Vitis vinifera - a chemotaxonomic approach: Seed storage proteins

A. Scienza [1]), R. Anzani [1]), O. Failla [1]), F. Mattivi [1]), P. L. Villa [1]), G. Tedesco [2]), P. G. Righetti [3]), C. Ettori [3]), S. Magenes [3]) and E. Gianazza [3])

[1]) Istituto di Coltivazioni Arboree, via Celoria 2, I-20133 Milano, Italy

[2]) Dipartimento di Biologia, Sezione di Botanica Sistematica, via Celoria 26, I-20133 Milano, Italy

[3]) Dipartimento di Scienze e Tecnologie Biomediche, Sezione di Biochimica, via Celoria 2, I-20133 Milano, Italy

Summary: The IEF pattern of the constituent peptides for the storage protein from *Vitis vinifera* endosperm is used for the construction of a dendrogram relating 74 seed specimens.

Key words: seed, protein, analysis, Vitis vinifera, variety of vine, clone, ampelography, Italy.

Introduction

In a previous report (Gianazza *et al.* 1989), we investigated the major endosperm proteins of *Vitis vinifera sativa* utilizing seeds from cv. Chardonnay. The storage protein was found to be a globulin, homogeneous by size (M_r > 400 kDa after PAGE) and highly heterogeneous by charge, 23 bands being resolved by IEF, with pIs 4.8-5 for the major components. From SDS-PAGE under reducing and non-reducing conditions, the native structure appeared to be assembled from non-covalently bound subunits, with M_r ca. 65 kDa, which in turn were composed of disulfide-bridged peptides, M_r = 19-21 kDa and 38-44 kDa. The focusing pattern of the denatured protein (8 M urea after –S–S–reduction) included 15 acidic (pIs = 4.25-4.80) and 2 alkaline (M_r = 26 kDa, pIs = 6.8-6.9) components.

We compare here the subunit composition of the storage proteins from a number of cultivars grown across Italy. This data base is then used for the construction of a dendrogram relating the various cultivars to one another.

Materials and methods

The seeds from 54 cvs of *V. vinifera sativa* from the following Italian regions: Val d'Aosta (AO), Piemonte (TO), Liguria (GE), Lombardia (PV), Trentino (TN), Veneto (VE), Friuli (TS), Emilia (BO), Toscana (FI), Sardegna (CG), Puglia (BA), as well as from different clones of cvs Schiava (SC, N = 5), Malvasia (MAL, 6) and Trebbiano (TB, 7) and of two *V. vinifera silvestris* specimens (Reppi2 and Reppi3, SI), were collected at vintage 1988. For each sample, the endosperm dissected from 30-35 seeds was ground and extracted with 10 volumes of 0.2 M glycine (Gianazza *et al.* 1989).

Isoelectric focusing was performed on immobilized pH gradients (IPGs) (Bjellqvist *et al.* 1982) in the pH range 4-5.5 (1-D) or 4-6 (2-D experiments). The IPG plates were polymerized according to standard procedures (Righetti and Gianazza 1987) with Immobiline monomers purchased from LKB; the washed and dried gel slabs were reswollen in 8 M urea – 0.5 % carrier ampholytes (0.25 % 4-6 Ampholine and 0.25 % 4-6.5 Pharmalyte, from Pharmacia-LKB Biotechnology, Uppsala, S). Prior to loading, the protein samples were diluted 1 : 1 with 8 M urea – 2 % 2-mercaptoethanol. Gels were run overnight at 500 V, followed by 1 h at 1,300 V. For 2-D mapping, the gel strips from the 1st d run were equilibrated for 15 min in electrode buffer

(according to LAEMMLI 1970) with 3 % SDS and 2 % 2-mercaptoethanol added. The 2nd d separation was on a 7.5-17.5 % T polyacrylamide gradient, at 50 mA/gel (140 x 140 x 1.5 mm).

A total of 57 bands were analyzed in the 1-D pattern of the various samples. Data reduction was for presence/absence of any given component – disregarding quantitative variations. A dendrogram using average linkage was constructed with the statistical program SPSS-X run on a VAX-VMS computer (Istituto Agrario Provinciale, San Michele all'Adige, TN).

