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**Wall proteins of *Vitis vinifera* pollen  
II. Influence of environment and rootstock on the  
electrophoretic pattern<sup>1)</sup>**

by

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**Proteine der Pollenwand bei *Vitis vinifera***

**II. Einfluß von Umwelt und Unterlage auf das elektrophoretische Muster**

**Zusammenfassung:** Die aus der Wand von *Vitis-vinifera*-Pollen extrahierbaren Proteine sind genotypisch festgelegt, und ihre Expression — möglicherweise auch deren Intensität — ist unabhängig von Umwelteinflüssen. Nachdem schon zuvor gezeigt werden konnte, daß der Zeitpunkt der Probenahme sich nicht auf das IEF-Muster auswirkt, wird nunmehr nachgewiesen, daß auch der jeweilige Standort einer Rebe sowie die Unterlage des Edelreises keinen Einfluß auf das Muster der Pollenwandproteine ausüben.

**Key words:** pollen, wall, protein, analysis, environment, rootstock, variety of vine, clone, biotype, ampelography

**Introduction**

Biochemical methods are increasingly applied to plant taxonomy (HARBORNE 1984), mostly when the morphological traits alone leave ambiguity. In several instances biochemical parameters do help classification (PAYNE and FAIRBROTHERS 1973; SCIENZA *et al.* 1986), but the relevant characters need to be searched for (STEGEMANN 1983).

Our research project means to recognize specific and constant biochemical properties for the many biotypes belonging to *Vitis vinifera* species. Allocation based on biochemical criteria is expected to be more dependable than standard ampelographic analyses relying on morphological traits, as the latter may be heavily affected by the growing conditions, including climatic vagaries.

We have focused our search for biotype-specific markers on pollen. In fact, this material — which can be easily collected and fully preserves its properties during storage (JOHNSON and FAIRBROTHERS 1975) — may be singled out as representing a well-

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defined step in the vegetable cycle. The pattern of the pollen wall proteins is most significant owing to their involvement in the pollen-stigma interaction (KNOX and HESLOP-HARRISON 1971; LAWRENCE *et al.* 1985; GIBBS 1986), and they were anticipated to feature stable and specific plant traits.

In a previous report (CARGNELLO *et al.* 1988), we had shown for two vine cultivars — Merlot and Cabernet franc — that the soluble protein fraction from pollen wall was clone-specific and independent from location and time of sample collection. Along this line, we test in the present work whether other variables, known to influence several phenotypic properties, also affect the electrophoretic pattern of pollen wall proteins.

We thus compared 24 samples collected from the cvs Merlot, Cabernet Sauvignon and Cabernet franc. They could be grouped as follows:

- (i) Same clone, grown in the same environment, but on different rootstocks;
- (ii) same clone, same rootstock, different environments;
- (iii) same clone, different environments, different rootstocks;
- (iv) different clones, same rootstock, same environment.

### Materials and methods

#### Samples

24 pollen samples of *Vitis vinifera*, listed on the opposite page, were collected; 15 from cv. Merlot (M), 3 from cv. Cabernet Sauvignon (CS) and 6 from cv. Cabernet franc (CF).

#### Protein extraction

The pollen was stirred at 4–6 °C for 17 min with a tissue : buffer ratio of 1 : 20 either in a 0.15 M Tris/HCl buffer, pH 8.8, or in isoelectric 0.2 M glycine, in presence of 44 fg/ml PMSF (phenyl-methyl-sulfonyl fluoride). The suspension was spun for 3 min at 3,200 *g* (5,000 rpm with the JA20 rotor in a Beckman J2-21 centrifuge). Glycine is known not to hamper the isoelectric focusing process (RIGHETTI 1983), while high concentrations of ions from the sample do interfere. For this reason a solubilization protocol different from that adopted in our previous report (CARGNELLO *et al.* 1988) was tried. With either extraction medium the same classes of proteins were solubilized, and in the same proportions (not shown). Glycine should be preferred, though, as it allows application of larger sample volumes.

