

A chemotaxonomic investigation on *Vitis vinifera*

I. Within-cultivar population analysis¹⁾

by

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Chemotaxonomische Untersuchung von *Vitis vinifera*

I. Populationsanalyse innerhalb von Rebsorten

Zusammenfassung: Anhand eines umfangreichen Samenmaterials aus Selbstungen der Rebsorten Chardonnay, Sangiovese und Traminer wurde die Variationsbreite biochemischer Merkmale zwischen den Einzelsamen einer Sorte ermittelt. Hierzu wurden mit Hilfe der isoelektrischen Fokussierung sowohl die Reserveprotein-Untereinheiten als auch die Isoenzyme der sauren Phosphatase (AcP), Esterase (EST) und Phosphoglucomutase (PGM) ausgewertet. Die Extrakte aus 35 zufällig ausgewählten Samen je Sorte lieferten reproduzierbare Proteinstreifenmuster; sie können somit als repräsentativ für die durchschnittliche genetische Zusammensetzung eines bestimmten Biotyps gelten.

Key words: systematics, chemotaxonomy, variety of vine, population, analysis, seed, protein, enzyme, electrophoresis, zymogram, clustering.

Introduction

A prerequisite to any taxonomical study dealing with relatedness, either between different species or among various populations within a given species, is the evaluation of all morphological and biochemical traits that vary from a subject to another (HEYWOOD 1967; SNEATH and SOKAL 1973). Seed proteins feature useful reference parameters for cultivar characterization (LARKINS 1981). They are usually polymorphic, either by size or by surface charge (STEGEMANN 1983); both physicochemical parameters were successfully applied to cultivar identification in many species (mainly of Gramineae genera (reviewed in: COOKE 1984)). Moreover, wealth of evidence in the literature shows that seed protein make-up is under genetic control, with little or no influence from the environment (for Gramineae: NELSON 1980; for Leguminosae: BOULTER 1981).

Protein polymorphism is currently made out by electrophoretic techniques (STEGEMANN and PIETSCH 1983; COOKE 1984). The most abundant seed components — which

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may be readily identified as storage proteins — can be assessed with general staining procedures, while by zymogram techniques (incubation with the appropriate substrates and cofactors) the activity of a number of enzymes can be tested. Use of isozyme patterns in different organs (TANSKLEY and ORTON 1983) was found very informative not only in taxonomy and its evaluation of variability (HARBORNE 1984), but also in systematics with its search for hierarchical relationships (e.g., GOTTLIEB *et al.* 1985; OLMSTEAD 1989; for review: CRAWFORD 1985; CRONQUIST 1987).

In the present investigation, the extension of the above concepts and techniques to the characterization of *Vitis vinifera* L. biotypes was validated through the analysis of a large number of individual specimens. Information about the extent of variability was then instrumental to select the most informative parameters as well as to detail a representative sample for a given biotype in taxonomical studies. However, advantage of the same knowledge on the individual genetic variability within different cultivars could also be taken in programs of crop selection and breeding.

In this phase, the panel of data we evaluated did not include any morphological parameters, because these are influenced by environment and growing conditions more often than biochemical features (DAVIS 1983; HILLIS 1987). As representative of diversified situations, we selected for our study two cultivars (Chardonnay and Sangiovese), in Italy grown on a wide area, and another (Traminer) from a small region. Moreover, cv. Chardonnay stands for a rather constant phenotype while the morphology of Sangiovese is highly variable.

Materials and methods

Grape seeds were obtained: for cv. Chardonnay from Centro Vitivinicolo Provinciale, Brescia; for cv. Traminer from Istituto Agrario Provinciale, San Michele all'Adige, TN; for Sangiovese from Panerai (samples from individual vines in different plots, on the basis of bunch size — CS 1, 2, 3, 4, 5, 6, 8; CN 2, 4, 8, 12, 15; CP 5, 6), Nipozzano (NF 3, 6, 7) and Pomino farms (PR 3, 7), Siena.

For cvs Sangiovese and Traminer only self-pollinated, for cv. Chardonnay both self- and open-pollinated specimens were analyzed. Individual seeds were selected by size (in order to get ≥ 5 mg endosperm). After dissection of the wooden testa, each kernel was ground in a mortar with 10–20 vol. of 0.2 M glycine; the extracts were then clarified by centrifugation (GIANAZZA *et al.* 1989). For cv. Chardonnay, three samples each including 35 seeds were also compared.

After protein fractionation by isoelectric focusing on immobilized pH gradients (BJELLQVIST *et al.* 1982; RIGHETTI 1990), total native proteins and dissociated subunits were stained with Coomassie Blue, whereas the isozyme patterns of esterase (EST) (COATES *et al.* 1984), acid phosphatase (AcP) (SWALLOW and HARRIS 1972), phosphoglucosmutase (PGM) (SPENCER *et al.* 1964), alcohol dehydrogenase (ADH) (SMITH *et al.* 1971), gluconate-6-phosphate dehydrogenase (G6PDH) (STUBER and GOODMAN 1980), malic dehydrogenase (MDH) (DAVIDSON and CORTNER 1967) and peroxidase (POD) (TAKETA 1987) were detected by specific zymograms.

For each sample, a binary chart was compiled to include distinctive bands on the basis of their presence (1) or absence (0). The statistical analysis of the results was run with package NTSYS-pc 1.3 on a Olivetti M250 microcomputer. Dendrograms relating the different samples were built with the UPGMA (unweighted pair-group method using arithmetic averages) clustering method, then evaluated through cophenetic correlation coefficients. Individual sets of data were compared through congruity tests (SPIEGEL 1961; CAVALLI-SFORZA 1965; SNEATH and SOKAL 1973; ORLÓCI 1975; SOKAL 1986).

Results

The results of the electrophoretic separation under native conditions of the total proteins from 20 open-pollinated Chardonnay seeds are shown in Fig. 1 (a summary of common and non-common Coomassie-stained bands, and their numbering) and in Table 1 (a 0—1 array for absence-presence of distinctive bands in individual samples). The statistical analysis of non-common band distribution shows no correlation among the various samples (Fig. 2).