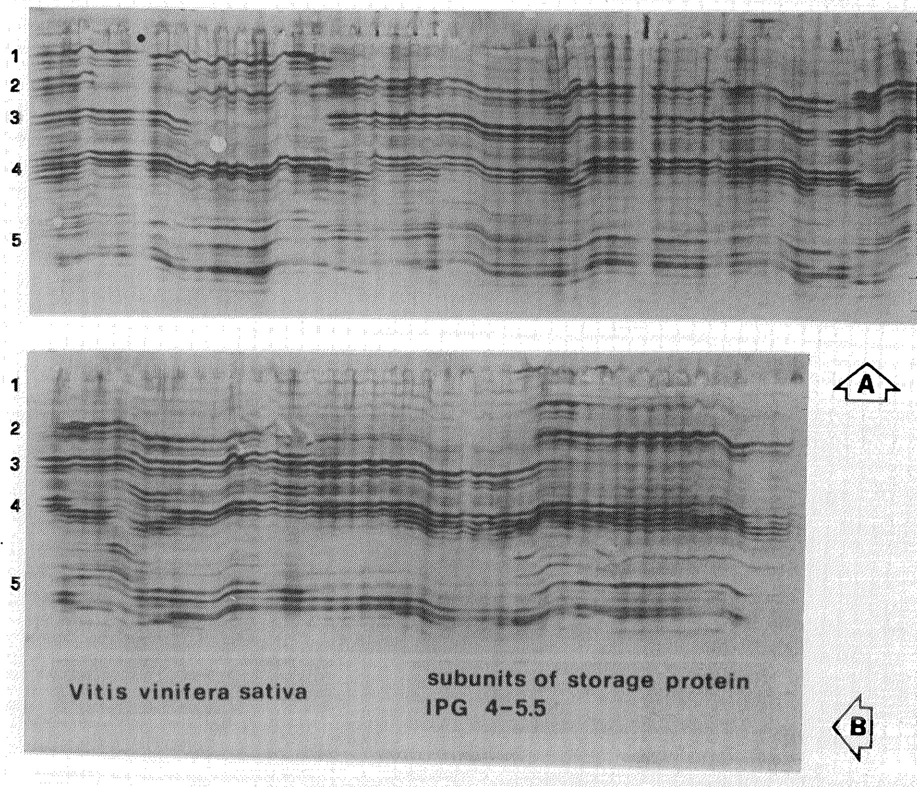

Fig. 1: Isoelectric focusing on immobilized pH gradients (IPG) 4-5.5 of the endosperm proteins from *Vitis vinifera* seeds. 15 μl of a glycine extract diluted 1 : 1 with 8 M urea – 2 % 2-mercaptoethanol were applied per lane. The gel had a polyacrylamide matrix T % = 4, C % = 4 and was made to contain 8 M urea and 0.5 % carrier ampholites in the pH range 4-6.5. Gels were run at 15 °C for 12 h at 500 V, then for 1 h at 1,300 V. Coomassie stain according to RIGHETTI and DRYSDALE (1974). Samples: gel A = Reppi 2 (*V. vinifera silvestris*), Cagnina, Ribolla Nera, Canina, Cannonau, Lambrusca d'Alessandria, Malvasia Brindisi, Verdicchio, Trebbiano Valtenesi, Tb. Soave, Tb. Lugano, Torbiana Soave, Carignano, Malvasia Casorso, Ancellotta, Schiava Grigia, Coda di Volpe di Labico, Lambrusco Salamino, Lambrusco Sorbara, Fumat, Lambrusco Grasparossa, Malvasia Candia, Malv. Candia N. A., Lambrusco Oliva, Lambrusco Maestri, Brunello, Prugnolo, Sangiovese, Aglianico, Grechetto Bianco, Malv. Lecce, Malv. Lunga Chianti, Croatina, Freisa, Bonarda, Pignola, Sc. Lombarda, Uva d'Oro, Tb. Romagnolo, Sc. Gentile, Trollinger, Lambrusco F. F., Neyret, Reppi3 (*V. vinifera silvestris*); gel B = Trollinger, Lambrusco F. F., Neyret, Reppi3 (*V. vinifera silvestris*), Malv. Asolo, Lagrein, Vermentino Spoletino, Pigato, Vermentino Finale, Favorita, Colorino, Marzemino, Teroldego, Mammolo, Vernaccia San Giminiano, Sc.Grossa, Tb. Toscano, Nebbiolo, Barbera Bs., Barbera, Ribolla Spizade, Croà Rosso, Sc. Grossa, Vernaccia, Rossetta di Montagna, Dindarella, Cabrusina, Cividino, Picolit, Corvina Cl. 7, Corvina Cl. 47, Rondinella, Rondinella Cl. 77, Timoraccio, Refoscone, Vien de Nus, Canaiolo, Malvasia.

Results

Fig. 1 A and B shows the isoelectric focusing pattern of the storage protein subunits from the seeds of various *V. vinifera* cvs. The sequence of the samples is based on pattern similarity as evaluated by visual inspection for the presence (from left to right) of cathodal to anodal major components. 10-15 prominent bands in 5 clusters and a number of minor components can be recognized in each lane. None of the major components is present in all samples, i. e. a typical pattern includes only 2-4 band clusters.

