Research on the meaning of the enzymatic systems (GPI and PGM) as parameters for the definition of varieties (*Vitis* sp.): The Italian case of Cabernet franc

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Summary: Several studies carried out at Davis and Conegliano showed that isozyme analysis of the GPI and PGM enzymatic systems agrees with the conventional definition of the variety in ampelography.

Differences were reported among varieties but not among biotypes of the same varieties. The only exception recorded was in the population of Cabernet franc in which GPI and PGM reveal two different types (A – the traditional type encountered in France and B – type encountered in the Italian region of Veneto).

Further ampelographic, ampelometric, phenological and chemical studies on the polyphenolic and aromatic substances in fruits have shown considerable differences between the two types. Such differences demonstrate that the type B is a different variety and not a clone of Cabernet franc. Preliminary ampelographic analysis and the equality in GPI and PGM patterns lead to the conclusion that the type B very probably is Carmenère.

Therefore, the hypothesis of variety discrimination based on the analysis of GPI and PGM is valid and this method is useful to help to characterize the varieties.

Key words: variety of vine, biotype, Italy, ampelography, biometry, analysis, morphology, leaf, berry, enzyme, pyrazine, polyphenol, phenol.

Introduction

Generally, ampelography provides the definition of vine varieties according to morphological, phenological and chemical features that may differ in more or less significant ways. Conventionally, when the basic morphological features of a variety's population are the same, clones are distinguished according to the genotypical variations of the physiological and/or phenological and/or chemical features.

Studies carried out at Davis and Conegliano over a 2-year period (1987-1988), at first on 225 varieties and subsequently on 9 variety populations and 63 clones of these (8), showed that isozyme analysis of the GPI and PGM enzymatic systems by starch gel electrophoresis of leaf extracts agrees with the conventional definition of the variety in ampelography.

Differences were reported among varieties but not among biotypes of the same varieties, probably because the genes of which these enzymes are the main expression, did not cause any significant variations of the morphological, physiological and chemical features, practically and conventionally considered in the variety's definition.

So far, the only exception recorded was the population of Cabernet franc, widely employed in Italy, in which the GPI and PGM reveal two different types, i. e. A – the traditional type encountered in France and B – the type mainly encountered in the Italian region of Veneto. The aforementioned types have significantly different features requiring further ampelographic, ampelometric, phenological and chemical studies on the polyphenolic and aromatic substances in fruits in order to provide objective differences that the operative world is also suggesting.

The Cabernet varieties seem to date back to the Biturica mentioned by Plinius and Columella, in relation to the ancient synonyms Viduve and Vidure, according to Petit-Lafitte (who relied on the authority of Vinet, an expert of the 18th century). Although impossible to prove, it is quite evident that the varieties date back to a very early age, a fact of which trustworthy evidence is found in Gironde, where Cabernet franc was well-known at the times of the Cardinal of Richelieu.

During the diffusion of these varieties, started in the 17th century, several populations spread out with different synonymities or distinctions, still existing at the present time. The distinctions involved varieties, i. e. Cabernet franc, Cabernet Sauvignon, Carmenère and then a series of synonyms recalled by the different ampelographies (14, 15, 17, 20). It may be worth underlining the fact that in the 19th century, the ampelographical expert, Count ODART, mistook Cabernet franc and Cabernet Sauvignon, since the latter was still not very well-known in French regions other than Gironde.

In Italy (10, 11, 16), apparently Cabernet franc was imported for the first time by the Count of SAMBUY in Piedmont at the beginning of the 19th century. By the end of the century, the variety was cultivated in approximately 45 provinces, from Piedmont to Southern Italy. Noteworthy cultivations were present in Veneto, where the variety had come directly from France through BORTOLO CLEMENTI, the Count of SCHIO, Count CORINALDI and other vine-growers.

The origins of the vines and thus of the populations were naturally diverse, as still can be noticed in some vineyards propagated with this material.

The Istituto Sperimentale per la Viticoltura has been performing selections since 1980 on the above-mentioned populations that led to the identification and now to the characterization of the types examined in this report.

Materials and methods

Research was conducted in 1987-1988 by the Istituto Sperimentale per la Viticoltura in two fields situated on the Eastern Venetian plain in entirely different pedoclimatic conditions. In each field of comparison the two types of Cabernet franc were represented by 24 vines per biotype with 6 repetitions each.

Examination involved the following features:

1. Biochemical features

The starch gel electrophoretic analysis of leaf extracts was performed for the enzymatic systems glucose phosphate isomerase (GPI) and phosphoglucomutase (PGM). The methods and procedure employed were those previously reported (3, 18).

2. Morphological features

Testing of these features was performed according to the instructions of the international O.I.V. descriptor list. Features are described in Table 1. The following informations were also recorded:

- phenological ages considering the average time of the phenomenon's appearance;
- potential and actual fertility of the buds along the fruit bearing tendrils (7).

3. Ampelometric measurements

These tests analyze the features provided by measurements and the relations that define the leaf, in particular:

- ratios between veins L_1/L, L_2/L_1
- depth of petiole sinus S_1/L_1, S_2/L_2
- angles between veins: α between L and L_1, β between L_1 and L_2, γ between L_2 and L_3
- leaf length/width
- petiole/leaf length

Measurements were performed on adult leaves selected in summer from the 5th to the 8th node of the main shoots.

The following grape bunch characteristics were also considered:

- berry weight in g
- berry number per bunch
- bery volume in ml
- petiole length in cm
- berry dimensions (length and width) in cm
- bunch size (width and length) in cm

The data were gathered upon ripening from 10 bunches and 100 grapes.

4. Berry samples were taken weekly from veraison to ripening to establish the following juice components:
   - total acidity (g/l)
   - malic acid (g/l)
   - pH
   - tartaric acid (g/l)
   - sugar (% ml)
   - potassium (g/l)

5. Volatile berry components and berry skin phenolic components

5.1. Berry pyrazines

1 kg of berries were homogenized and steam distilled to establish the amount of pyrazines. The distillate was extracted 3 times with 25 ml of CH_2Cl_2. After solvent distillation, the extract was analyzed by gas chromatography-mass spectrometry by means of the S.I.M. programme with acquisition of ions 123-138, 124-137, 124-151, respectively, related to 2-ethyl-3-methoxy pyrazine, 2-isopropyl-3-methoxy pyrazine, 2-isobutyl-3-methoxy pyrazine.

