Recent developments in biochemical characterization of *Vitis vinifera* L. varieties in Hungary

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Summary: Isoelectric focusing is an effective and well reproducible method to provide information for identification of various plant species and clones if breeding or other genetic modification(s) for a given species are reflected in changes of an isozyme pattern. The method has been used for characterization of plant proteins and enzymes and for identification of various species and varieties. Our aim was to continue our several-year-work carried out on a wide variety of grapevine varieties and to reveal whether analyses of esterase and peroxidase isozyme patterns are suitable to distinguish various grapevine varieties. Therefore we compared esterase and peroxidase isozyme patterns of various species. Plant samples were obtained from Szigetcsép, Kecskemét, Tokaj and Eger. The following samples were analyzed: Pinot gris, noir, blanc, Chardonnay, Riesling Theses, Chasselas from Szigetcsép, Bianca and his parents Eger2 and Bouvier from Kecskemét, Furmint and Hárslevelű from Tokaj, Kékfrankos and Zweigelt from Eger. To identify various species according to their esterase isozyme patterns the after blooming phenological phase while according to their peroxidase isozyme pattern the dormant phenological phase was found as optimal sampling time.

Key words: identification of grapevine varieties, esterase isozymes, peroxidase, isozymes, isoelectric focusing

Introduction

It has been known for a long time that the morphological characters and the chemical compositions of different plant species are affected by many factors including environment, age, physiological state of plants, etc. Therefore, to evaluate the real taxonomic relations of plants one has to supplement morphological methods by more sophisticated ones.

Plant analysis at protein level combined with genetics can provide information for selection of plants having desired characteristics. Although plants contain a lot of various proteins only a few of them are targets of genetic modifications. Since the protein patterns are characteristic for plant taxa *(Tanksley* 1983, *Helentjaris* et al. 1988), this information combined with genetics may serve as a tool for selection of plants having desired features. Nowadays different analytical methods are used for identification of different species and for qualitative control *(Bergmann* 1987, *Patterson and Payne* 1989).

In viticulture like in any horticultural or agricultural production there is an increasing demand for improving quality of cultivated varieties. That demand in turn evokes the use and development of up-to-date taxonomic methods to guarantee the quantitative and qualitative controls of different varieties to help breeding, plant biology, population genetics and quality assurance in processing.

Ge lelectrophoresis or isoelectric focusing are effective and well reproducible methods, applicable to provide information for identification of different species and clones if breeding or other genetic modification(s) are reflected in changes of an isozyme pattern of a given species. Those methods have been used for species identification in viticulture too. Different parts – berries *(Wade* 1976), leaves *(Kalchgruber* 1994, *Martinelli* 1993), seeds *(Gianazza* 1989, *Scienca* 1994), shoot tips *(Walters* 1989), phloem of dormant canes *(Bachmann* 1994), etc. – of grapevine and different enzymes – esterase *(Wade* 1976, *Gianazza* 1989, *Martinelli* 1993) and peroxidase *(Wade* 1976, *Gianazza* 1989, *Bachmann* 1994) – were analyzed to discriminate cultivars or clones.

The aim of our several-year-work was to determine characteristic differences in esterase and peroxidase isozyme patterns of some grapevine species by isoelectric focusing. Our previous experiments were complemented with analyses and comparison of several Hungarian grapevine varieties

from the different parts of Hungarian area, and those of the same species collected from different areas.

Material and method

Plant samples were obtained from Szigetcsép and Kecskemét, cultivated in sandy soil where the growing conditions were the same, and from Tokaj and Eger, where the plants were grown in mountain region. The following *Vitis vinifera* L. samples were analysed: Pinot gris, noir, blanc, Chardonnay, Riesling Theses, Chasselas from Szigetcsép, Bianca and his parents Eger2 and Bouvier from Kecskemét, Furmint and Hárslevelű from Tokaj, Kékfrankos and Zweigelt from Eger.