The extracts were concentrated by ultrafiltration, either in an Amicon Cell Model M3 or, for small volumes, in a Micropartition System MPS-1, equipped with YM2 membranes, cut-off at 2,000 daltons. Protein concentration on the raw extracts was evaluated according to MARKWELL *et al.* (1978), in order to adjust sample loads (to ca. 20–30 ng/lane). To check the integrity of the pollen grains, the specific activity of MDH (enzyme units/protein mass) was assessed (MCNEIL *et al.* 1984; CARGNELLO *et al.* 1988).

#### Protein fractionation

Isoelectric focusing was performed on a non-linear gradient spanning the pH range 3.5–10 (0.66 % w/v LKB Ampholine 3.5–10, 0.33 % LKB 5–7, 1 % Kem-En-Tec Polyampholyte 3.5–6, in presence of 0.2 M taurine and glycine), on a T % = 5 C % = 4 polyacrylamide matrix (HJERTÉN 1962). The slabs — 125 × 260 × 0.5 mm — were run for 80 min at 4–6 °C in a Desaphor HF chamber (Desaga, Heidelberg, FRG) while delivering a constant power of 15 W with a Desaga Desatronic power supply 3000/200. The focused bands were stained with silver nitrate according to MERRIL *et al.* (1981).

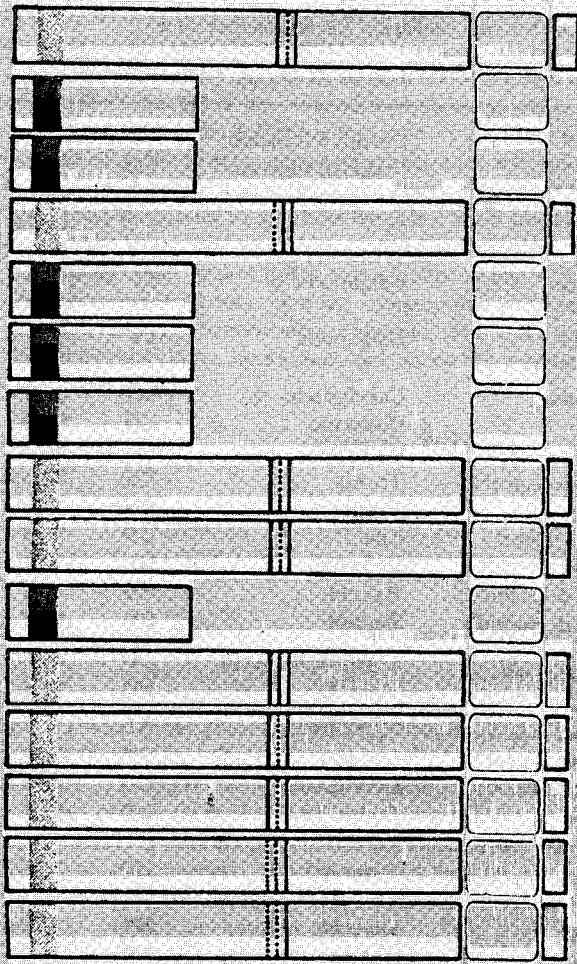
Samples investigated  
 Untersuchungsmaterial  
 M = Merlot, CS = Cabernet Sauvignon, CF = Cabernet franc

#	Biotype	Origin			Stock
Ma	Toppani	Tilatti	Corno di Rosazzo	UD	5BB
Mb	Toppani	Tilatti	Corno di Rosazzo	UD	5C
Mc	Toppani	Tilatti	Corno di Rosazzo	UD	5BB
					Italia
Md	Toppani	Tilatti	Corno di Rosazzo	UD	SO4
Me	Toppani	Bortoluzzi	Salganeda	TV	SO4
	Presa # 20				
Mf	Rauscedo	Bortoluzzi	Salganeda	TV	SO4
	Presa # 21				
Mg	Toppani	Cervignano	Cervignano	UD	SO4
Mh	Toppani	Ersa	Codroipo	UD	5BB
Mi	Rauscedo	Ersa	Codroipo	UD	5BB
Mj	Rauscedo	Ist. Tecnico Agrario	Trento	TN	5BB
Mk	Rauscedo	Torregaia	Dugenta	BN	5BB
Ml	Toppani	Tenuta di Lison	Lison	UD	5BB
Mm	Rauscedo	Badoglio	Codroipo	UD	5BB
Mn	Maestrello	Dominio di Bagnoli	Bagnoli	PD	5BB
Mo	Toppani	Dominio di Bagnoli	Bagnoli	PD	5BB
CSa	Toppani	Tilatti	Corno di Rosazzo	UD	SO4
CSb	Toppani	Dominio di Bagnoli	Bagnoli	PD	SO4
CSc	Rauscedo	Bortoluzzi	Salganeda	TV	SO4
CFa	Toppani	Dominio di Bagnoli	Bagnoli	PD	5BB
CFb	Toppani	Cervignano	Cervignano	UD	5BB
CFc	Toppani	Ersa	Codroipo	UD	5BB
	Presa A				
CFd	Toppani	Ersa	Codroipo	UD	5BB
	Presa B				
CFe	Rauscedo	Torregaia	Dugenta	BN	5BB
CFf	Rauscedo	Latina	Latina	LT	5BB