5.2. Volatile juice components

250 ml of juice was extracted with C_5H_{12} : CH_2Cl_2 in a 60 : 40 ratio for 12 h. After the solvent's evaporation by distillation, the extract was subjected to gas chromatography.

5.3. Phenolic berry skin components

Anthocyanins: These were extracted from 10 berries with ethanol + 0.1 % of concentrated HCl and with mixture ethanol : H_2O : concentrated HCl in the ratio 70 : 30 : 1.

Phenolic acids: The ethanol extract of skins was employed for the analysis of the hydroxy cinnamoyl, tartaric acids.

6. Organoleptic tests were performed on wines produced by the microvinification of the two types of Cabernet franc and tasting the grapes by means of the statistical duo-trio test.

Results and discussion

1. Isoenzymatic analysis

As may be seen in Fig. 1, the two types of Cabernet franc have different GPI and PGM patterns.

2. Morphological features

Of all the features examined (see Table 1), the following proved to be repeatedly different and steady in the two types of Cabernet franc (Table 2).

Type B has higher anthocyanic pigmentation levels in the shoot tip, more blisters on the upper leaf side, a larger amount of hairs between veins on lower leaf side and a looser cluster. These features will be emphasized in further detail by analyzing the ampelometric tests.

Moreover, a particular feature is the special shape of stamen that may be spiral-shaped in type B, as previously illustrated (7) on this population (see Fig. 10).

3. Ampelometric tests

Table 3 illustrates the features that proved to be significantly different among those examined.

The analysis of the results concerning the leaves shows that the dimension of the apex lobe determined by the ratios L_1/L and S_1/L_1 are major in type A than in type B.

With regard to the bunch, type B, as already mentioned, is looser (longer bunch, fewer berries and less compact) and it has larger berries (size and volume).

4. Berry juice components

The course of sugar development in the berries, of the degradation of total tartaric and malic acidity, and of the pH increase does not differ considerably from one type to the other (Fig. 2).

Type B, however, ripens earlier and thus it attains higher sugar in berries earlier and, at the same time, has a significant degradation of total acidity, related to the decrease of both malic and tartaric acid, whereas the pH is higher.

In the juice, there is also a considerable difference in potassium concentrations: higher in type A, an aspect that is probably interesting in enological terms.

Table 1: Morphological features examined

O.I.V. CODE	FEATURE
001	young shoot: form of tip
003	" " : intensity of anthocyanin coloration of tip
004/005	" " : hair density ot tips
007/008	shoot: color of internodes
009/010	" : color of nodes
011/013	" : density of hairs of nodes
068	mature leaf: number of lobes
075	" " : blistering of upper side
076	" " : shape of teeth
079	" " : general shape of petiole sinus
081	" " : particularities of petiole sinus
084/085	" " : density of hairs between the veins (lower side)
088/089	" " : density of hairs on main veins (upper side)
090/091	" " : density of hairs on petiole
151	inflorescence: sex of flower
153	" : number of inflorescence per shoot
204	bunch: density

Table 2: Features proved to be repeatedly different and steady

OIV CODE	FEATURE	EXPRESSION LEVEL	
		TYPE A	TYPE B
003	young shoot: intensity of anthocyanin coloration of tip	3	5
075	mature leaf: blistering of upper side	5	7
084	" " : density of hairs between the veins (lower side)	5	7
204	bunch : density	6,65	5,42

Table 3: Ampelometric measurements proved to be significantly different among those examined

FEATURE	CABERNET FRANC TYPE A	CABERNET FRANC TYPE B
L_1/L (in cm)	0,893	0,842
S_1/L_1 (in cm)	0,544	0,497
S_2/L_2 (in cm)	0,628	0,591
α (in degrees)	48,62	53,5
β (in degrees)	51,52	55,27
γ (in degrees)	48,32	53,25
berry weight (in grammes)	1,54	1,90
berry volume (in ml)	1,39	1,67
berry length (in cm)	1,43	1,52
berry width (in cm)	1,33	1,45
average number of grapes	99,44	85,32
bunch length (in cm)	12,26	13,04

5. Volatile grape components and berry skin phenolic components

5.1. Berry pyrazines

The two types (A and B) differ in the 124 ion chromatogram profile and partly the 151 ion profile, related to 2-isobutyl-3-methoxy pyrazine, the content of which seems to be considerably higher in type B (Fig. 3). The presence of a great number of peaks leads to the conclusion that the preparation system generates adulterations.

5.2. Volatile berry juice components

The profiles of the samples are practically similar (Fig. 4). There are C_6 components originated by the enzymatic attack of linoleic and linolenic acids, besides other unidentified components and a considerable amount of benzylic alcohol.

5.3. Phenolic berry skin components

Anthocyanins: The analysis of the chromatogram HPLC of the anthocyanins (Fig. 5 and 6) revealed that the two types of Cabernet franc differ in the percentage of peonidin monoglucoside (higher in type B), acetates (higher in type B), and p-cumarates (higher in type A). The anthocyanins of both varieties are trisubstituted. The amount of anthocyanins in the skins of type B is approximately double the amount of type A (Table 4).

