Assessment of the Native Electrophoretic Analysis of Total Grape Must Proteins for the Characterization of *Vitis vinifera* L. Cultivars

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The findings of an ampelographic analysis of vines belonging to a Germoplasm Bank were compared to the results of native electrophoresis of the total proteins in their musts. Cluster analysis of the data from the morphological description produced correct groupings, in terms of variety, for all samples. When cluster analysis was performed on the electrophoretic data, 10 of the 11 musts studied were grouped correctly. Electrophoresis was also performed on 30 musts made from a mixture of grapes from large vineyards. In the cluster analysis of the electrophoretic data on the proteins of the 41 musts studied, all the musts are grouped correctly in terms of variety. Electrophoretic analysis of proteins is a simple technique that can be used routinely, provides complementary information to morphological analysis for varietal characterization of vines, and in the majority of cases, makes it possible to ascertain the grape variety from which musts originate.

INTRODUCTION

The characterization and identification of the varieties of the *Vitis vinifera* L. species involves great difficulty and is a matter of concern both to vine growers and enologists. Varietal characterization of the plants is traditionally carried out using descriptive ampelographic methods. Despite attempts to standardize them, the results obtained from these methods depend to a large extent on the subjective assessment of the person drawing up the description; on the variability resulting from the influence of the climate, soil, plant age, type of pruning, etc.; on the characters used; and on the imprecision of the definitions adopted. For this reason, it is often difficult to compare ampelographic descriptions of the same variety drawn up by different authors. Until now no other methods have been found to completely replace these descriptive morphological techniques, but great efforts are being made to find nonsubjective methods, chemical or biochemical, that will at least complement them.

A widely used alternative to descriptive ampelographic methods is the electrophoretic analysis of the isoenzymes of different parts of the plant, for example, grapes (Wolfe, 1976) and vine shoots (Altube et al., 1991; Benin et al., 1988; Cabello and Ortiz, 1995). This technique also presents difficulties since it is necessary to prepare a large number of buffers and stainings. Isoelectrofocusing of the soluble proteins in root apical meristemes has been used by Tedesco et al. (1997). According to these authors, this technique is suitable for characterizing species but not varieties. DNA sequence is also used as a varietal character in grapes

(Costacurta et al., 1996; Meredith et al., 1996) and in leaves (Sensi et al., 1996).

Among the chemical compounds used for varietal identification of musts and wines are phenolic compounds (Estrella et al., 1984; González-San José et al., 1990; Di Stefano, 1996), terpenes (Cravero et al., 1994), and amino acids and aroma compounds (Polo et al., 1983; Martín-Alvarez et al., 1987). Native electrophoresis of total grape must proteins is an easy technique and, according to several authors (Bayly and Berg, 1967; Correa et al., 1988; González-Lara et al., 1989; Polo et al., 1990; Pueyo et al., 1993), different grape varieties have different electrophoretic profiles. To our knowledge, up to the present moment no comparison has been made between the results obtained by this method and those obtained by descriptive ampelographic methods, which would make it possible to verify its validity both for characterizing grape varieties and for ascertaining the variety from which grape musts originate. It was for this reason that the present study was carried out, where the two methods were applied to plants and to grape musts of the Macabeo, Xarel.lo, and Parellada varieties and their synonyms Viura (synonym of Macabeo) and Pansa Blanca and Viñate (synonyms of Xarel.lo). These are very important varieties in Spain, since they are the varieties used to manufacture *cava*—high-quality sparkling wine manufactured by the Champenoise method in a specific region.

MATERIALS AND METHODS

Plant Material. The ampelographic and electrophoretic studies of total grape must proteins were performed on plants from the Germoplasm Bank Collection of the "El Encín" estate belonging to the Agricultural Research Service of the Autonomous Regional Community of Madrid (Table 1). An electrophoretic study of total proteins was also performed on monovarietal musts of grapes originating from large plantations in Navarra and El Penedès (Spain) (Table 2).