Table 1

Variability of seed total protein within cv. Chardonnay (open-pollinated samples) · Presence-absence of the bands in Fig. 1 are entered as 1—0

Variabilität der Gesamtreserveproteine innerhalb der Sorte Chardonnay (nicht-selbstbefruchtete Samen) · Vorhandensein oder Fehlen der Banden in Fig. 1 sind als „1“ oder „0“ eingegeben

ch1	1	1	1	0	1	0	0	0	1	1	0	0	0	0
ch2	0	0	1	0	0	0	1	1	1	1	0	0	1	1
ch3	0	0	0	0	0	1	0	0	1	1	0	0	0	0
ch4	0	0	0	0	0	0	0	0	1	1	0	0	0	0
ch5	0	0	1	0	1	1	1	1	1	1	0	1	1	1
ch6	0	0	1	0	0	0	0	1	1	1	1	1	1	1
ch7	0	0	0	0	0	1	1	1	1	1	0	1	1	1
ch8	1	1	0	0	0	0	0	1	0	0	0	0	1	1
ch9	0	0	0	0	0	0	1	1	1	1	1	1	1	1
ch10	0	0	0	0	0	1	0	1	1	1	0	0	1	1
ch11	0	0	1	0	1	0	0	1	1	1	1	0	1	1
ch12	1	1	0	1	0	0	1	1	1	1	0	0	1	1
ch13	1	1	0	1	1	0	1	1	1	1	0	1	1	1
ch14	1	1	0	0	0	0	0	1	1	1	0	0	0	0
ch15	1	1	0	0	1	1	1	1	1	1	0	0	0	0
ch16	0	0	1	0	0	1	1	1	1	1	0	1	1	1
ch17	1	1	1	0	0	0	1	1	1	1	0	1	1	1
ch18	0	0	1	1	0	0	1	1	1	1	0	1	1	1
ch19	0	0	1	0	0	1	0	1	1	1	0	0	0	0
ch20	1	1	1	0	0	0	1	1	1	1	1	0	1	1
ch21	1	1	1	0	0	0	1	1	1	1	1	0	1	1
ch22	1	1	0	1	0	0	0	1	1	1	0	0	0	0
ch23	1	1	0	1	0	1	1	1	1	1	0	0	1	1
ch24	0	0	0	0	0	0	1	1	1	1	0	0	1	1
ch25	0	0	0	0	0	1	0	0	0	0	0	0	1	1

Similar evidence came from the analysis of the native proteins on 25 self-pollinated Chardonnay seeds (Fig. 3). Samples including 2—3 kernels from the same berry, collected at different positions on the bunch, also resulted in a large variability (not shown).

21 self-pollinated seeds from cv. Chardonnay gave: for storage protein subunits, 22 stable and 14 changing bands (Fig. 4 a and Table 2 a); for EST, 13 common and 19 non-common isozymes (Fig. 4 b and Table 2 b); for AcP, no constant and 13 variable compo-

total protein

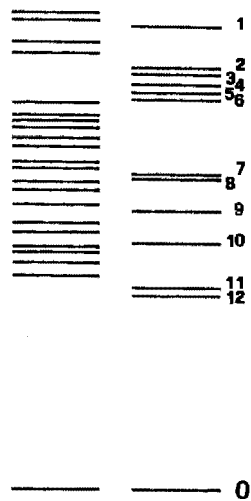


FIG.1

subunits

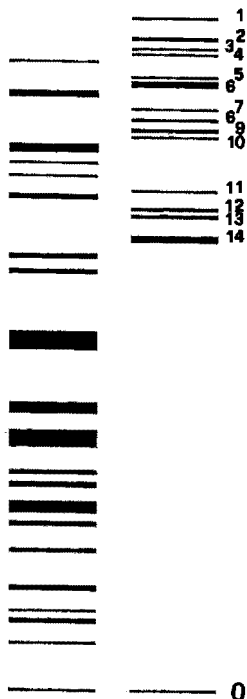


FIG.4a

EST

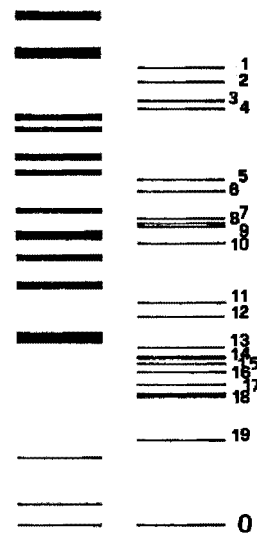


FIG.4b

ACP

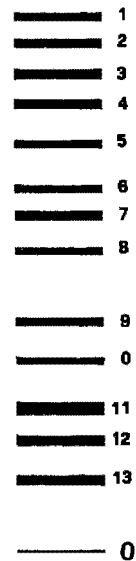


FIG.4c

PGM

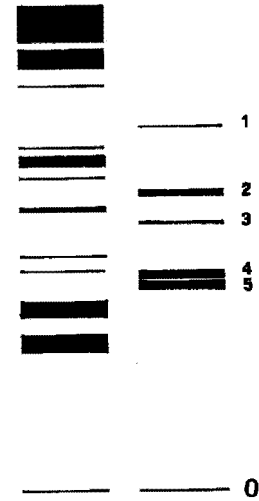


FIG.4d

Fig. 1: Drawing of common (left) and non-common (right) bands in the IEF pattern of total seed proteins for open-pollinated individual seeds from cv. Chardonnay, run on a non-linear 4—10 IPG (GIANAZZA *et al.* 1984) and Coomassie-stained. Anode is uppermost.

Fig. 4: Drawing of common (left) and non-common bands (right) of seed proteins for self-pollinated cv. Chardonnay samples: a) Storage protein subunits, run on a non-linear 4—10 IPG in presence of 8 M urea—2% 2-mercaptoethanol and Coomassie-stained; b) EST, stained according to COATES *et al.* (1984), on a non-linear 4—10 IPG; c) AcP, stained according to SWALLOW *et al.* (1964), on a 4.5—7 IPG (GIANAZZA *et al.* 1985); no common bands; d) PGM, stained according to SPENCER *et al.* (1964), on a non-linear 4—10 IPG. In all instances, the anode is uppermost.

Abb.1: Gemeinsame (links) und nicht-gemeinsame Banden (rechts) im IEF-Muster der Gesamtsereproteine von nicht-selbstbefruchteten Einzelsamen der Sorte Chardonnay; nichtlinearer 4—10 IPG (GIANAZZA *et al.* 1984), Coomassie-Färbung, Anode oben.