Table 4: Anthocyanins %

ANTHOCYANINS	CABERNET FRANC TYPE A	CABERNET FRANC TYPE B
Delphinidin monoglucoside	6,57	6,02
Cyanidin monoglucoside	0,67	1,37
Petunidin monoglucoside	6,96	5,96
Peonidin monoglucoside	3,66	10,84
Malvidin monoglucoside	41,45	38,77
Delphinidin monoglucoside acetate	1,50	1,63
Cyanidin monoglucoside acetate	0,11	0,31
Peonidin monoglucoside + Malvidin monoglucoside acetate	12,99	20,58
Delphinidin monoglucoside p.Cumarate	1,48	0,87
Cyanidin monoglucoside p.Cumarate	0,06	0,07
Petunidin monoglucoside p.Cumarate	1,83	0,51
Peonidin monoglucoside + Malvidin monoglucoside p.Cumarate	21,16	11,28

Table 5: Differences between type B of Cabernet franc and type A

Morphological differences

1. Major anthocyanic pigmentation of shoot tip
2. Major blistering of adult leaf upper side
3. Major presence of hairs between veins of adult leaf lower side
4. Spiral shaped flower stamen
5. Minor bunch compactness

Ampelometric

1. Lower ratio L_1/L
   Lower ratio S_1/L_1
   Lower ratio S_2/L_2
2. Major angle α (between L and L_1)
   " " β (between L_1 and L_2)
   " " γ (between L_2 and L_3)
3. Looser berry bunch:
   * longer bunch
   * lower number of berries
4. Major berry dimension:
   * size
   * weight
   * volume

Biochemical and chemical

1. Different isoenzymes for enzyme systems GPI and PGM
2. Grape pyrazines: higher content of 2-isobutyl-3methoxy pyrazine
3. Skin anthocyanins: higher content of peonidin monoglucoside
   higher content of acetates
   lower content of p-cumarate
   double quantity of total anthocyanins
4. Phenolic acids: higher content

Organoleptic

Clear identification of the two types and a more herbaceous taste in type B.

Phenolic acids: The chromatogram at 320 nm shows that type B has a higher incidence of these components. The same test performed at 280 nm (Fig. 7 and 8) shows that type B contains a large amount of an unidentified component with a characteristic spectrum (Fig. 9), of which there are no traces in type A or in wine.

6. Organoleptic tests

Continuous organoleptic research on wines has allowed the preparation of a merit list in which Cabernet franc type B appears to be more appreciated and with a specific individuality. Furthermore, grape tasting performed by means of the duo-trio test indicated that the two types may be distinguished quite clearly (80 %), with a major mark of the herbaceous taste of type B.

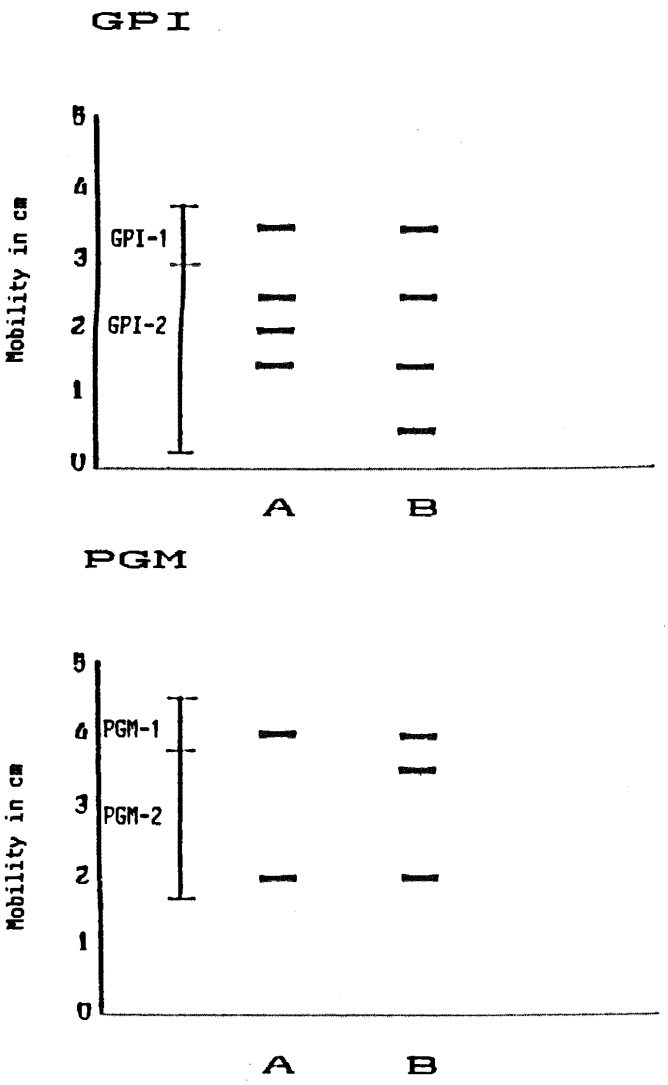


Fig. 1: GPI and PGM patterns in Cabernet franc type A (A) and type B (B).

