

Inheritance of isoenzymes and soluble proteins in grape varieties and F₁ hybrids

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S u m m a r y : The aim of our experiments was to verify the existence of a genetically interpretable molecular polymorphism in several grape varieties and their F₁ hybrids, which we can employ for genetical and ampelographical characterization. In addition, we also programmed the progress of investigation methods.

The authors present protein and enzyme analysis of two pairs of parents, Pearl of Csaba x S. V. 12375 and Saperavi x Blaufränkisch, and of ten other cultivars and several F₁ progenies.

The best experimental results for genetic markers can be gained when shoot and callus samples are collected at the end of winter, in February, at the same time. Spring shoot collection is less effective because of high chlorophyll contents, and must and wine samples are less suitable due to their microbial contamination.

The esterase enzyme group gives well reproducible, characteristically differentiated patterns. The enzyme patterns of parental varieties typically segregate in individual progenies, hence they prove to be good markers.

K e y w o r d s : genetics, ampelography, protein, enzyme, analysis, variety of vine, hybrid, Hungary, shoot, callus, must, wine.

Introduction

After developing investigation methods for plant proteins and enzymes in the last two decades, these methods are widely used for the ampelographical and genetical characterization and segregation of grape varieties. The proteins and isoenzymes are the most acceptable and simple gene markers which are co-dominant in hereditary processes.

For the analysis of protein and enzyme samples in the grape, leaves (SCHAEFER 1969, 1970; WOLFE 1976; DAL BELIN PERUFFO *et al.* 1981; FALLOT *et al.* 1985; ARULSEKAR and PARFITT 1986; BENIN *et al.* 1988), pollen grains (SAMAAN and WALLACE 1981; LOUKAS *et al.* 1983; STAVRAKAKIS and LOUKAS 1983; CARGNELLO *et al.* 1988), shoots (SUBDEN *et al.* 1987), internodal phloem of shoots (BACHMANN and BLAICH 1988), juice of betty (DRAWERT and GÖRG 1974; CORREA *et al.* 1988) have been used. From the samples, proteins and enzymes were separated and determined with gel electrophoresis and isoelectronic focusing methods. Among enzymes, α -esterase, acidic phosphatase, glutamate-oxaloacetate transaminase, catechol oxydase, phenol oxydase, indophenol oxydase, leucine aminopeptidase, alcohol dehydrogenase, peroxydases, etc. have been detected.

The results of our experiments made possible ampelographical and genetical characterization of a great number of grape varieties.

Materials and methods

The experiments were carried out in 1987 and 1988. Our task was to resolve protein and enzyme analysis first on several typical grape varieties of three convarietates of *Vitis vinifera* L. and next on parental varieties of two hybrid families and their F₁ progenies. We also decided to detect genetically interpretable molecular polymorphism.

Grapevine material

Vitis silvestris GMEL.convarietas *occidentalis*:

Pinot gris B. 10

Welschriesling B.20

Traminer

Müller-Thurgau

convarietas *pontica*:

Noble Furmint

Noble Kadarka

convarietas *orientalis*:

Chasselas blanc

Afuz Ali

Other varieties:

S. V. 18315

Berlandieri x Riparia T 5C

CsFT 3166 (composed species hybrid)

Hybrid families:

S.V. 12375 x Pearl of Csaba (KOZMA P.)

CsFT 175

CsFT 194

CsFT 195

Saperavi x Blaufränkisch (= Limberger)
(KOZMA P.)

CsV 463

CsV 525

CsV 528

Pearl of Zala (CSIZMAZIA, BEREZNAI)

Hindognü x Blaufränkisch (KOZMA P.)

CsV 420

Sampling

In 1987 the following samples were used: at the end of winter, shoots from field-grown vine-stocks; shoots collected in June; in the autumn freshly made and preserved must; in March fermented new wine. Shoots were collected on 6th November, 1987; 6th February, 9th March, 11th April, 1988. Two-bud cuttings of the latter sample were sprouted at 26 °C and fresh callus tissues were also used.