Abb. 4: Gemeinsame (links) und nicht-gemeinsame Banden (rechts) von Reserveproteinen aus selbstbefruchteten Samen der Sorte Chardonnay: a) Reserveprotein-Untereinheiten; nichtlinearer 4—10 IPG, 8 M Harnstoff — 2 % 2-Mercaptoethanol, Coomassie-Färbung. b) EST; Anfärbung nach COATES *et al.* (1984), nichtlinearer 4—10 IPG. c) AcP, Anfärbung nach SWALLOW *et al.* (1964), 4,5—7 IPG (GIANAZZA *et al.* 1985). Keine gemeinsamen Banden. d) PGM; Anfärbung nach SPENCER *et al.* (1964), nichtlinearer 4—10 IPG. Anode stets oben.

nents (Fig. 4 c and Table 2 c); for PGM, 11 neuter and 5 informative bands (Fig. 4 d and Table 2 d). Data from Table 2 a—d were evaluated through Jaccard's coefficient to build a similarity matrix, then used by UPGMA clustering procedures in order to shape the dendrograms of Fig. 5 a—d.

Since congruity tests between the similarity matrices for each group of variables showed every set of parameters to be independent from any other, a statistical analysis was also performed on the whole data collection (Fig. 6).

For 96 individual samples from cv. Sangiovese, the distribution between common and non-common bands was: for storage protein subunits, 30 vs 4; for EST, 28 vs 5; for AcP, 10 vs 3; for PGM, 5 vs 2 (Fig. 7 a—d). The whole set of data is listed in Table 3 a—d and the results of their statistical analysis are shown in Fig. 8.

For cv. Traminer, the electrophoretic fractionation of protein subunits from 14 seeds showed 30 constant and 25 variable bands (Fig. 9 and Table 4); the statistical analysis of the results gave the dendrogram depicted in Fig. 10.

The comparison between three extracts, each from 35 randomly selected Chardonnay seeds, shows that both the qualitative and the quantitative distribution among the protein bands is constant with either non-specific or specific gel stains (Fig. 11).

For cvs Sangiovese and Traminer, POD, MDH and G6PDH and for cv. Traminer, AcP zymograms gave a constant pattern throughout.

Discussion

We started our investigation by analysing of total proteins from single kernels or from the couple of seeds in individual berries. Then we selected a number of enzymes with high specific activity in seed extracts along with the subunits from storage proteins (GIANAZZA *et al.* 1989). The latter offer the advantage of a higher solubility, hence of a better resolution and easier evaluation of the results; moreover, the approach to the building blocks of the quaternary assembly, $M_r = 400$ kDa (GIANAZZA *et al.* 1989) was expected to account more directly for the primary gene products and possibly to disclose some underlying uniformity. However, this was not the case: for (self-poll-

Table 2

Variability of the seed protein pattern within cv. Chardonnay (self-pollinated samples) · Presence-absence of the bands in Fig. 4 are entered as 1—0
 Variabilität des Reserveproteinmusters innerhalb der Sorte Chardonnay (selbstbefruchtete Samen) · Vorhandensein oder Fehlen der Banden in Fig. 4 sind
 als „1“ oder „0“ eingegeben

	Protein subunits	EST	ACP	PGM
Ch1	1 0 1 1 1 1 1 0 0 0 0 1 0 1	1 0 1 0 0 1 1 0 1 0 1 0 1 1 1 1 0 1 1	0 1 0 0 1 0 0 0 1 1 1 0 1	0 0 0 1 0
Ch2	1 0 1 1 1 1 1 0 0 0 1 0 0 0	1 0 1 0 0 0 0 0 0 0 1 1 1 1 1 1 1 1 1	0 1 0 0 1 0 0 0 1 1 1 0 0	1 1 0 1 0
Ch3	1 1 1 1 1 1 1 1 1 0 1 0 0 0	1 0 1 0 0 1 0 1 0 1 1 0 1 1 1 1 1 1 1	1 0 1 0 0 1 0 1 1 1 1 0 1	1 1 1 1 0
Ch4	0 1 1 0 1 1 1 1 1 0 0 1 0 1	1 1 0 1 1 1 0 1 0 1 1 0 1 1 0 1 1 1 1	1 0 1 0 0 1 0 1 1 0 1 0 1	1 1 1 1 0
Ch5	0 1 1 0 1 0 0 1 0 0 1 1 0 0	1 0 1 0 1 0 0 0 1 1 0 0 0 1 1 1 1 1 1	0 1 0 1 1 0 1 0 0 1 1 1 0	0 1 1 1 0
Ch6	0 1 1 0 1 1 1 1 0 0 0 1 0 0	0 0 1 0 0 0 0 1 0 1 0 0 0 1 1 1 1 1 1	1 1 1 1 0 1 0 1 0 1 1 1 0	1 1 1 1 0
Ch7	1 1 1 0 1 1 1 1 1 1 1 1 0 0	0 0 1 0 0 1 0 1 0 1 1 1 1 1 1 1 1 1 1	1 0 1 0 0 1 0 1 0 1 1 1 0	1 1 1 1 0
Ch8	1 1 0 0 0 0 1 0 1 1 1 0 0 0	1 0 1 0 0 0 0 1 0 0 1 0 0 1 0 1 1 1 0	1 0 1 1 0 1 0 1 0 1 1 0 1	0 1 1 0 0
Ch9	1 1 1 1 1 1 1 0 1 1 1 0 0 1	1 0 1 0 0 1 0 1 0 1 1 1 1 1 0 1 1 1 1	0 1 0 0 0 1 0 1 1 0 1 0 0	0 1 1 1 1
Ch10	0 0 0 0 0 1 1 1 1 1 1 1 0 0	1 0 1 0 0 1 0 0 1 0 1 0 0 1 0 0 1 1 1	1 0 1 0 0 1 0 1 1 1 1 1 0	0 0 1 1 0
Ch11	1 1 0 0 1 1 1 1 1 1 1 1 1 1	1 0 1 0 0 1 0 0 0 1 0 1 0 1 0 1 1 1 1	1 0 1 0 0 1 0 1 0 1 1 1 0	0 1 1 1 0
Ch12	0 1 1 0 1 1 1 1 1 1 1 1 0 0	1 0 1 0 0 1 0 0 0 1 1 0 0 1 1 1 1 1 1	1 0 1 1 0 1 0 1 0 1 1 1 0	1 1 1 1 0
Ch13	1 1 1 0 0 1 1 1 1 0 0 1 0 0	1 0 1 0 0 0 0 1 0 1 1 0 0 1 0 0 1 1 1	1 0 1 1 0 1 0 1 0 1 1 1 0	0 0 1 1 0
Ch14	1 1 0 0 1 1 1 1 0 0 1 1 0 0	0 0 1 0 0 0 0 0 1 0 0 0 0 0 0 0 1 1 1	0 0 1 1 0 1 0 1 0 1 1 1 0	0 0 1 1 0
Ch15	1 0 1 0 1 1 1 0 0 1 0 1 1 1	1 0 1 0 1 1 0 1 1 1 0 0 1 1 1 1 1 1 1	1 0 1 0 0 1 0 1 1 0 1 0 0	1 1 1 1 0
Ch16	1 0 1 0 1 1 1 0 0 1 1 1 1 1	1 0 1 0 1 1 0 0 0 1 0 1 1 1 0 1 0 1 1	1 1 1 0 0 1 0 1 1 0 1 0 0	0 1 0 1 0
Ch17	1 0 1 0 1 1 1 1 1 1 1 1 0 1	1 1 0 1 1 0 1 1 0 0 0 0 0 1 0 1 1 1 1	0 1 0 0 0 1 1 0 1 0 1 0 0	0 1 1 1 0
Ch18	0 1 0 0 0 1 1 1 1 1 1 0 0 0	1 0 1 0 0 1 0 0 0 1 0 1 0 1 0 0 0 1 1	1 0 1 0 0 1 0 1 0 1 1 1 0	0 1 1 1 0
Ch19	1 0 1 1 1 1 1 0 0 0 1 0 0 1	1 0 1 0 0 1 0 0 1 0 1 0 0 1 0 1 1 1 1	0 1 0 0 0 1 1 0 0 1 1 1 0	0 1 1 1 0
Ch20	1 0 1 1 1 1 1 0 0 0 1 0 0 1	1 0 1 0 0 1 0 1 0 1 0 0 0 1 0 1 1 1 1	1 0 1 0 0 1 0 1 1 0 1 0 0	0 0 0 1 0
Ch21	1 1 1 1 1 1 0 0 0 1 1 0 0 1	1 0 1 0 1 1 0 1 0 1 0 0 1 1 1 0 1 1 1	1 0 1 1 0 1 0 1 0 1 1 1 0	1 1 1 0 0
a)		b)	c)	d)