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Table 1. List of Cultivars Studied Belonging to the Germoplasm Bank

key	variety	origin area	vintage
PAR-G26	Parellada	El Penedés (Barcelona)	1996 and 1997
PAR-G34	Parellada	El Penedés (Barcelona)	1996 and 1997
PAR-G35	Parellada	El Penedés (Barcelona)	1996 and 1997
PAR-G48	Parellada	Tárrega (Lérida)	1996 and 1997
MAC-H30	Macabeo	Brafim (Tarragona)	1996 and 1997
MAC-H41	Macabeo	Casas Ibánez (Albacete)	1996 and 1997
VIU-A02	Viura (Macabeo)	Rioja Alavesa (Álava)	1996 and 1997
XAR-G28	Xarel.lo	El Penedès (Barcelona)	1996 and 1997
XAR-H35	Xarel.lo	El Vendrell (Tarragona)	1996 and 1997
PAB-H13	Pansa Blanca (Xarel.lo)	La Selva (Tarragona)	1996 and 1997
VIÑ-H16	Viñate (Xarel.lo)	Reus (Gerona)	1996 and 1997

Table 2. List of Must Produced from a Mixture of Grapes from a Large Number of Plants

key	variety	origin area	vintage
PAR-G01	Parellada	El Penedès (Barcelona)	1985
PAR-E01	Parellada	El Penedès (Barcelona)	1988
PAR-E02	Parellada	El Penedès (Barcelona)	1988
PAR-E03	Parellada	El Penedès (Barcelona)	1988
PAR-V01	Parellada	El Penedès (Barcelona)	1993
PAR-V02	Parellada	El Penedès (Barcelona)	1994
PAR-V03	Parellada	El Penedès (Barcelona)	1995
MAC-G01	Macabeo	El Penedès (Barcelona)	1985
MAC-G02	Macabeo	El Penedès (Barcelona)	1985
MAC-E01	Macabeo	El Penedès (Barcelona)	1988
MAC-E02	Macabeo	El Penedès (Barcelona)	1988
MAC-E03	Macabeo	El Penedès (Barcelona)	1988
MAC-E04	Macabeo	El Penedès (Barcelona)	1988
MAC-E05	Macabeo	El Penedès (Barcelona)	1988
MAC-E06	Macabeo	El Penedès (Barcelona)	1988
MAC-V01	Macabeo	El Penedès (Barcelona)	1993
MAC-V02	Macabeo	El Penedès (Barcelona)	1994
MAC-V03	Macabeo	El Penedès (Barcelona)	1995
VIU-516	Viura (Macabeo)	Olite (Navarra)	1985
XAR-G01	Xarel.lo	El Penedès (Barcelona)	1985
XAR-G02	Xarel.lo	El Penedès (Barcelona)	1985
XAR-E01	Xarel.lo	El Penedès (Barcelona)	1988
XAR-E02	Xarel.lo	El Penedès (Barcelona)	1988
XAR-E03	Xarel.lo	El Penedès (Barcelona)	1988
XAR-E04	Xarel.lo	El Penedès (Barcelona)	1988
XAR-E05	Xarel.lo	El Penedès (Barcelona)	1988
XAR-E06	Xarel.lo	El Penedès (Barcelona)	1988
XAR-V01	Xarel.lo	El Penedès (Barcelona)	1993
XAR-V02	Xarel.lo	El Penedès (Barcelona)	1994
XAR-V03	Xarel.lo	El Penedès (Barcelona)	1995

Table 3. Ampelografic Characters Used (O.I.V., 1984)

001	young shoot: form of tip
002	young shoot: ditribution of anthocyanin coloration of tip
003	young shoot: intensity of anthocyanin coloration of tip
004	young shoot: density of prostrate hairs of tip
006	shoot: attitude
007	shoot: color of dorsal side of intenodes
011	shoot: density of erect hairs of the nodes
012	shoot: density of erect hairs on internodes
016	tendrils: distribution on the shoot
017	tendrils: length
065	mature leaf: size
067	mature leaf: shape of blade
068	mature leaf: number of lobes
070	mature leaf: anthocyanin coloration of the main veins on upper side of the blade
075	mature leaf: blistering of upper side
076	mature leaf: shape of teeth
077	mature leaf: length of teeth
078	mature leaf: length of teeth compared with their width at the end of the base
079	mature leaf: general shape of petiole sinus
080	mature leaf: shape of base of petiole sinus
081	mature leaf: particularities of petiole sinus
084	mature leaf: density of prostrate hairs between the veins (lower side)
085	mature leaf: density of erect hairs between the veins (lower side)
086	mature leaf: density of prostrate hairs on main veins (lower side)
087	mature leaf: density of erect hairs on main veins (lower side)
090	mature leaf: density of prostrate hairs on petiole
091	mature leaf: density of erect hairs on petiole
102	woody shoot: surface
151	inflorescence: sex of flower
202	bunch: size
204	bunch: density
206	bunch: length of peduncle
220	berry: size
223	berry: shape
225	berry: color of skin
230	berry: color of flesh
236	berry: particular flavor
241	berry: presence of seeds
244	berry: transversal ridges on dorsal side of seed
301	time of bud burst

