Wall proteins of *Vitis vinifera* pollen
II. Influence of environment and rootstock on the electrophoretic pattern

by

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Proteine der Pollenwand bei *Vitis vinifera*
II. Einfluß von Umwelt und Unterlage auf das elektrophoretische Muster


**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-
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 (Carnelillo et al. 1988), we had shown for two vine cultivars — Merlot and Carbernet 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 μg/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 (Carnelillo 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; Carnelillo 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 (Hjerten 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).
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Samples investigated

Untersuchungsmaterial

M = Merlot, CS = Cabernet Sauvignon, CF = Cabernet franc

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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 < pH < 6.5). The other set includes the remaining samples (a, b, c, d, e, g, h, i, 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 pl's, besides the 27 acidic components distinctive of the previous group.
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.


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 samples 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 h) 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).

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 -— MI— Mo, Md — Mg, Mj — Mk;
(iii) different environment and different rootstock for samples Ma — Mg and for samples Mb, Mc, Md versus Mg, Mh, MI, Mo;
(iv) different biotypes grown under identical conditions, samples Me — Mf, Mh — Mi, Mn — Mo.
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**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 Toppani (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 grape-vine 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.

**Literature cited**


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