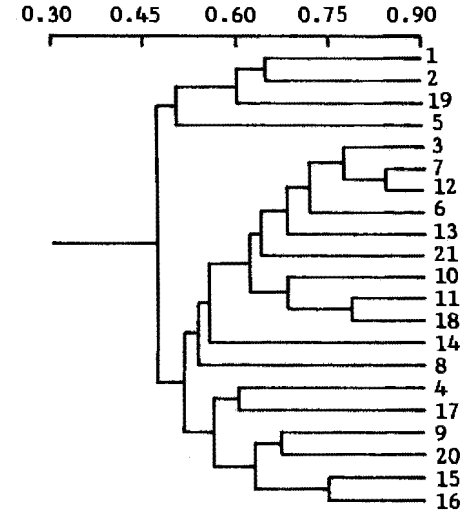
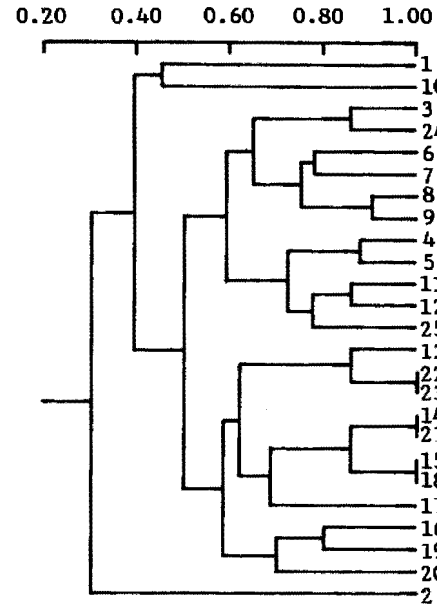
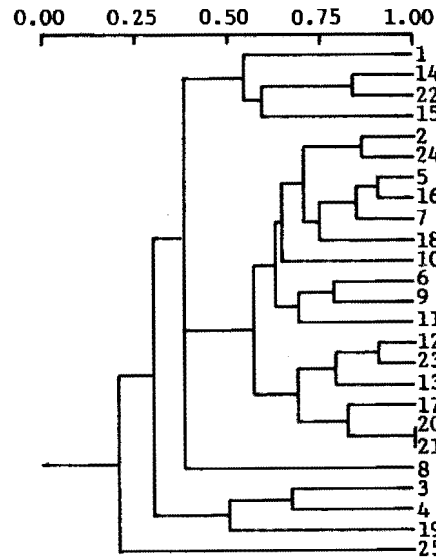


Fig. 2 (left): Dendrogram describing extent of similarity and grouping for open-pollinated cv. Chardonnay samples. Each specimen included a single seed; on glycine extracts presence-absence was evaluated for electrophoretically resolved bands of total proteins (Fig. 1 and Table 1).

Links: Dendrogramm, das den Ähnlichkeitsgrad und die Gruppierung nicht-selbstbefruchteter Samen der Sorte Chardonnay zeigt. Jede Probe stammt von einem Einzelsamen. Die mit Glycin extrahierten Gesamtproteine (Fig. 1 und Table 1) wurden auf das Vorhandensein oder Fehlen elektrophoretisch trennbarer Banden hin ausgewertet.

Fig. 3 (middle): Dendrogram for self-pollinated cv. Chardonnay samples from total protein data.

Mitte: Dendrogramm selbstbefruchteter Samen der Sorte Chardonnay für die Gesamtproteine.

Fig. 6 (right): Dendrogram for self-pollinated cv. Chardonnay samples from the whole set of data (Fig. 4 a—c and Table 2 a—d).

Rechts: Dendrogramm selbstbefruchteter Samen der Sorte Chardonnay aus dem gesamten Datenmaterial (Fig. 4 a—c und Table 2 a—d).