Extraction

a) Shoots deep-frozen at -30 °C, vine-stocks and callus tissues derived from them were sliced into 5 mm pieces and broken in a special disc grinder. Extraction buffer solutions coded to below 0 °C were added at concentrations of 1 : 5 (w/v). The composition of buffers was the following:

- | | |
|---------------------------------|-------------------------------------|
| 1. 0.2 M HEPES buffer, pH = 6.5 | 2. 0.05 M Tris-HCl buffer, pH = 6.5 |
| 0.02 M MgCl ₂ | 0.5 mM NaCl |
| 1 % (v/v) NP-40 | 2 % (v/v) LiDS |
| 0.005 M 2-mercapto-ethanol | 5 % (v/v) 2-mercapto-ethanol |

After blending, the mixture for enzyme detection was frozen to -20 °C for 24 h and the samples that contained ionic detergent were kept in water bath at 50 °C for 3 h.

The samples were then centrifuged, and after dissolving them in 20 % (v/v) saccharose they were stored at -20 °C until electrophoresis.

b) Preparation of wine and must samples: Into 1 ml centrifuged must and wine the following components were measured: 20 mg LiDS, 50 ml 2-mercapto-ethanol, 200 mg saccharose. After solution of the components, the samples were kept in water bath for 3 h at 50 °C. Afterwards the samples were stored at -20 °C until electrophoresis.

Electrophoresis, staining

a) Separation and detection of enzymes: 8 x 8 x 0.2 cm polyacrylamide gel slabs were used. The monomer concentration was 30 % (stock solution), the concentration gradient of polymer was

4-10 %. Solidity pH = 6.0, separation pH = 7.0, electrode buffer: 0.01 M Tris, 0.03 M 5,5'-diethylbarbituric acid. The electrophoresis has run for 1.5 h at 200 V. The hydrolase activity possessing protein components, the esterase isoenzymes, were made visible in 0.01 M phosphate buffer (pH = 6.5) by applying α -naphthyl-acetate as substrate, and diazonium salt dye.

b) Separation of proteins on the basis of molecular weight: The separations were performed on 8 x 8 x 0.2 cm polyacrylamide gel slabs. The monomer concentration was 30 % (stock solution), the gel contained 0.2 % ionic detergent (LiDS). Solidity pH = 6.5, separation pH = 8.9, the composition of electrode buffer: 0.05 M Tris, 0.4 M glycine, 0.1 % (v/v) SDS. The relative molecular masses were determined by parallel running of Pharmacia's molmass standard. The molecular masses of the calibration series were as follows:

	Molecular mass (kD)
phosphorylase-b	94
albumine	67
ovalbumine	43
carboxylic acid anhydrase	30
trypsin inhibitor	20.1
α -lactalbumine	14.4

Results

Esterase isoenzymes of shoot samples

The enzyme patterns originated from different samples of a certain variety were the same, consequently they can be considered as an adequate biochemical characteristic.

Fig. 1 shows enzyme patterns of the parental varieties: (1) Pearl of Csaba, (2) S. V. 12375; of their F_1 progenies: (3) CsFT 194, (4) CsFT 195, (5) Pearl of Zala. We could establish that parental patterns appear in additive manner in hybrids and that hybrids also differ from each other. It is recognizable that: 1. sample (3) (CsFT 194) is similar to sample (1) (Pearl of Csaba) in band number; 2. the bands of S.V. 12375 sample dominate in all hybrid samples, although less intensively; 3. the characteristic bands of Pearl of Csaba can be detected in samples (4) and (5).

Fig. 2 presents enzyme patterns from shoot samples of seven grape varieties. Significant alterations could also be found among them. Pinot gris B. 10 (11) and Welschriesling B. 20 (12) which belong to convar. *occidentalis* group, are characteristic of identical intensive upper (anodic) bands, while the others are different. Noble Furmint (14) and Noble Kadarka (15) originated from convar. *pontica* have both identical and divergent bands. Chasselas (13) (convar. *orientalis* subconvar. *caspica*) has less intensively stained bands, but the pattern corresponds to (11) or more to (12) sample. The enzyme patterns of (10) and (16) samples are markedly different from those of the others. A possible explanation may be that these hybrids are descendants of North American *V. vinifera* varieties.

Esterase isoenzymes of callus samples

Enzyme patterns of callus origin are generally more complicated, differentiated and have more bands than those of shoot samples. But in our opinion, they are more suitable for isoenzyme analyses, because they more accurately show the heredity of parental features in F_1 progenies. The alterations among varieties and their descendants are well recognizable as is the additivity of the markers in the hybrids.