Description of Morphological Characters. Forty characters, belonging to the minimum list for the establishment of gene collections and the minimum list for distinction of varieties, as adopted by the IWO (1984), were described (Table 3). A description was prepared for four plants of each clone in the 1996 and 1997 harvests. The results shown in the table are the modes of 20 determinations of each character (10 determinations each year).

Preparation of Samples for Electrophoretic Study. The musts from the grapes of the Germoplasm Bank vines (Table 1) were produced from a mixture of grapes from four plants of each clone. The musts from the large plantation vines were produced industrially from a mixture of grapes from a large number of vines. A total 200 mL of each must was centrifuged at 10000*g* for 20 min. The supernatants were collected and dialyzed against running water in Cellu-Sep T1 membranes (3500 Da) (Membrane Filtration Products, Inc., San Antonio, TX) for 48 h. The dialyzed liquid was lyophilized, and the resulting residue was dissolved in 2 mL of pH 8.3 buffer (0.6 g of tris(hydroxymethyl)aminomethane + 2.9 g of glycine/L of water).

Polyacrylamide Gel Electrophoresis (PAGE). Polyacrylamide gel electrophoresis was performed as described by Hillier (1976). The sample was applied to a polyacrylamide gel (140 × 140 × 0.75 mm) containing 9.0 g of acrylamide and 400 mg of *N,N*-methylenebisacrylamide in 100 mL of pH 8.9 buffer. Electrophoresis was performed at a constant current setting of 20 mA/gel. The gel was stained with Coomassie Brilliant Blue R-250 (Winter et al., 1977).

Statistical Methods. Cluster analysis was used to examine the natural linkage of the samples on the basis of the information provided by the variables analyzed. The morphological data matrix was prepared from the characters evaluated, which were discrete data. For the electrophoretic data, data matrixes were prepared with values 0 and 1 to indicate absence or presence of each of the bands on the corresponding protein electrophoregram. The percent disagreement was taken as a measure of the proximity between the samples, and Ward's method was used as the linkage ruler. Cluster analysis
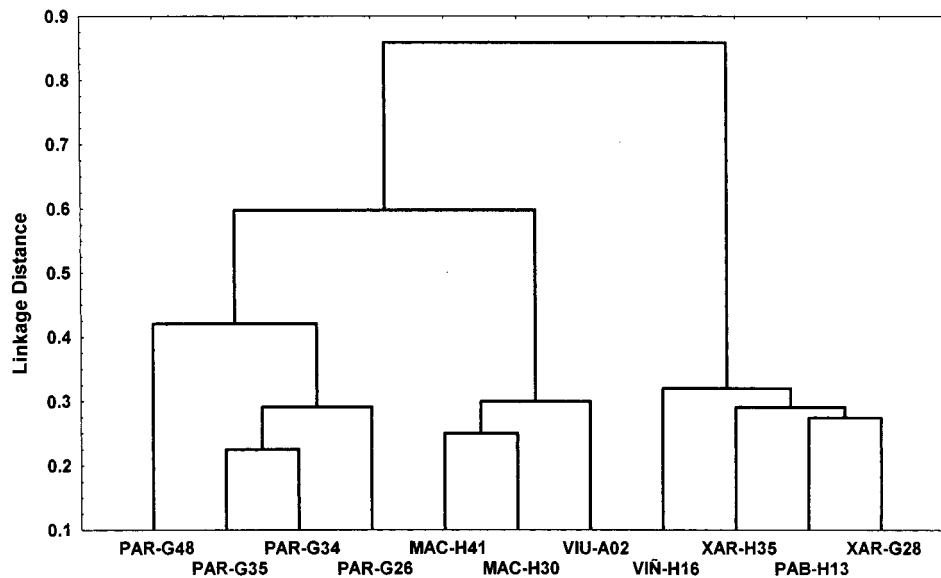
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Figure 1. Dendrogram of the Germoplasm Bank cultivars obtained from the morphological data (see Table 4).