## Results

The protein patterns of the pollen wall components for the 15 specimens from cv. Merlot (M) are compared in Fig. 1.

27 protein bands are shared by all specimens; otherwise, the patterns can be sorted in either of two sets. One set includes samples f, i, j, k, m, n — all of which belong to biotype Rauscedo — with identical electrophoretic profiles, only featuring the 27 common bands, clustered in the acidic pH range ( $3.5 < \text{pI} < 6.5$ ). The other set includes the remaining samples (a, b, c, d, e, g, h, l, o, belonging to biotype Toppani) the patterns of which coincide only in qualitative terms. They amount to 55 protein bands evenly spaced across the entire width of the gradient — 28 more elements, with neutral and alkaline pIs, besides the 27 acidic components distinctive of the previous group.



1) M.a M.b M.c M.d M.e M.f M.g M.h M.i M.j M.k M.l M.m M.n M.o

2) T T T T T T R T R T R T R R R R R T

3) 58B 5C 58B1 S04 S04 S04 S04 S04 58B 58B 58B 58B 58B 58B 58B 58B 58B

4) [Blacked out]



Type T  
Type R  
Type T  
Type R

1) Sample 2) Biotype 3) Stock 4) Same origin

Fig. 1: IEF pattern of the pollen wall proteins from 15 samples of cv. Merlot. From left to right: outline of the distinctive protein bands for the 2 biotypes being compared; picture of the actual gel; schematic representation of the differences among individual samples. Shaded and solid lines or blocks mean, respectively, minor or major quantitative variations for single bands or groups thereof. Abbreviations: R = Rauscedo; T = Toppani; otherwise, as listed in the table. Experimental: IEF on a non-linear pH 4—10 gradient; the anode is uppermost.

IEF-Muster der Pollenwandproteine von 15 Proben der Rebsorte Merlot. Von links nach rechts: Gezeichnete Wiedergabe der ausgeprägten Proteinbanden bei den beiden miteinander verglichenen Biotypen; tatsächliches Erscheinungsbild der Gele; schematische Darstellung der Unterschiede zwischen den einzelnen Proben. Schraffierte und dunkle Linien oder Blöcke bedeuten schwächere bzw. stärkere Anfärbung einzelner Banden oder Gruppen von Banden. R = Rauscedo; T = Toppani; weitere Abkürzungen s. Tabelle. Auftrennung: IEF mit nichtlinearem pH-Gradienten (pH 4—10), Anode oben.

Some of the 27 common protein bands (group I in Fig. 1) show higher intensity in biotype Rauscedo than in biotype Toppani samples. Further quantitative variation among the different specimens from biotype Toppani are observed for the neutral protein bands (marked as II, Fig. 1).