It can be seen in Fig. 3 that the bottom and upper bands of Pearl of Csaba (1) are similar to those of CsFT 194 (3). S. V. 12375 (2) upper band and middle band of (1) and (3) are equivalent

Parental polypeptide components of Pearl of Csaba and S. V. 12375 hybrids in shoot extracts

parent hybrid	Polypeptides /kD/	
	Pearl of Csaba	S.V. 12375
194	19.8	59.5
	19.2	58.5
	18.5	31.0
	--	30.5
175	30.5	59.5
	19.8	58.5
	19.2	--
	18.5	--
Pearl of Zala	30.5	59.5
	19.8	58.5
	19.2	--
	18.5	--

with Pearl of Zala (5) bands. Bands of Pearl of Csaba appear also in CsFT 195 (4) sample, but less intensively.

In Fig. 4 shoot callus esterase patterns are shown from varieties Blaufränkisch (6) and Saperavi (7) with those of their hybrid, CsV 525 (8) and of Hindognü x Blaufränkisch hybrid, CsV 420 (9). The patterns of parents are essentially different but in hybrids they appear additive.

Fig. 5 presents esterase patterns of four *V. vinifera* cultivars. All of them have identical and non-identical bands.

Results of protein analyses

We separated proteins by their molecule masses isolated from shoots and calli with ionic detergents, and investigated the protein composition of enzymatic isolates. The results of shoot analyses in 1987 and 1988 are shown in the table and Figs. 6 and 7.

Saperavi and Blaufränkisch have easily distinguishable polypeptides of molecular masses at 58.5 and 59.0 kD, respectively. In their hybrids, these two components appear in additive manner and form two-banded patterns in the region in question, as Fig. 6 shows. This is also characteristic of CsV 463, CsV 525 and CsV 528.

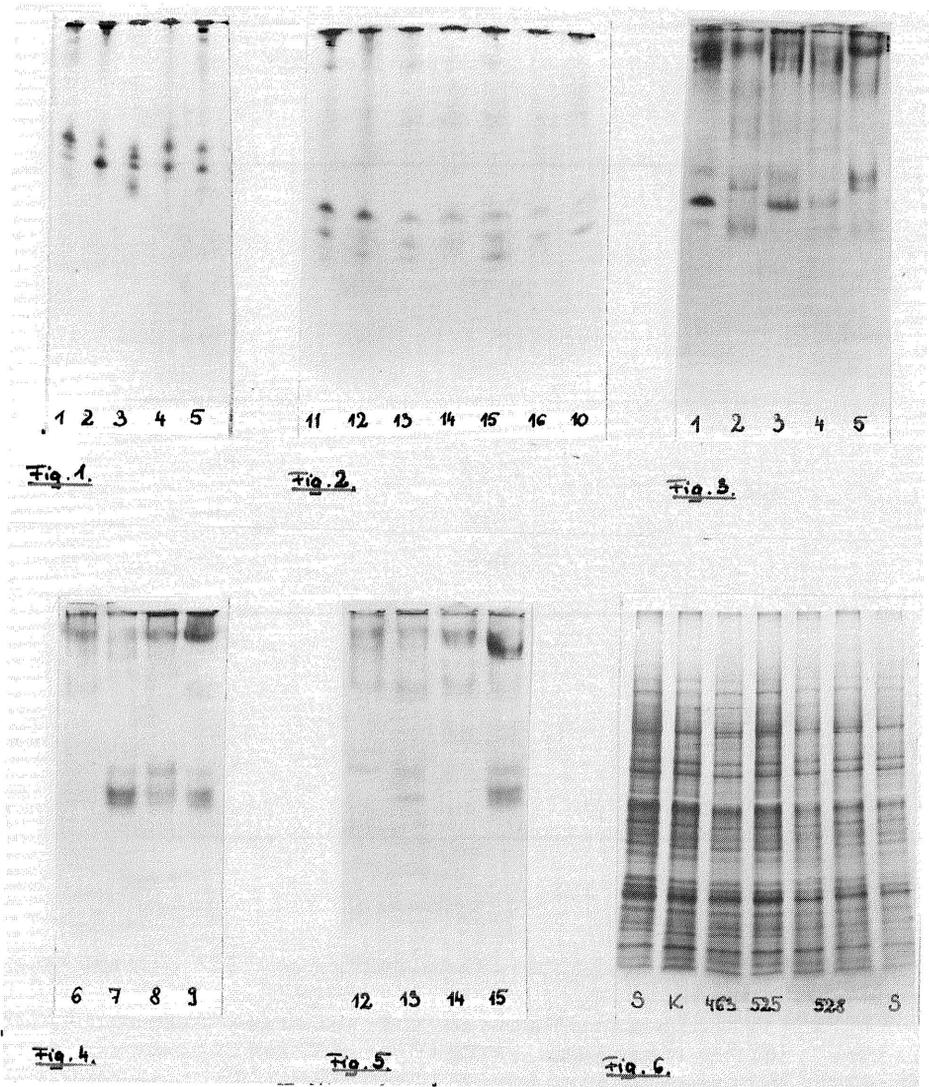


Fig. 1: Zymogram for shoot esterases of Pearl of Csaba (1) and S.V.12375 (2) parental varieties and CsFT 194 (3), CsFT 195 (4), Pearl of Zala (5) hybrids.