Table 4. Ampelographic Descriptions in Accordance with the O.I.V. (1984) (see Table 3)

	PAR-G26	PAR-G34	PAR-G35	PAR-G48	MAC-H30	MAC-H41	VIU-A02	XAR-G28	XAR-H35	PAB-H13	VIÑ-H16
001	7	7	7	7	7	7	7	7	7	7	7
002	2	2	2	2	1	1	1	3	3	3	2
003	5	3	5	3	1	1	1	7	1	5	5
004	7	3	7	6	7	8	7	7	6	5	5
006	5	6	4	6	6	6	1	6	7	6	5
007	2	2	2	2	1	1	2	3	3	2	1
011	1	1	1	1	1	1	1	1	1	1	1
012	1	1	1	1	1	3	3	1	1	1	1
016	1	1	1	1	1	1	1	1	1	1	1
017	4	2	2	4	3	5	3	4	4	4	4
065	3	4	4	4	7	6	5	7	5	5	5
067	4	4	4	4	3	3	3	3	3	3	3
068	3	3	3	3	3	3	3	3	3	3	3
070	1	1	1	1	1	1	1	3	3	4	1
075	3	3	1	4	3	3	1	7	9	7	1
076	3	3	3	3	3	3	3	2	2	2	2
077	1	1	5	4	3	1	5	7	5	5	7
078	1	1	3	3	1	3	3	6	7	5	7
079	4	3	3	4	4	2	3	2	2	2	2
080	1	1	2	2	1	1	1	2	2	2	1
081	3	3	3	1	1	1	1	1	1	1	1
084	4	3	4	6	4	5	5	3	6	5	3
085	4	1	1	1	1	1	1	1	1	1	1
086	1	3	3	2	3	3	7	3	5	4	3
087	1	1	1	1	1	1	3	1	1	1	1
090	1	1	1	3	1	1	1	1	1	1	1
091	1	1	1	1	1	1	1	1	1	1	1
102	3	3	3	3	3	3	3	3	3	3	3
151	3	3	3	3	3	3	3	3	3	3	3
202	5	4	5	1	3	3	5	3	1	3	3
204	9	7	7	1	8	7	5	7	7	3	3
206	1	1	1	1	1	1	1	1	1	1	1
220	5	5	5	5	5	5	5	4	5	3	7
223	3	3	3	3	3	3	3	3	3	3	2
225	1	1	1	1	1	1	1	1	1	1	1
230	1	1	1	1	1	1	1	1	1	1	1
236	1	1	1	1	1	1	1	1	1	1	1
241	3	3	3	3	3	3	3	3	3	3	3
244	1	1	1	1	1	1	1	1	1	1	1
301	3	3	3	1	3	1	3	1	3	1	3

was performed with the Statistica program (StatSoft, Inc., 1996). This program was run on a PC-Pentium personal computer.

RESULTS AND DISCUSSION

Table 4 shows the results obtained from the ampelographic study of the vines from the Germoplasm Bank (Table 1), and Figure 1 shows the dendrogram obtained from these data. In the figure, it can be observed that two clearly distinguished groups appear. The first group is formed, in turn, by two subgroups. The first subgroup is all the clones of the Parellada variety, and the second subgroup is the two clones of the Macabeo variety and that of the Viura variety, the Macabeo synonym. The

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Table 5. 0/1 Entries for Absence/Presence of Must Protein Bands Separated by Polyacrylamide Gel Electrophoresis[a]

must	electrophoretical mobility										
	0.30	0.33	0.36	0.37	0.39	0.45	0.50	0.53	0.55	0.59	0.65
PAR-G26	0	1	1	1	0	0	1	0	1	0	0
PAR-G34	0	1	1	1	0	0	1	0	1	0	0
PAR-G35	0	1	1	1	0	0	1	0	1	0	0
PAR-G48	0	0	0	1	0	0	1	0	1	0	0
MAC-H30	0	1	0	1	0	1	1	1	1	0	0
MAC-H41	0	1	0	1	0	1	1	1	0	0	0
VIU-A02	0	1	0	1	0	1	1	1	0	0	0
XAR-G28	1	1	1	1	1	1	1	1	0	1	0
XAR-H35	0	1	0	1	0	1	1	1	0	1	0
PAB-H13	0	1	1	1	1	1	1	1	0	1	0
VIÑ-H16	0	1	1	1	1	1	1	1	0	1	1

[a] For identification of the musts, see Table 1.

second group is made up of the clones of the Xarel.lo variety and those of its synonyms Viñate and Pansa Blanca.