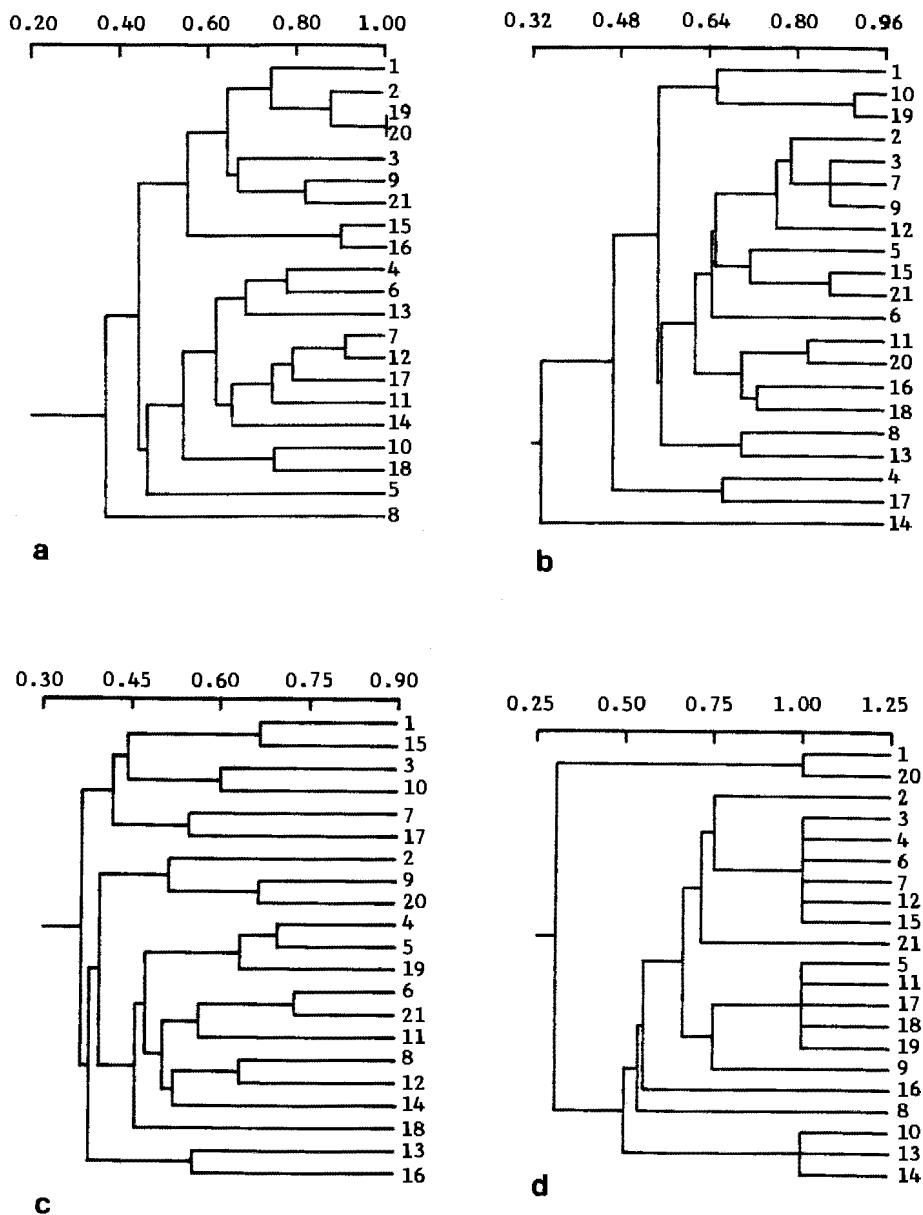


Fig. 5: Dendrograms for self-pollinated cv. Chardonnay samples; a) Storage protein subunits (Fig. 4 a and Table 2 a); b) EST (Fig. 4 b and Table 2 b); c) AcP (Fig. 4 c and Table 2 c); d) PGM (Fig. 4 d and Table 2 d).

Dendrogramme selbstbefruchteter Samen der Sorte Chardonnay: a) Reserveprotein-Untereinheiten (Fig. 4 a und Table 2 a). b) EST (Fig. 4 b und Table 2 b). c) AcP (Fig. 4 c und Table 2 c). d) PGM (Fig. 4 d und Table 2 d).

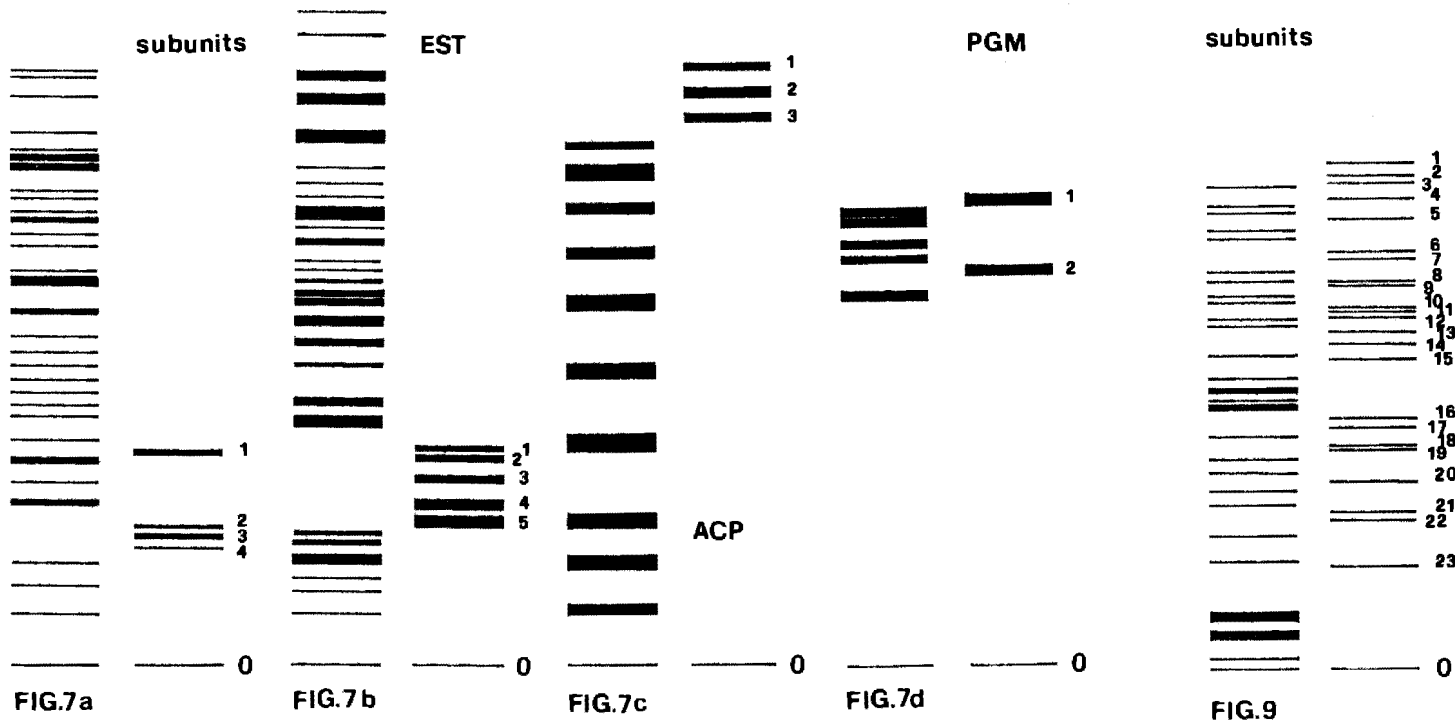


Fig. 7: Drawing of common (left) and non-common bands (right) of seed proteins for self-pollinated cv. Sangiovese samples; a) Storage protein subunits; b) EST; c) AcP; d) PGM. The anode is uppermost.