The relationships between the different clusters are better evaluated from the 2-D maps of Fig. 2 A-D. The M_r of the polypeptide chains tend to increase with their pI, but clusters 1-2 and 3-4 share essentially the same size.

The dendrogram in Fig. 3 depicts the dissimilarity hierarchy between the banding patterns for the various cultivars. The same results were obtained when specifying either the squared Euclidean or the city block measure as the agglomeration method. Within the widely grown cultivars, 5 out of 7 Trebbiano clones are virtually identical, while the specimens from cvs Schiava and Malvasia are found more scattered across the tree. From the point of view of the geographical origin, the cultivars from Veneto appear the most, and those from Val d'Aosta the least homogeneous.

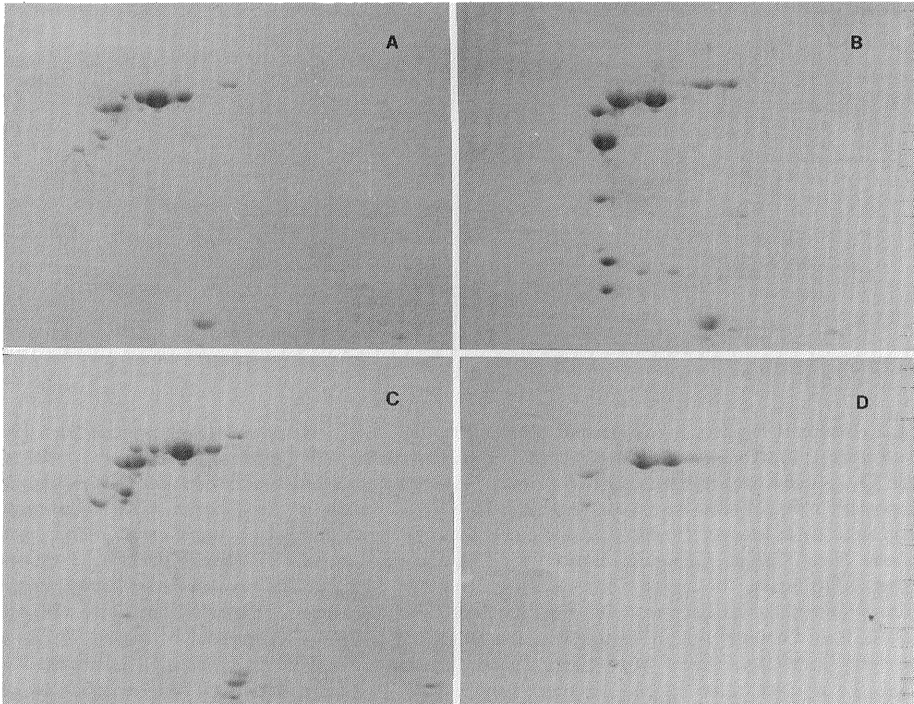

Fig. 2: Two dimensional mapping of the endosperm proteins from *Vitis vinifera* seeds. 1st d: isoelectric focusing in presence of 8 M urea under reducing conditions on a 4-6 IPG; 2nd d: SDS-PAGE according to LAEMMLI(1970) on a polyacrylamide gel T % = 7.5-17.5 %, after reduction and denaturation of the sample. Coomassiestain. A = Canina, B = Trebbiano Lugano, C =Prugnolo, D = Schiava Grossa.