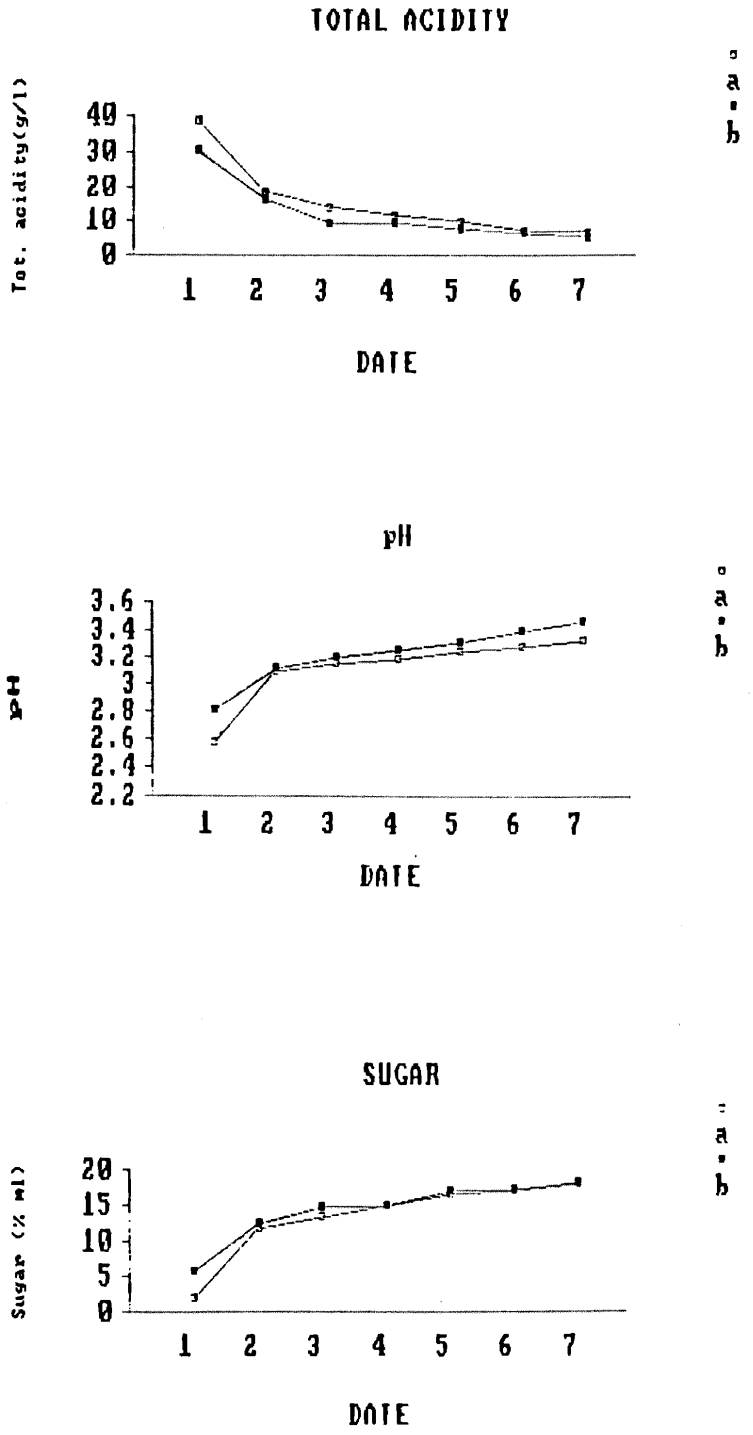
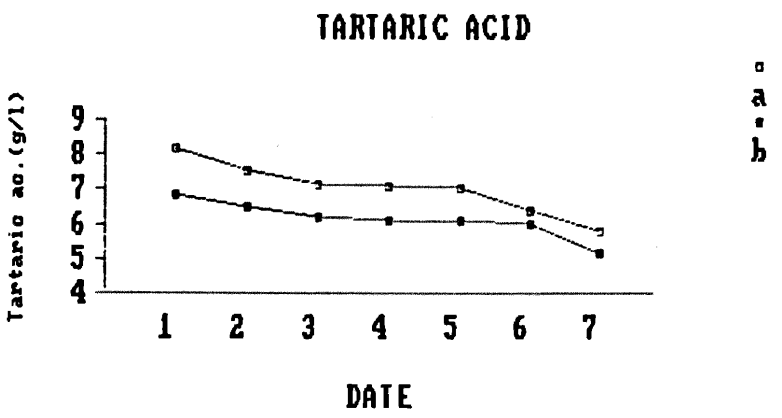
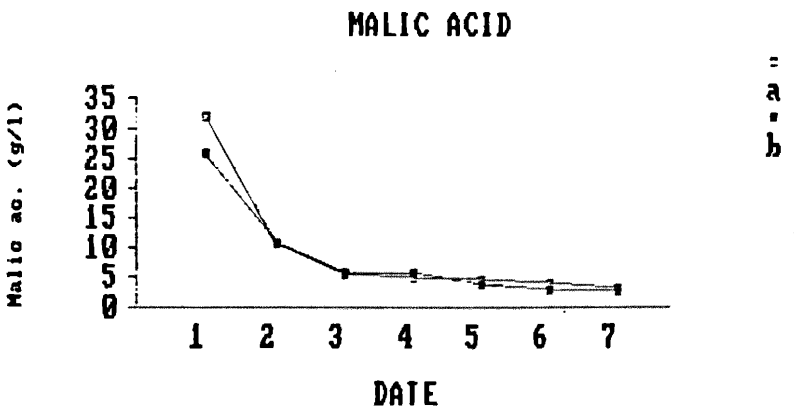


Fig. 2: Berry components of Cabernet franc type A (A) and type B (B). (Continued overleaf.)





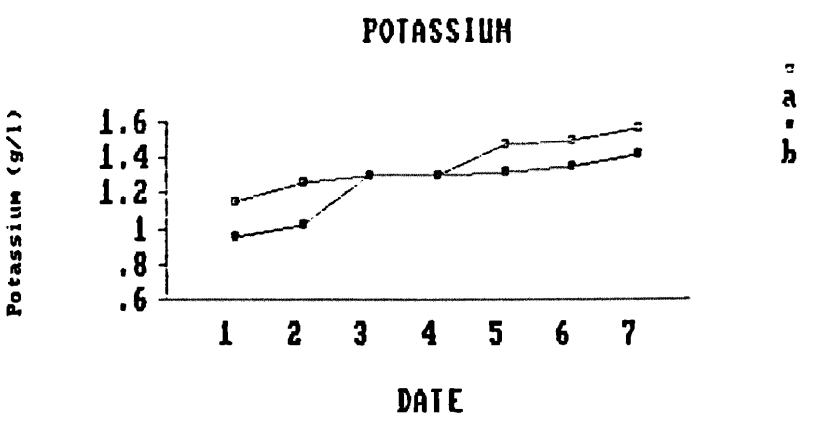


Fig. 2 (continued).