Plant samples were collected at the same phenological phase. The youngest leaves were analyzed after blooming and at the fruit set. In all cases esterase and peroxidase isozyme patterns were determined by isoelectric focusing (IEF).

Sample preparation and isoelectric focusing were carried out as described earlier *(Stefanovits-Bányai* et. al 1998).

Results and discussion

In our previous work *(Stefanovits-Bányai* et. al 1998) the various species were compared according to their esterase isozyme patterns obtained at after sprouting phenological phase. However, certain well detectable differences were found among various species in our recent work. In order to refine resolution of differentiation the blooming phenological phase was used as sampling time.

Our results are summarized in *Figs. 1–5*. Esterase isozyme patterns are presented only after blooming, when the best characteristic differences in the isozyme patterns were obtained.

Esterase isozyme patterns of Pinot gris, Chardonnay, Riesling Theses and Chasselas obtained by isoelectric focusing are shown in *Fig. 1*. We have found both qualitative and quantitative differences among different varieties collected after blooming. The characteristic isozyme bands can be found not only in the basic pH range (pH ≅ 8.0–8.5) but in the neutral (pH ≅6.0) and the acidic (pH ≅ 4.0–5.0) pH ranges too.

In the basic pH range differences in the esterase isozymes of Pinot gris, Chardonnay and Riesling Theses were found while a very close relation was found between Pinot gris and Chasselas. In the neutral pH range Pinot gris, Chardonnay and Riesling Theses exhibited similar isozyme patterns.

When we compared the esterase isozyme patterns of interspecific hybrid Bianca and its parents Eger2 and Bouvier we have found characteristic bands both in the basic (pH ≅ 8.0–9.0) and acidic (pH ≅ 4.5–5.5) pH ranges *(Fig. 2)*. The esterase isozyme pattern of interspecific hybrid Bianca is similar to Eger2 in the acidic and neutral pH ranges, but in the basic range it is a hybrid of its parents'.

We compared the esterase isozyme patterns of Pinot gris, Pinot noir and Pinot blanc samples collected after blooming from the same cultivation area Szigetcsép. The results are shown in *Fig. 3*. It is worthwhile mentioning that characteristic esterase isozyme fractions were the same in all the three samples.

Figure 4 shows the esterase isozyme patterns of Furmint and Hárslevelű collected after blooming from another part of Hungary, where the grapevines are growing in mountainside of Tokaj. The two characteristic species of *Vitis vinifera* L. of this area can well be distinguished on the basis of their esterase isozyme patterns. Furmint samples have more isozyme fraction than the Hárslevelű ones both in the basic (pH ≅ 8.0–9.0), neutral (pH ≅ 6.0–7.0) and the acidic (pH ≅ 4.5–5.5) pH ranges.

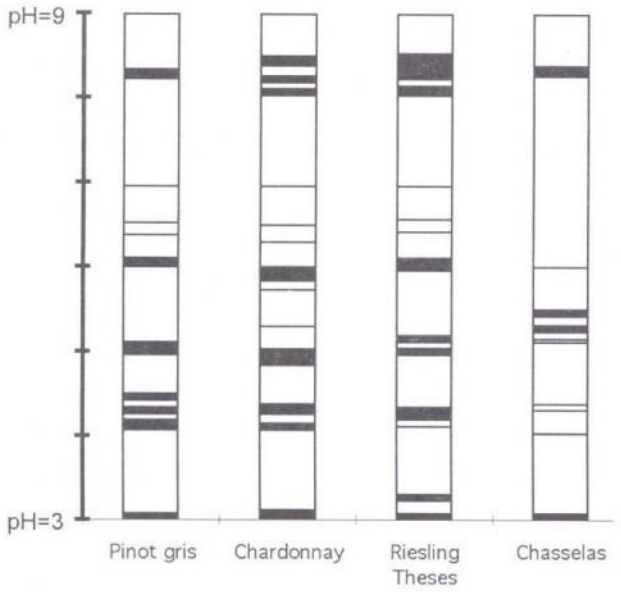

Figure 1 Characteristic esterase isozyme patterns obtained by isoelectric focusing in a linear pH gradient gel of Pinot gris, Chardonnay, Riesling Theses and Chasselas samples collected after blooming in Szigetcsép