Fig. 9: Drawing of common (left) and non-common bands (right) of storage protein subunits for self-pollinated cv. Traminer samples. The anode is uppermost.

Abb. 7: Gemeinsame (links) und nicht-gemeinsame Banden (rechts) der Reserveproteine selbstbefruchteter Samen der Sorte Sangiovese; a) Reserveprotein-Untereinheiten, b) EST, c) AcP, d) PGM. Anode oben.

Abb. 9: Gemeinsame (links) und nicht-gemeinsame Banden (rechts) der Reserveprotein-Untereinheiten selbstbefruchteter Samen der Sorte Traminer. Anode oben.

Table 3

Variability of the seed protein pattern among 20 biotypes from cv. Sangiovese · Presence-absence of bands in Fig. 7 a—d are entered as 1—0
 Variabilität des Reserveproteinmusters zwischen 20 Biotypen der Sorte Sangiovese · Vorhandensein oder Fehlen der Banden in Fig. 7 a—d sind als „1“ oder „0“ eingegeben

	Subunits	EST	ACP	PGM		Subunits	EST	ACP	PGM
CN2	1 0 0 1	1 0 1 1 1	0 1 1	0 0	CS2	1 0 0 1	1 0 1 1 1	1 1 1	1 0
CN2	1 0 0 0	1 1 1 0 1	1 1 1	0 0	CS4	1 0 0 1	0 1 1 1 1	1 1 1	0 0
CN2	1 0 0 1	1 0 1 1 1	1 1 1	0 0	CS4	1 0 0 1	1 1 1 1 1	1 1 1	1 1
CN2	1 0 0 1	1 0 1 1 1	1 1 1	0 0	CS4	1 0 0 0	1 1 1 0 1	1 1 1	0 1
CN2	1 0 0 0	1 1 0 1 1	0 1 1	0 1	CS4	1 1 0 1	1 0 1 1 0	1 1 1	0 0
CN4	1 0 0 1	1 0 1 1 1	1 1 1	0 0	CS4	1 0 0 1	1 0 1 1 1	1 1 1	1 1
CN4	1 0 0 1	1 1 1 1 1	1 1 1	0 0	CS5	1 0 0 1	0 0 0 0 0	1 1 1	1 0
CN4	1 0 0 1	1 1 1 1 1	1 0 1	0 0	CS5	1 0 0 1	1 1 1 1 1	1 1 0	1 1
CN4	1 0 0 1	1 1 1 1 1	1 1 1	0 0	CS5	1 0 0 1	1 1 1 1 1	0 0 1	1 1
CN4	0 0 0 1	1 1 1 1 1	1 1 1	0 0	CS5	1 0 0 1	1 1 1 1 1	1 1 1	1 1
CN8	1 0 0 1	1 1 1 1 1	1 1 0	0 0	CS5	1 0 0 1	1 1 1 1 1	1 1 1	1 1
CN8	1 0 0 1	1 1 1 1 1	0 1 1	0 1	CS6	1 0 0 1	1 1 1 1 1	1 1 1	1 1
CN8	1 0 0 1	1 1 1 1 1	1 1 1	1 1	CS6	1 0 0 1	0 1 1 1 1	1 1 1	0 1
CN8	1 0 0 1	1 1 1 1 1	1 1 1	0 0	CS6	1 0 0 1	1 1 1 1 1	1 1 1	1 1
CN8	1 0 0 1	1 1 1 1 1	0 1 1	1 0	CS6	1 0 1 1	1 0 1 1 0	1 1 0	0 1
CN12	1 1 0 1	1 0 1 1 0	0 0 0	1 0	CS6	1 0 0 1	1 1 1 1 1	0 0 0	1 1
CN12	1 1 0 1	1 0 1 1 1	1 1 1	1 0	CS8	1 0 0 1	1 0 1 1 0	1 1 1	0 0
CN12	0 0 0 1	1 0 0 1 1	0 0 1	0 0	CS8	1 0 0 1	1 0 1 1 0	1 1 1	1 1
CN12	1 0 0 1	1 0 1 1 0	0 0 0	0 0	CS8	1 0 0 1	1 0 1 1 0	0 0 0	1 0
CN12	1 0 0 1	1 0 0 1 1	0 0 0	0 0	CS8	1 0 0 1	1 1 1 0 1	1 1 1	1 0
CN15	1 0 0 1	1 1 1 1 1	0 0 1	1 0	CS8	1 0 0 1	1 0 1 1 0	1 1 1	1 1
CN15	1 1 0 1	1 1 1 1 1	0 0 1	1 0	NF2	1 0 0 1	1 1 1 1 1	0 1 0	1 1
CN15	1 0 0 1	1 1 1 1 1	0 1 1	0 0	NF2	1 0 0 1	1 1 1 1 1	0 0 1	0 1
CN15	1 0 0 1	1 1 1 1 1	0 1 1	0 0	NF2	1 0 0 1	0 1 1 1 1	0 0 1	0 0

CN15	0 0 0 1	1 1 1 1 1	0 1 1	0 0
CN16	1 0 0 1	1 1 1 1 1	0 0 1	0 0
CN16	1 0 0 1	1 1 0 1 1	0 1 0	0 0
CN16	0 0 0 0	0 1 0 1 1	0 0 1	0 0
CN16	1 0 0 1	1 0 1 1 1	0 1 1	0 0
CN16	0 0 0 0	1 1 1 1 1	0 1 1	0 0
CP5	1 0 0 1	0 1 0 1 1	0 1 1	0 0
CP5	1 0 0 1	0 1 0 1 0	1 1 0	0 1
CP5	0 0 0 1	0 1 0 1 0	0 0 1	0 1
CP5	1 0 0 1	0 1 1 1 1	0 0 1	0 1
CP6	1 0 0 1	0 1 1 1 1	1 1 1	0 0
CP6	1 0 0 1	1 1 1 1 0	1 0 1	0 1
CP6	1 0 0 1	1 1 0 1 1	0 0 1	0 1
CP6	1 0 0 1	0 1 0 1 1	0 0 0	0 1
CP6	1 0 0 1	0 1 0 1 1	0 0 1	1 0
CS1	1 0 0 1	1 0 1 1 1	1 1 1	0 0
CS1	1 0 0 1	1 0 1 0 1	0 0 1	1 0
CS1	0 0 0 1	1 1 1 1 1	1 0 1	1 1
CS1	0 0 0 1	1 0 0 1 1	1 1 1	0 0
CS1	1 0 0 1	1 0 0 1 0	0 1 1	1 0
CS2	1 0 0 1	1 0 1 1 1	0 0 0	1 1
CS2	1 1 0 1	1 0 1 1 1	0 0 0	0 0
CS2	0 0 0 1	1 0 1 1 1	1 1 1	1 1
CS2	1 0 0 1	1 0 1 1 1	0 0 0	1 1

a) b) c) d)