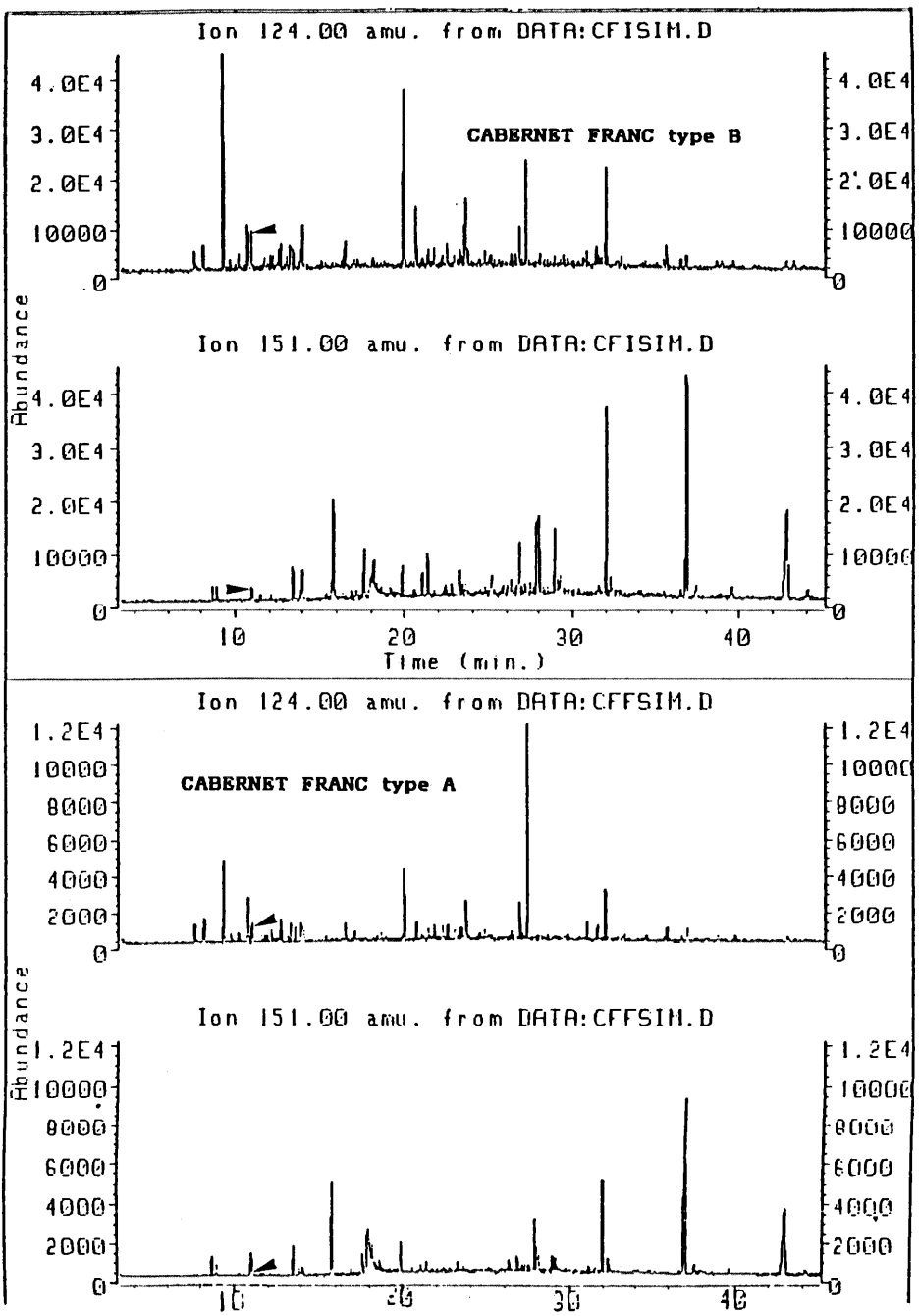


Fig. 3: Ion chromatogram of the CH_2Cl_2 extract of the homogenizated grapes. ◄ = Ions 124 and 151 are the ones related to 2-isobutyl-3-methoxy pyrazine.