Fig. 2: Esterase enzymes of Pinot gris B.10 (11), Welschriesling B.20 (12), Chasselas (13), Noble Furmint (14), Noble Kadarka (15), S. V. 18315 (16) and Berlandieri x Riparia T 8B (10) shoot samples.

Fig. 3: Esterase patterns of calli from Pearl of Csaba (1) and S.V.12375 (2) parental varieties and CsFT 194 (3), CsFT 195 (4) and Pearl of Zala (5) hybrids.

Fig. 4: Esterase patterns of shoot calli from Blaufränkisch (6) and Saperavi (7) parental varieties and CsV 525 (8) and CsV 420 (9) hybrids.

Fig. 5: Esterase patterns of shoot calli from Welschriesling (12), Chasselas (13), Noble Furmint (14) and Noble Kadarka (15).

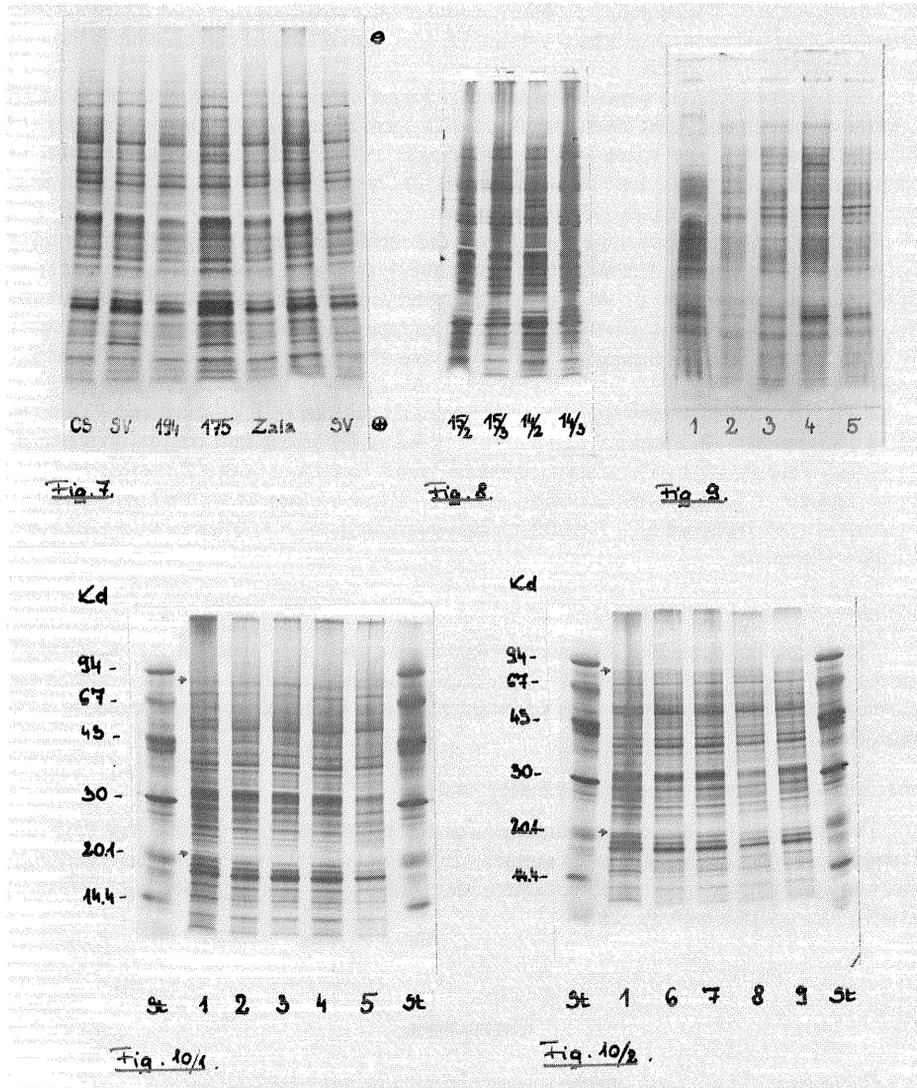


Fig. 6: Polypeptide range of shoot extract from Saperavi and Blaufränkisch varieties, as well as of their hybrids.