NF2	1 0 0 1	1 0 1 1 1	1 1 1	0 0
NF3	1 0 0 1	1 1 1 1 1	0 0 1	1 1
NF3	1 0 0 1	0 1 0 0 1	0 0 0	0 0
NF3	1 0 0 1	1 1 1 1 1	0 0 0	0 1
NF3	1 0 0 1	1 1 1 1 1	1 1 0	1 1
NF6	1 0 0 1	1 1 1 1 1	0 1 1	0 0
NF6	1 0 0 1	1 1 1 1 1	0 1 1	0 0
NF6	1 0 0 1	0 1 1 1 1	0 1 1	1 0
NF6	1 0 0 1	1 0 0 1 1	0 1 1	0 0
NF6	1 0 0 1	0 1 1 1 1	0 1 1	1 0
NF7	1 0 0 1	1 1 1 1 0	1 1 0	1 0
NF7	1 0 0 1	1 1 1 1 1	1 1 1	1 0
NF7	1 0 0 1	1 0 1 1 0	0 0 1	0 0
NF7	1 1 0 1	1 0 1 1 0	1 1 1	0 0
PR3	0 0 0 0	1 0 1 1 0	1 1 1	0 1
PR3	1 0 0 1	0 1 1 1 1	0 1 0	1 1
PR3	1 0 0 1	0 1 1 1 1	1 1 1	1 0
PR3	1 0 0 1	1 1 1 1 1	0 1 1	1 0
PR3	1 0 0 1	1 1 1 1 0	0 1 1	0 0
PR7	1 0 0 1	0 1 1 1 1	0 0 1	0 0
PR7	1 0 0 1	1 0 1 1 1	0 0 1	0 0
PR7	1 0 0 1	0 1 1 1 1	0 1 1	1 0
PR7	1 1 0 1	1 1 1 1 1	0 1 1	0 0
PR7	1 1 0 1	1 0 1 1 0	0 1 1	1 0

a) b) c) d)

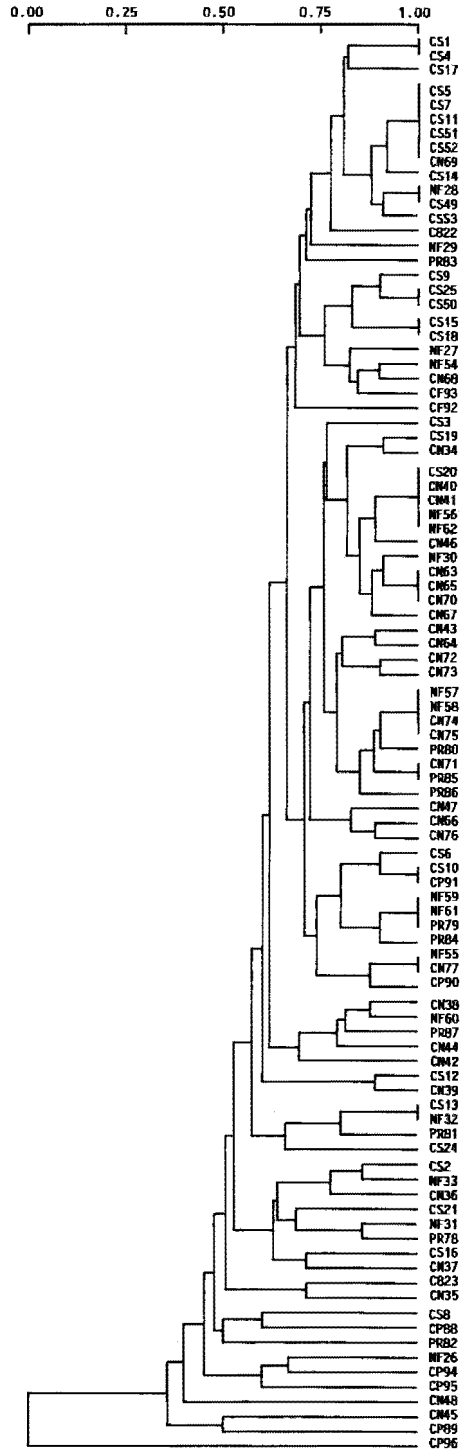


Fig. 8: Dendrogram for self-pollinated cv. Sangiovese samples from the whole set of data (Fig. 7 a—c and Table 3 a—d).

Dendrogramm selbstbefruchteter Samen der Sorte Sangiovese aus dem gesamten Datenmaterial (Fig. 7 a—c und Table 3 a—d).

Table 4

Pattern variability of the storage protein subunits in cv. Traminer · Presence-absence of the bands in Fig. 9 are entered as 1—0

Variabilität im Muster der Reserveprotein-Untereinheiten bei der Sorte Traminer · Vorhandensein oder Fehlen der Banden in Fig. 9 sind als „1“ oder „0“ eingegeben