In terms of degrees of similarity, the G26, G34, and G35 Parellada clones are very similar. The G48 Parellada clone is somewhat different from its homonyms, although related to them. From a morphological perspective, the following differences are observed: G48 lacks the tooth at the base of the petiole sinus present on the other Parellada clones, the size of the bunch is smaller, and its density is very scattered as compared to the compact bunches of the other clones. The bud of the G48 clone also bursts earlier than the other Parellada clones studied.

The H30 and H41 Macabeo clones, in the second subgroup, are similar to each other and also to the Viura clone. This grouping confirms the synonymy between the Macabeo and Viura varieties. The first referenced mention of the synonymy of these two varieties was by Martínez-Zaporta (1965). The G28 Xarel.lo clone in the second group is more related morphologically to the H13 Pansa Blanca and the H35 Xarel.lo clones, which are in turn related to the H16 Viñate clone. This grouping confirms the synonymies of Xarel.lo, Pansa Blanca, and Viñate proposed by Mestre in Marcilla (1954) and Hidalgo (1988).

The results obtained from the electrophoretic study of the total proteins in the musts of the Germoplasm Bank vine grapes (Table 1) are shown in Table 5. Figure 2 shows the electrophoretic patterns of some of these grape musts. The results of the cluster analysis of these data are shown in Figure 3. In this case also, as in the morphological study, two clear groups can be distinguished. The first one, highly differentiated from the rest, is made up of the musts of the Parellada variety clones. The second one is formed by two subgroups. In the first subgroup, the H41 and H30 Macabeo musts and the A02 Viura must form a group that can be considered as made up of highly related clones. Also related to these is the H35 Xarel.lo clone must. In the morphological study, this clone was correctly grouped with the other Xarel.lo variety clone and its synonyms. In the electrophoregram of the H35 Xarel.lo clone must proteins, mobility bands 0.36 and 0.39 do not appear (Table 5), although they are present in the G28 Xarel.lo and its synonyms Pansa Blanca and Viñate. These bands do not appear in the musts of the Macabeo variety studied either. The second subgroup is formed by the G28 Xarel.lo must and its synonyms H12 Pansa Blanca and H16 Viñate.

Varietal identification of grape musts on the basis of PAGE electrophoretic total protein data had already
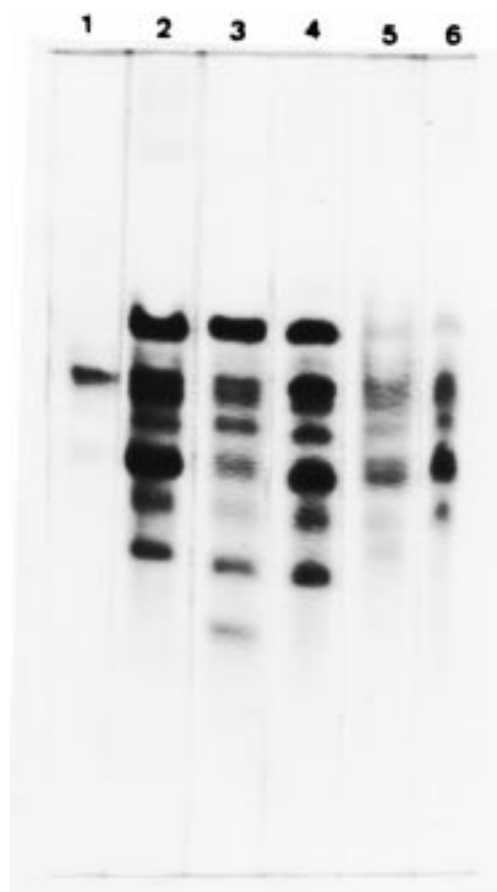

Figure 2. PAGE profiles obtained for proteins from the grape musts. Lane 1, PAR-G26; lane 2, PAB-H13; lane 3, VIÑ-H16; lane 4, XAR-G28; lane 5, MAC-H30; lane 6, VIU-A02.

been performed in previous studies with favorable results (González-Lara et al., 1989; Pueyo et al., 1993). Electrophoretic analysis of total grape must proteins is a simple, less subjective determination than descriptive methods. To corroborate its validity for varietal identification and to rule out the influence of the clones, we performed it on samples of monovarietal musts originating from a mixture of grapes from a large number of vines. The varieties analyzed, the area in which the vineyards were located, and the year of harvest are shown in Table 2. Table 6 shows the data obtained from electrophoretic analysis of these samples.