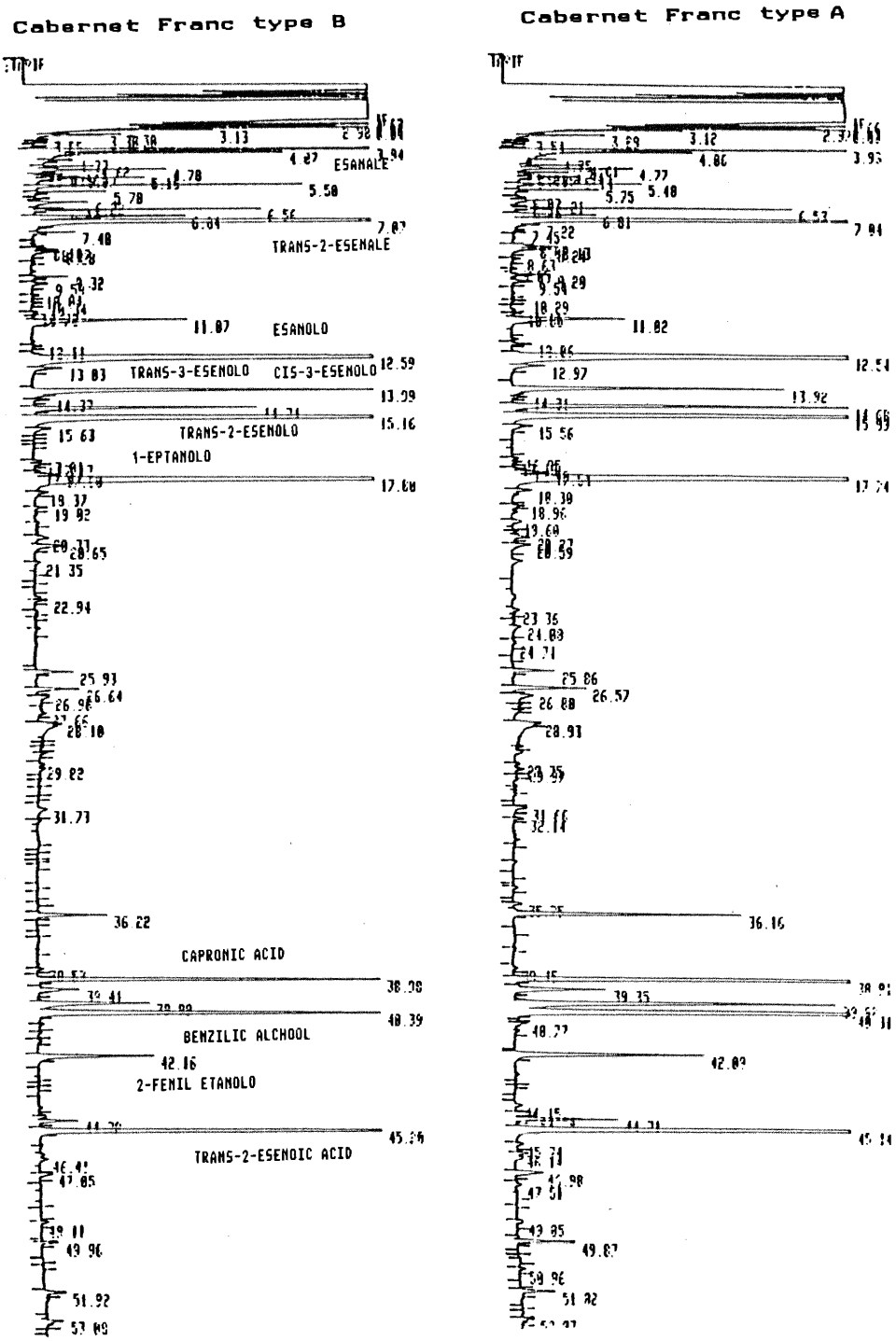


Fig. 4: Chromatogram of grape juice volatile components.

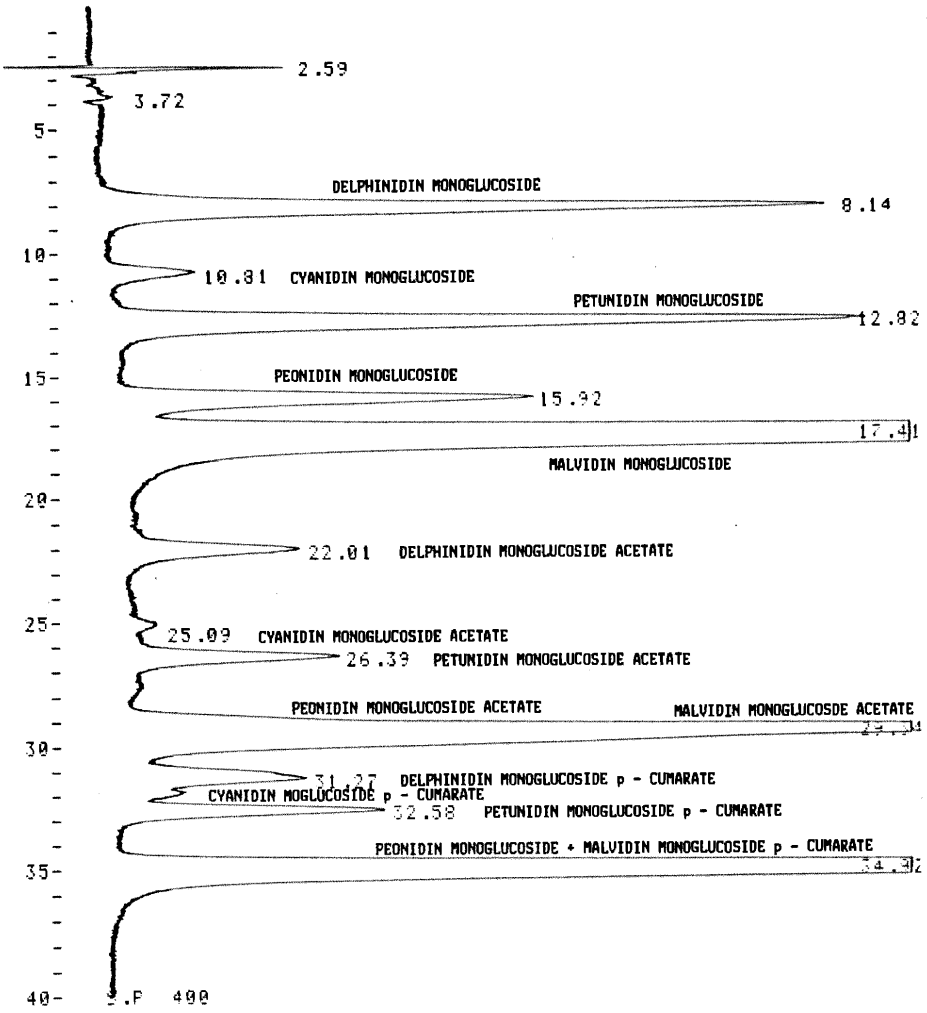


Fig. 5: HPLC chromatogram of the anthocyanins of the skins of Cabernet franc type A. $\lambda = 520$ nm.

