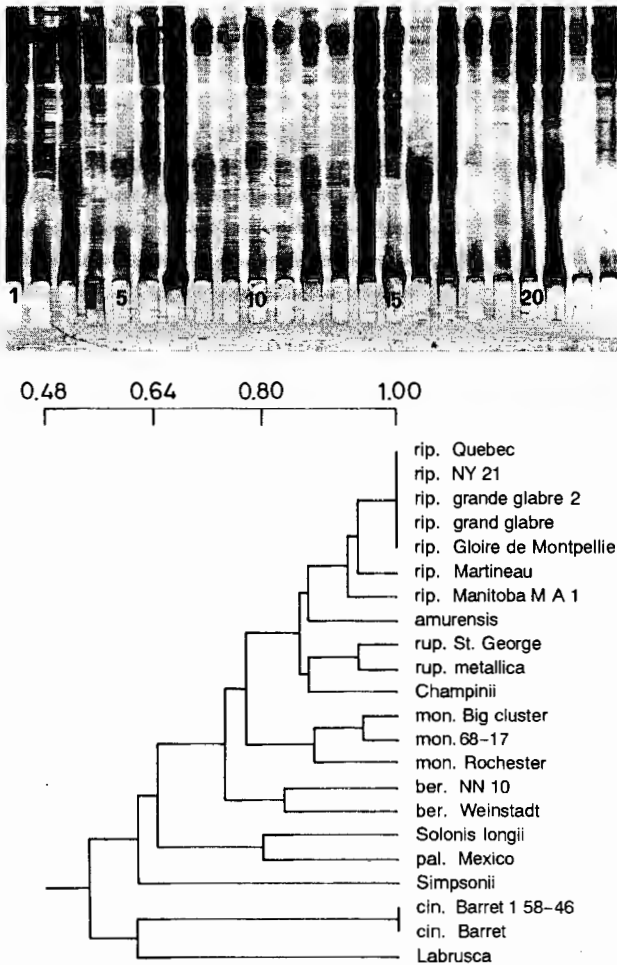


Fig. 3: The IEF of a non-linear pH gradient for proteins obtained from radical apices of *Vitis* species (top) and a dendrogram showing the relative similarity between the samples (bottom).

sity of wooly hairs of opening buds and leaves (of particular importance with regard to the taxonomy of the Vitaceae (GALET 1988)) is correlated with the increase of distance in the dendrogram. The only exception is the species included in the Candicansae (*V. Champinii*, *V. solonis Longii* and *V. Simpsonii*) which are scattered in the dendrogram.

A comparison of the hybrids (crossings of the species *V. riparia*, *V. rupestris* and *V. Berlandieri*) by the total soluble protein separation is not informative, while, on the other hand, the use of AcP isoenzymes (Fig. 4) shows the groups being correlated with the parents.

By comparison of *V. silvestris* with a number of *V. vinifera* biotypes (Fig. 5) using the total protein component distribution, it was possible to verify that the biotypes can be separated solely by berry colour into two main groups: vines with light- and vines with dark-coloured berries. *V. silvestris* belongs to the latter group. On the contrary, a comparison among the above mentioned biotypes proves no direct parental correlation.

The results presented in this paper indicate that the analysis of the soluble proteins from root apical meristem is an appreciable method for the identification of very different biotypes, while the use of isoenzymes is necessary to describe less different biotypes, e.g. parental relationships.

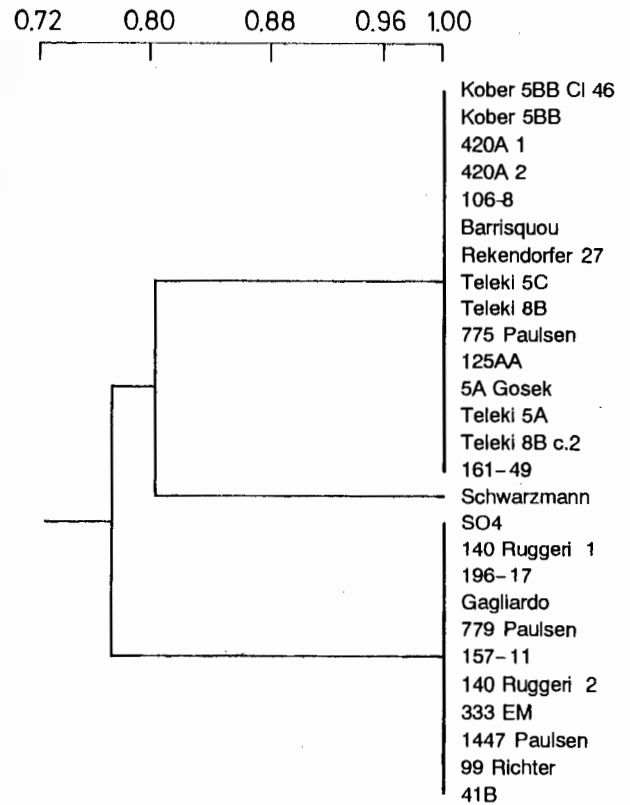


Fig. 4: Dendrogram showing the relative similarity between samples analysed for AcP obtained from root tips of interspecific *Vitis* genotypes.

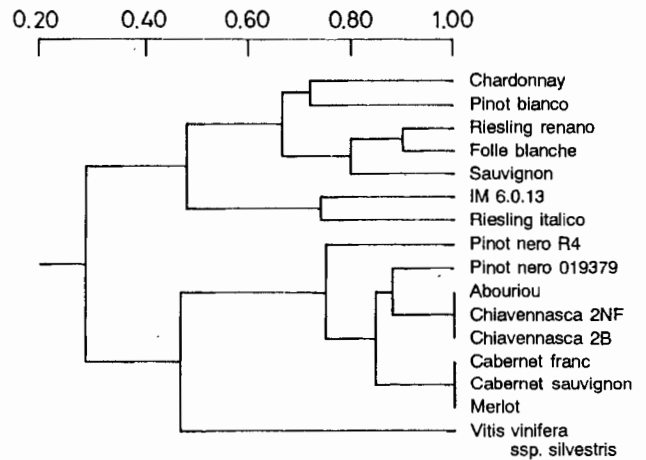


Fig. 5: Dendrogram showing the relative similarity between samples analysed for soluble proteins obtained from root tips of *Vitis vinifera* ssp. *sativa* and *silvestris*.

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