The dendrogram in Figure 4 was obtained by performing a cluster analysis on the data of Tables 5 and 6. Two groups can be observed: a smaller one made up of all the Parellada variety musts, and another that is,
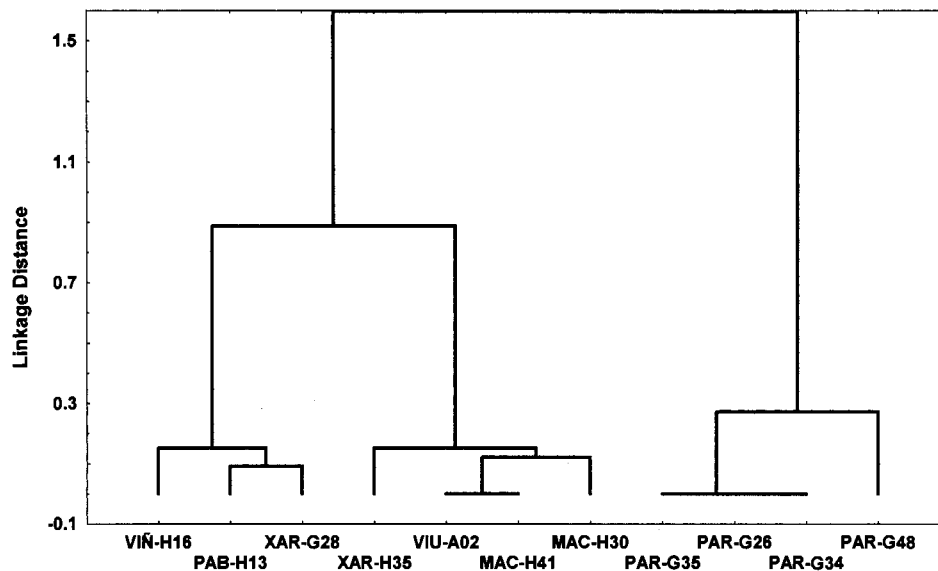
Figure 3. Dendrogram of the Germoplasm Bank cultivars, obtained from the electrophoretic study of the total proteins (see Table 5).

Table 6. 0/1 Entries for Absence/Presence of Must Protein Bands Separated by Polyacrylamide Gel Electrophoresis[a]

	electrophoretical mobility										
must	0.30	0.33	0.36	0.37	0.39	0.45	0.50	0.53	0.55	0.59	0.65
PAR G01	0	0	1	1	0	0	1	0	1	0	0
PAR-E01	0	1	1	1	0	0	1	0	1	0	0
PAR-E02	0	1	1	1	0	0	1	0	1	0	0
PAR-E03	0	1	1	1	0	0	1	0	1	0	0
PAR-V01	0	1	1	1	0	0	1	0	1	0	0
PAR-V02	0	1	1	1	0	0	1	0	1	0	0
PAR-V03	0	1	1	1	0	0	1	0	1	0	0
MAC-G01	0	1	0	1	0	1	1	1	0	0	0
MAC-G02	0	1	0	1	0	1	1	1	0	0	0
MAC-E01	0	1	0	1	0	1	1	1	0	0	0
MAC-E02	0	1	0	1	0	1	1	1	0	0	0
MAC-E03	0	1	0	1	0	1	1	1	0	0	0
MAC-E04	0	1	0	1	0	1	1	1	0	0	0
MAC-E05	0	1	0	1	0	1	1	1	0	0	0
MAC-E06	0	1	0	1	0	1	1	1	0	0	0
MAC-V01	0	1	0	1	0	1	1	1	0	0	0
MAC-V02	0	1	0	1	0	1	1	1	0	0	0
MAC-V03	0	1	0	1	0	1	1	1	0	0	0
VIU-516	0	1	0	1	0	1	1	1	0	0	0
XAR-G01	0	1	0	1	0	1	1	1	0	1	0
XAR-G02	0	1	0	1	0	1	1	1	0	1	0
XAR-E01	0	1	0	1	0	1	1	1	0	1	0
XAR-E02	0	1	0	1	0	1	1	1	0	1	0
XAR-E03	0	1	0	1	0	1	1	1	0	1	0
XAR-E04	0	1	0	1	0	1	1	1	0	1	0
XAR-E05	0	1	0	1	0	1	1	1	0	1	0
XAR-E06	0	1	0	1	0	1	1	1	0	1	0
XAR-V01	0	1	0	1	0	1	1	1	0	1	0
XAR-V02	0	1	0	1	0	1	1	1	0	1	0
XAR-V03	0	1	0	1	0	1	1	1	0	1	0