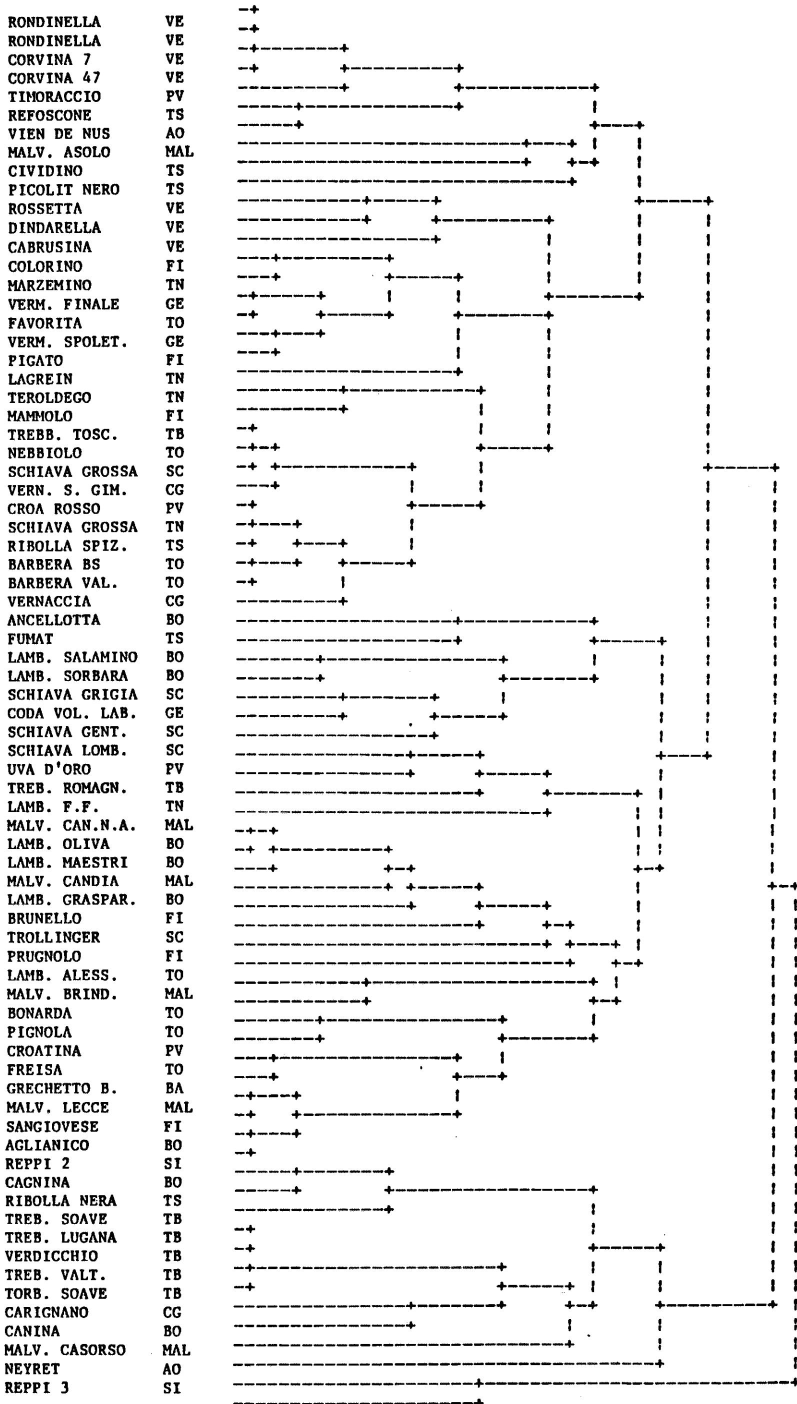

Fig. 3: Dendrogram relating various *Vitis vinifera* sativa cvs.

Discussion

Our current investigation on the seed proteins from *V. vinifera* addresses several problems:

a) the biochemistry of the storage protein (structure and biosynthesis),

b) the ability of various sets of biochemical parameters – i. e. the IEF banding pattern of different endosperm proteins, with or without enzymatic activity – to characterize a given cultivar, or clone, for identification purposes, and

c) their significance in constructing genealogic trees for taxonomy studies.

As for the first point, the present results generalize the findings of GIANAZZA *et al.* (1989) on cv. Chardonnay, and assign pI-M_r relationships between the 5 major protein clusters. Further studies will include: thorough purification of the storage protein from cytosolic components, separation of individual peptides from the different clusters and comparison of their V8-protease fragments while searching for sequence homologies. The hypothesis of a proteolytic processing for the protein subunits from a 65 kDa polypeptide chain (GIANAZZA *et al.* 1989) will be tested by analyzing the set-up of the storage protein across seed development as well as by quantitating in a panel of samples the low- and high-M_r species resolved by SDS-PAGE under reducing vs. non-reducing conditions.

We have defined the size of our samples ($N = 30\text{-}35$) from the evidence of a variability (for presence/absence as well as for relative concentrations) for the various bands of the native storage protein in cv. Chardonnay (GIANAZZA *et al.* 1989). We could then show that this sampling was indeed representative of the average genetic make-up of a given cultivar (to be published). In order to get an impression of the data dispersion around the median, we are currently analyzing the extracts from single seeds in a number of clones. The banding pattern for storage protein subunits is usually fairly stable across a given clone and thus represents a useful parameter in systematic studies.

The dendrogram based on the Coomassie-stained pattern of the proteins focusing (under denaturing conditions) in the pH range 4-5.5 is often close to the ones in which more data, from the analysis of a number of enzymes, were also included (to be published). Thus, the simplest analytical approach – requiring no special chemical nor further processing of the gels – may provide sufficient information for correct cultivar clustering.

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