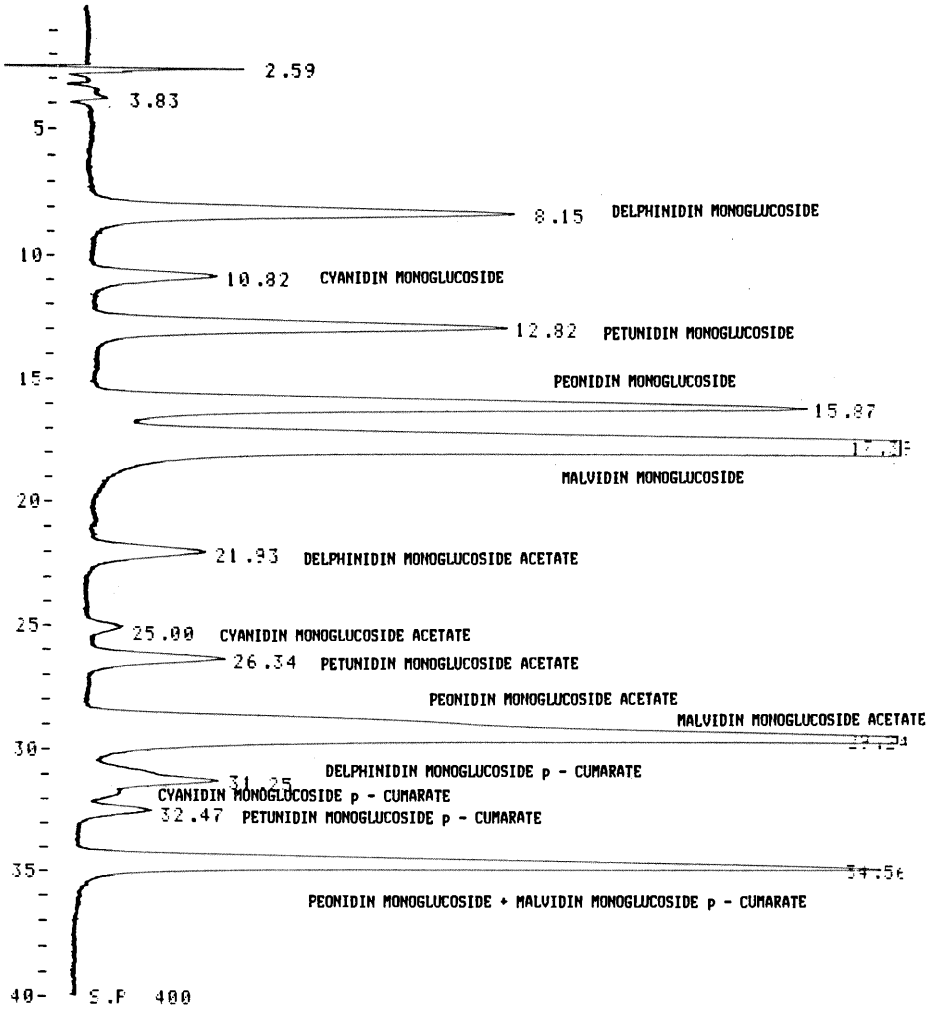


Fig. 6: HPLC chromatogram of the anthocyanins of the skins of Cabernet franc type B. $\lambda = 520$ nm.

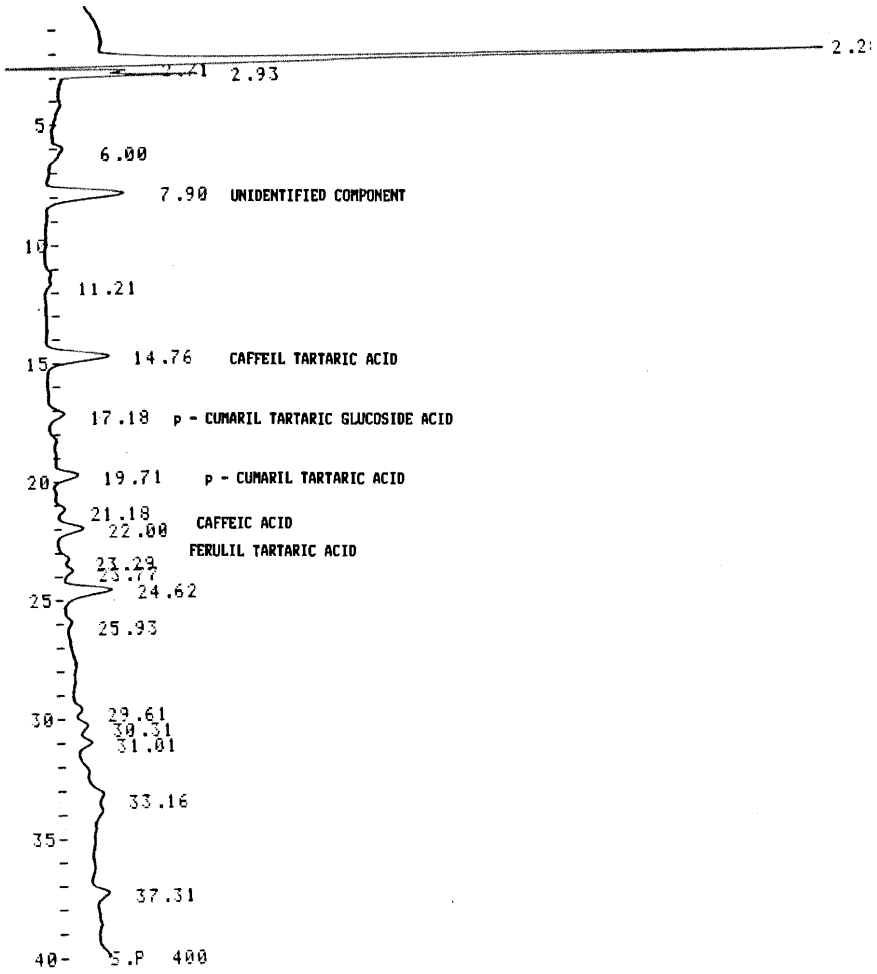


Fig. 7: HPLC Chromatogram of the phenolic acids of the skins of Cabernet franc type A. λ = 280 nm.