[a] For identification of the musts, see Tables 1 and 2.

in turn, divided into two subgroups—one of them corresponding to the musts of the Macabeo variety and its synonym Viura and the other one to those of the Xarel.lo varieties and its synonyms Pansa Blanca and Viñate. De la Presa-Owens et al. (1995) have also studied samples of Macabeo, Xarel.lo, and Parellada variety musts from El Penedès by analyzing their phenolic fraction and have also found two groups, the Parellada variety musts and all of the other musts.

In the Parellada variety group (Figure 4), all the musts form a group with a significance level of zero, except G48 and G01, which are linked in the figure and

slightly separated from the other musts of this variety. In the Macabeo and Viura varieties, all the musts are similar, with a significance level of zero, except H30 Macabeo. The greatest differences occur in the Xarel.lo variety. The musts originating from the large vineyards and the H35 must from the vine Germoplasm Bank of "El Encín" form a group with a significance level of zero. The Pansa Blanca variety must and those of H16 Viñate and G28 Xarel.lo are highly related to each other but separated from the other Xarel.lo variety musts.

In the cluster analysis of the proteins in the musts made from the Germoplasm Bank vine grapes (Figure
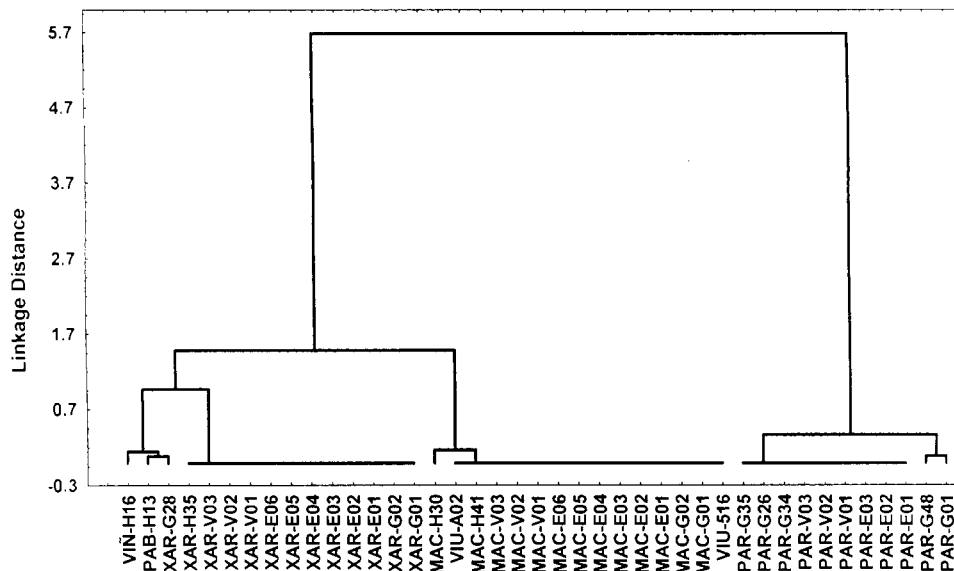
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Figure 4. Dendrogram of the 41 musts studied obtained from the electrophoretic data of the total proteins (see Tables 5 and 6).

3), the H35 Xarel.lo clone must differs slightly from that of G28 Xarel.lo and the Viñate and Pansa Blanca variety musts and so is grouped with the Macabeo variety musts. When a higher number of samples are used (Figure 4), it is grouped with the musts of its own variety.

Electrophoretic analysis of total grape must proteins is an objective, simple technique that can be performed routinely by an inexperienced analyst. The results obtained in this study as a whole confirm that with this technique it is possible to ascertain, in the majority of cases, the grape variety from which the musts originate and to reveal that the technique can be used as a complement to classical morphological descriptions for varietal characterization of vines. It is advisable that at least one reference sample should be included on the same plate on which the electrophoretic analysis of the unknown sample is performed. It is also recommended that, when performing the cluster analysis, the data of the unknown sample should be introduced together with those of other musts of the variety to which the sample is tentatively attributed.

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