The results for 3 samples from cv. Cabernet Sauvignon (CS) are shown in Fig. 2 A and B. The two extracts obtained from biotype Toppani (a and b) share the same protein pattern, with 56 bands. Sample c (from biotype Rauscedo) differs from the former

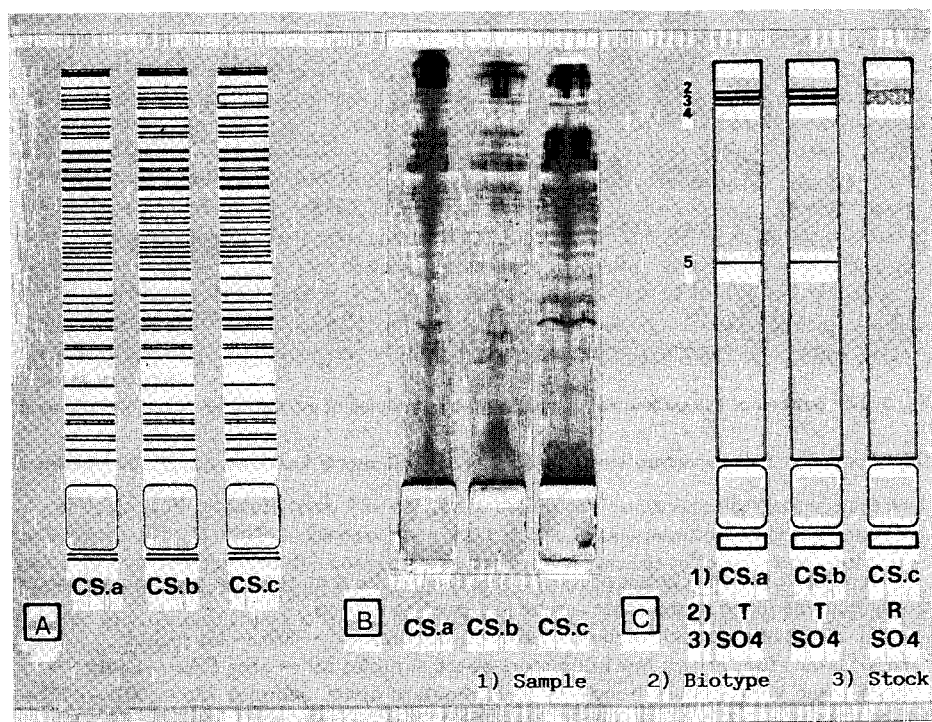


Fig. 2: IEF pattern of the pollen wall proteins from 3 samples of cv. Cabernet Sauvignon. Details as in Fig. 1.

IEF-Muster der Pollenwandproteine von 3 Proben der Rebsorte Cabernet Sauvignon. Einzelheiten s. Fig. 1.

in the acidic pH region by the lack of band 5 and the substitution of 2, 3 and 4 by a wide and diffuse band (Fig. 2 C). The common components among the 3 samples are thus 52.

Fig. 3 (A, B and C) compares the protein patterns of the wall components for 6 samples from *cv. Cabernet franc* — a to d (group 1) belonging to biotype Toppani, e and f (group 2) to biotype Rauscedo. The two groups share 53 bands. Just one component varies: qualitatively, between group 1 and 2 (absence of band 5), or quantitatively, among the samples of group 1 (5 is more distinct in a and d than in b and c).