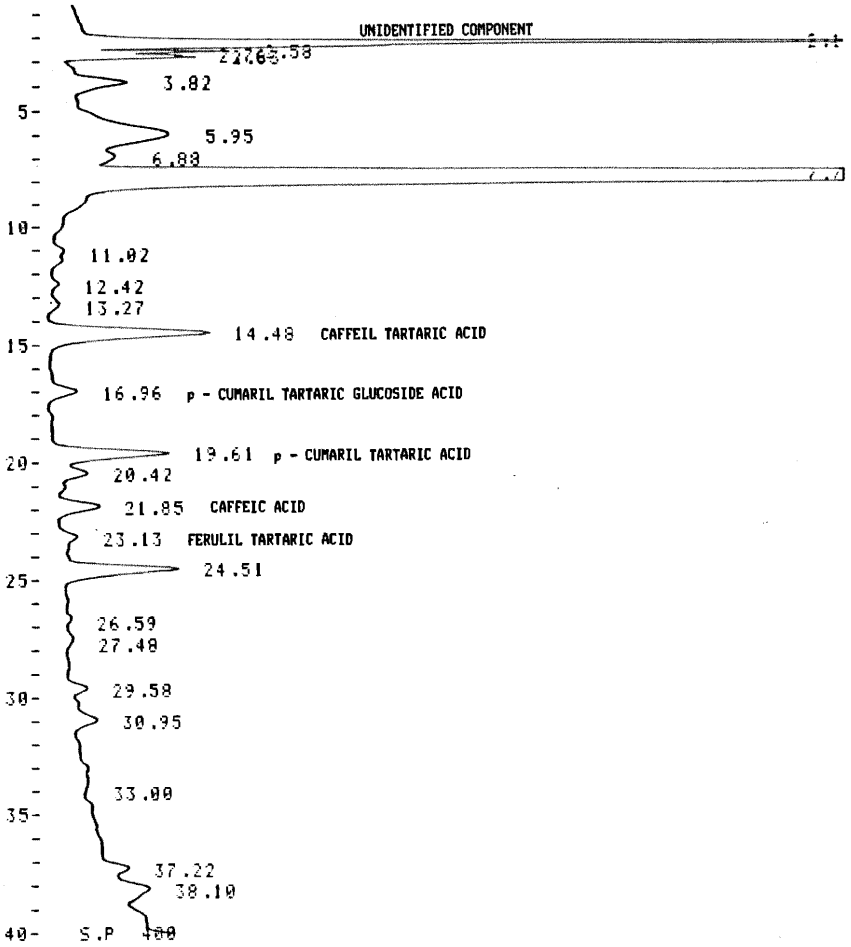


Fig. 8: HPLC chromatogram of the phenolic acids of the skins of Cabernet franc type B. $\lambda = 280$ nm.

Conclusions

A series of studies carried out on different varieties and on clones of the same varieties proved that isozyme analysis of the GPI and PGM enzymatic systems could be used as a discriminating factor when identifying varieties since different patterns may occur between varieties but not between the clones of the variety.

However, the population of Cabernet franc employed in Italy exhibited two different types (A and B) for GPI and PGM.

In this report the aforementioned types were carefully analysed for several morphological, ampelometric and chemical features. Considerable differences were recorded between type B (mainly diffused in Veneto) and type A (the traditional type popular in France). Such differences, illustrated in Table 5, led to the conclusion that it is a different variety and not a clone of Cabernet franc.

Type B is very probably Carmenère, whose population spread in Veneto since last century and has been frequently confused with Cabernet franc. This hypothesis is confirmed by preliminar ampelographic analysis as well as the equality in GPI and PGM patterns and it will be moreover examinated with further checks.

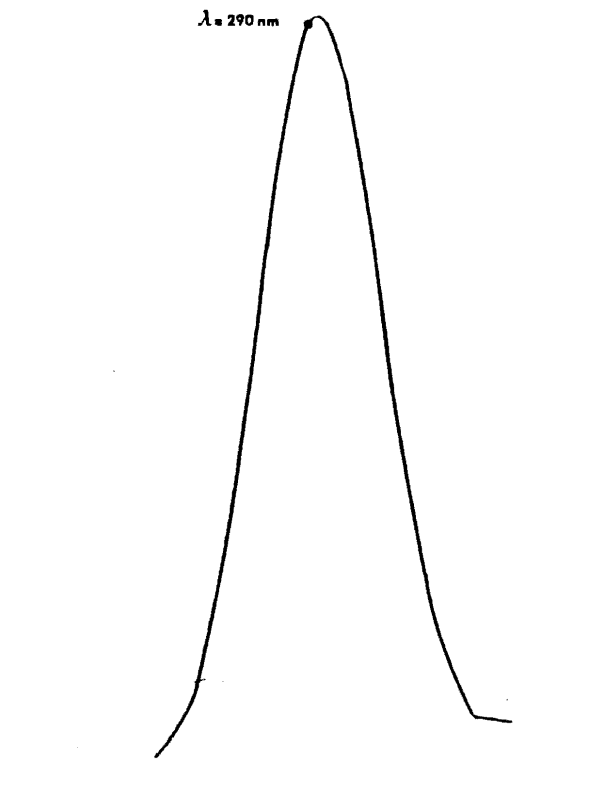


Fig. 9: Spectrum of the unidentified component present in the skins of Cabernet franc type B.

Fig. 10: Shape of the stamen in the two populations of Cabernet franc. Left: stamen of type A; right: spiral-shaped stamen of type B.

Therefore, the hypothesis of a variety discrimination based on the analysis of GPI and PGM is basically valid and thus the method is presently useful to help to characterize the varieties and in future will be suitable for the definition.

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Evaluation and utilization of *Vitis riparia* as a source of genes for extreme cold hardiness

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Abstract: The goal of the University of Minnesota grape breeding program is to develop high quality wine and table cultivars that tolerate winter temperatures as low as -35 °C and ripen in a relatively cool, short growing season. Northern clones of *Vitis riparia* have been collected, evaluated and used in the breeding program. Genotypes of *V. riparia* from the northern portion of the species range experience winter temperatures of -40 °C or lower. In addition to extreme cold hardiness, these genotypes also exhibit early ripening, high sugar levels, and resistance to powdery mildew and phylloxera. Deleterious traits of many *V. riparia* clones include high acidity, small cluster and berry size, very dark juice, strong characteristic flavor, and excessive vegetative growth.

Over 100 *V. riparia* clones have been selected and evaluated in Minnesota over the past decade. These clones exhibit substantial variability for the viticultural traits mentioned above. For example, sugar levels typically range from 23 to 28 °Brix; titratable acidity ranges from under 2 % to over 4 %; juice color varies from very dark purple to light red; time of leaf senescence can vary by 2 to 3 weeks. Controlled environment studies indicate that early acclimation in these northern clones is photoperiod-induced.

Superior clones crossed with *V. vinifera* and interspecific hybrid cultivars produced selections in the F_1 and subsequent generations that were viticulturally and enologically acceptable.

Some of these selections have suffered minimal injury after experiencing midwinter temperatures as low as -35 °C, both in the vineyard and in laboratory freezing studies.