TR1	0	1	0	0	1	0	0	1	1	0	0	1	1	1	1	1	0	1	0	1	1	1	1	1	0			
TR2	0	1	1	1	1	0	0	1	1	1	1	1	1	1	0	1	0	0	1	0	1	1	0	1	1	1	0	
TR3	0	0	0	0	1	0	0	1	0	1	0	1	1	0	0	0	1	0	1	0	0	1	0	1	0	0	0	
TR4	1	0	1	0	0	1	0	1	1	1	1	1	0	0	1	1	0	1	0	1	0	0	1	1	1	1	0	
TR5	1	0	1	1	0	1	1	1	1	1	1	1	1	0	1	0	0	1	0	1	0	0	1	1	1	1	0	
TR6	0	1	1	0	1	0	0	1	1	1	0	1	1	0	1	1	0	1	0	1	1	0	1	0	1	1	0	0
TR7	1	1	1	1	1	1	1	1	1	1	0	1	0	0	1	1	0	1	0	1	0	0	1	1	1	1	1	
TR8	0	1	0	0	0	0	0	0	1	0	0	1	1	0	1	0	0	1	0	1	1	0	1	1	0	1	1	0
TR9	0	1	1	0	1	0	0	1	1	0	1	1	1	0	0	0	1	0	1	0	0	1	1	1	1	0	0	
TR10	0	1	1	0	1	1	0	0	0	1	0	0	0	1	1	0	0	1	0	1	1	0	1	1	0	1	1	1
TR11	0	1	1	0	1	0	0	1	0	1	1	1	1	0	0	1	0	1	0	1	0	0	0	0	1	0	0	
TR12	0	0	0	1	1	1	0	0	1	1	0	1	1	0	1	0	0	1	0	1	0	1	1	1	1	1	1	
TR13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	1	0	1	0	1	1	0	1	0
TR14	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0	1	0	1	0	0	0	1	1	0	0

nated) Chardonnay the ratio of variable versus constant bands was 12 to 21 under native and 30 to 4 under denaturing conditions (although in the latter case most sample variability is accounted for by quantitatively minor components).

The large individual variability for all seed proteins described in the present investigation is in agreement with previous findings of a high degree of heterozygosity in *V. vinifera* (LEVADOUX 1956). Each of the parameters we have selected seems to be independently transmitted: from the analysis of individual variables no overlap between

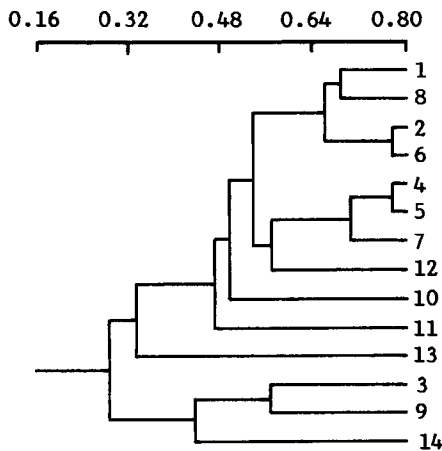


Fig. 10: Dendrogram for self-pollinated cv. Traminer samples from subunit data (Fig. 9 and Table 4). Dendrogramm selbstbefruchteter Samen der Sorte Traminer für die Reserveprotein-Untereinheiten (Fig. 9 und Table 4).

the different clustering patterns is observed (Fig. 4 a—d) and the congruity tests score a very low coefficient (SOKAL 1986). The ratio between constant versus variable bands differs both from a protein to another (compare the behavior of G6PDH and AcP in Chardonnay) and, for the same enzyme, from a cultivar to another (AcP in Chardonnay — 14, Sangiovese — 3, Traminer — no variable bands). For storage proteins, the pIs of the characteristic components vary from a cultivar to another (compare Figs. 4 a, 7 a and 9). No relationship is observed between the degree of morphological and biochemical variability; although diverse protein patterns are found in every distance, the extent of variability is higher for the phenotypically homogeneous cv. Chardonnay than for the heterogeneous cv. Sangiovese (14 vs 4 variable bands for storage protein subunits, 19 vs 5 for EST, 14 vs 5 for AcP, 7 vs 2 for PGM).

On the basis of the observed variability a large number of seeds must be sampled in order to get a representative picture of the average protein make-up for a given cultivar. With the most variable cultivar under investigation (Chardonnay) we could show that samples including 35 randomly selected seeds give highly constant protein patterns, both in qualitative and in quantitative terms (Fig. 11), and are thus adequate for the characterization of individual cultivars in taxonomical studies. This finding could be confirmed and extended by a broad survey on 20 Traminer clones from Italy, France, Germany, Austria, Yugoslavia and Hungary (SCIENZA *et al.*, in press).

We suggest the same kind of investigation on individual variability be undertaken for the parameters of interest before any genetic selection program is undertaken.

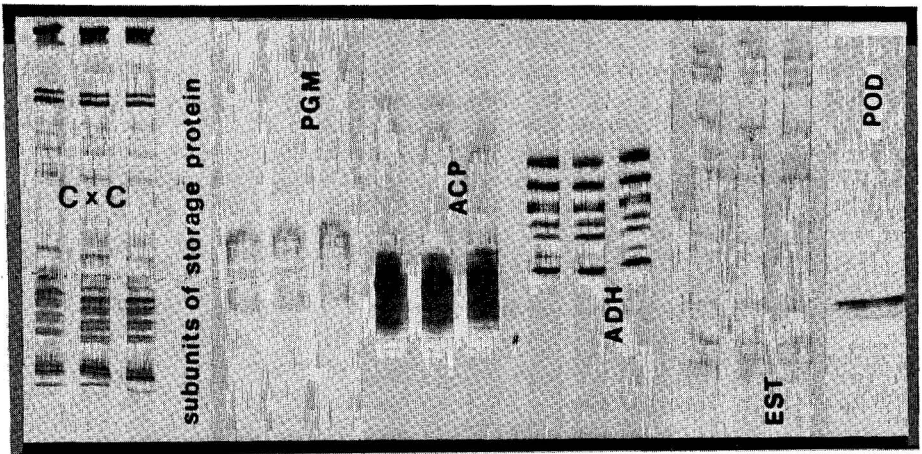


Fig. 11: Comparison of three protein extracts from 35 self-pollinated cv. Chardonnay seeds. From left to right, Coomassie-stained subunits of storage protein, and the zymograms for: PGM; AcP; ADH; EST; POD. Experiments run on a non-linear 4—10 IPG. The cathode is uppermost.

Vergleich zwischen drei Proteinextrakten aus 35 selbstbefruchteten Samen der Sorte Chardonnay. Von links nach rechts: Coomassie-gefärbte Untereinheiten des Reserveproteins und Enzymogramme für PGM, AcP, ADH, EST und POD. Nichtlinearer 4—10 IPG. Kathode oben.

Summary

The extent of variability for storage protein subunits as well as the isozymes of AcP, EST and PGM was evaluated by isoelectric focusing on a large number of individual self-pollinated seeds from cvs Chardonnay, Sangiovese and Traminer. Extracts from 35 randomly selected kernels gave reproducible protein patterns and may thus be taken as representative of the average genetic make-up in a given biotype.

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