Fig. 7: Polypeptide range of shoot extract from Pearl of Csaba, S. V. 12375, and of their hybrids.

Fig. 8: Polypeptide patterns of Noble Furmint (14) and Noble Kadarka (15) shoots collected on 6th February (2) and 9th March (3), 1988.

Fig. 9: Callus protein composition of Pearl of Csaba (1), S. V. 12375 (2) parents and CsFT 194 (3), CsFT 195 (4) and Pearl of Zala (5) hybrids.

Fig. 10: Callus proteins from *Vitis sivestris* species (1), Pinot gris B.10 (2), Welschriesling B.20 (3), Chasselas blanc (4), Afuz Ali (5), Noble Furmint (6), Noble Kadarka (7) varieties and S. V. 12375 (8), CsFT 3166 (9) species hybrids.

In the hybrid family of Pearl of Csaba x S. V. 12375 progenies (CsFT 175, 194, Pearl of Zala), the patterns are more complicated. The differences observable among polypeptides of two varieties appear in various degrees: some regions are similar to patterns of the one parent, some to those of the other parent (Fig. 7, Table).

Fig. 8 shows comparative electrophorograms of protein extracts coming from shoots sampled in February, March and April 1988. Significant differences in the polypeptide compositions are visible between winter and spring samples with regard to the relative amount and number of components. It can be stated therefore, that samples for characterization of some varieties, hybrid lines, clones, etc. must be collected at identical times.

The results on soluble proteins extracted from calli are demonstrated in Fig. 9 by examination of parents and their hybrids. In contrast with shoots, the protein range of parental calli turned to be considerably divergent. Hybrids are also well distinguishable from each other and from parents. Several characteristic bands of Pearl of Csaba (on upper part and lower third part of photo) come out in progenies: most intensively in CsFT 195 and Pearl of Zala. Nevertheless, generally S. V. 12375 is dominant.

In Fig. 10, protein patterns are seen belonging to six cultivars of *V. silvestris* and *V. vinifera* and two species hybrids. Pinot gris (2) and Welschriesling (3) belong into convar. *occidentalis*, Chasselas (4) and Afuz Ali (5) to convar. *orientalis*, Noble Furmint (6) and Noble Kadarka (7) to convar. *pontica*. *V. silvestris* has alterations in 80-85 kD interval, and in a 21 kD component in comparison to the other samples. The differences found among varieties are in number, width and intensity of the bands.

Results of spring collection

The samples collected had high chlorophyll contents which may affect the method applied. The protein ranges of green samples differ significantly from those of winter samples. It is probable that major photosynthetic components which are present in each of green plants should also be considered.

Polypeptide analysis of wines and musts

The results of our experiments indicate that the protein patterns of wine and must are divergent from those of shoot and callus samples. Considering the existence of microbial proteins in must and wine samples, these are less suitable for detection of varietal divergencies. For this reason, we will review our results on this topic.

Conclusions

On the basis of our 2-year investigations we can draw the following conclusions.

1. Genetically and ampelographically useful markers can be obtained from esterase isozymes and protein ranges from shoots and from calli originated from shoots.
2. Both the shoot and the callus samples result in well reproducible, characteristically different, variety specific enzyme patterns. On zymograms, the simple and additive appearances of parental markers can be traces in their hybrids. The 18 varieties examined show characteristically identical and definite bands related to their origin and taxonomic status.
3. On the basis of non-specific protein staining results, the protein patterns of shoot change very sensitively parallel with the physiological condition of the given plant, and the differences among varieties are less characteristic. For this reason, the method is applicable only to samples collected at the same point of physiological development. As for calli, protein composition separated by molecular mass may also be variety or clone specific.

4. For each type of analyses in comparative studies identical sampling (e. g. timing, physiological condition, localization on plant specimen) must be performed. Within a given time interval, the same day and, moreover, the same time of day is important for collection of samples and, in addition, equivalent storage conditions are also necessary. For callus samples, light and dark periods during its culture may also be important.
5. Must and especially wine samples are less suitable for the comparison of varieties because of microbial influences.

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