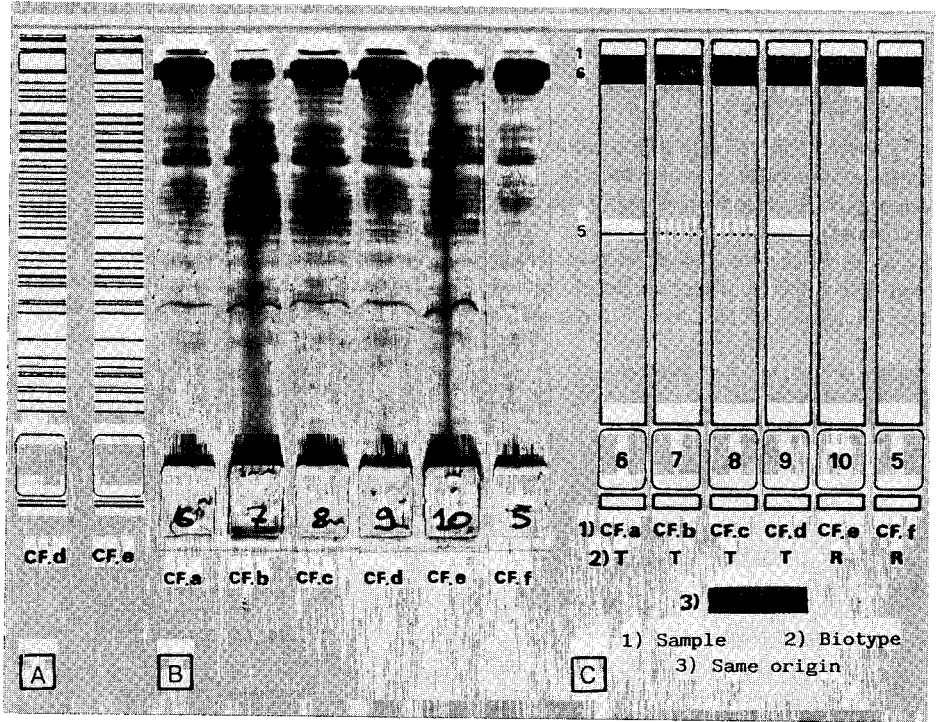


Fig. 3: IEF pattern of the pollen wall proteins from 6 samples of *cv. Cabernet franc*. Details as in Fig. 1.

IEF-Muster der Pollenwandproteine von 6 Proben der Rebsorte Cabernet franc. Einzelheiten s. Fig. 1.

According to the scheme proposed in the introduction, the five groups comparing the specimens listed in the table can be assigned as follows:

- (i) Effect of rootstock variation between samples Ma — Mb — Mc — Md;
- (ii a) same vines grown few kilometers apart, samples Mi — Mm, CSa — CSb;
- (ii b) same vines grown hundreds of kilometers apart, samples Ma — Mh — Ml — Mo, Md — Mg, Mj — Mk;
- (iii) different environment and different rootstock for samples Ma — Mg and for samples Mb, Mc, Md versus Mg, Mh, Ml, Mo;
- (iv) different biotypes grown under identical conditions, samples Me — Mf, Mh — Mi, Mn — Mo.

### Discussion

In the present investigation, we have introduced a slight variation to the technique proposed in the first paper of this series (CARGNELLO *et al.* 1988), i.e. we have tested the extraction of pollen wall components from vine by a glycine solution instead of treatment with Tris buffer. This simplifies the procedure for sample preparation and improves the quality of protein resolution. The coincidence of the final patterns from either extract — together with the disproof of cytoplasmic contamination afforded by the enzymatic assays — substantiates evidence that all proteins in the diffusate are indeed extrinsic wall components.

The main objective of the present investigation was to ascertain the influence on the electrophoretic pattern of the proteins in a pollen diffusate: (A) of the location where the vine is grown and (B) of the rootstock on which the scion is grafted.

With the technique proposed it is possible to arrange the 15 samples of cv. Merlot under investigation into two groups with distinct electrophoretic profiles.

Samples Mf, Mi, Mj, Mk, Mm, Mn — of the same origin, biotype Rauscedo, seedling nurseries Rauscedo and Maestrello — show an identical pattern of pollen wall proteins, with 27 bands, even when grown on various rootstocks or in different environments.

The results for the specimens belonging to the biotype Toppa (from seedling nursery Ferrari) are more complex. Their tracks exhibit the same number of bands, but quantitative variations are observed for the neutral components marked as group II in Fig. 1 B.

Similar results were obtained for Cabernet Sauvignon samples, in the pattern of which a panel of constant bands was observed. Again with Cabernet franc, the two biotypes investigated can be differentiated by the proposed technique, irrespective of the rootstocks or the environments where the scions from which pollen was collected were growing.

From these evidences, soluble proteins from pollen walls appear genotypically determined and their expression, if not the extent of their expression, is hardly influenced by any of the external factors tested so far (time of collection, as formerly shown, or growing conditions, as now demonstrated). Pollen proteins are thus effective markers for differentiating cultivars of *Vitis vinifera* and even of individual clones of a given cultivar. Further support to this conclusion is expected from follow-up studies already under way on a larger set of cultivars and on reference material from a grapevine collection.

### Summary

Soluble wall proteins from *Vitis vinifera* pollen are genotypically determined and their expression, if not the extent of their expression, is independent from external factors. After we had previously observed that the time of sample collection does not influence their electrophoretic pattern, we demonstrate in the present work that this is true also for environment where the vine is growing and for the rootstock on which the scion is grafted.

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