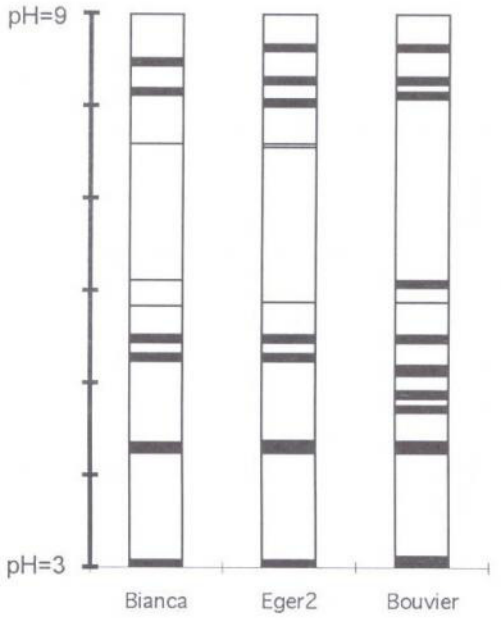

Figure 2 Characteristic esterase isozyme patterns of Bianca, Eger2 and Bouvier samples collected after blooming in Kecskemét

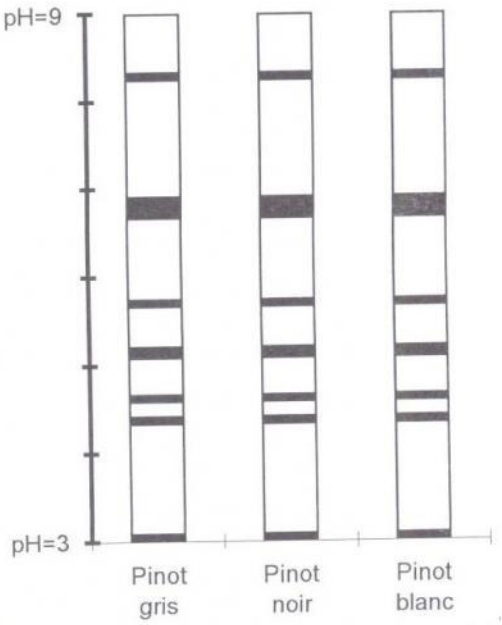

Figure 3 Characteristic esterase isozyme patterns of Pinot gris, Pinot noir and Pinot blanc samples collected after blooming in Kecskemét

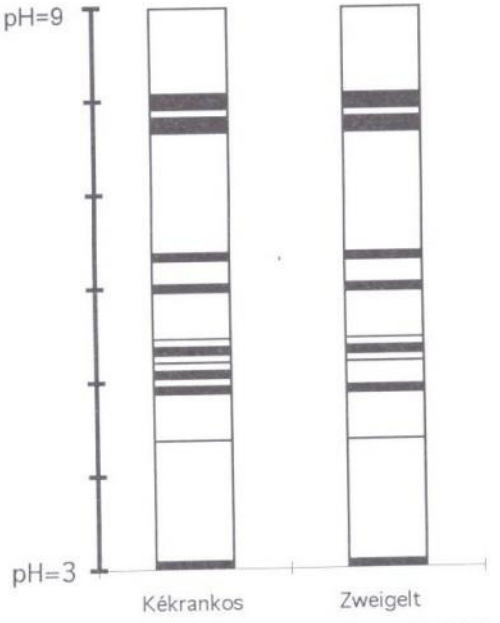

Figure 5 Characteristic esterase isozyme patterns of Kékfrankos and Zweigelt samples collected after blooming in Eger

Esterase isozyme patterns of Kékfrankos and Zweigelt are shown in the *Fig. 5*. The samples were collected after blooming in Eger. Esterase isozyme fractions were the same in the basic (pH ≅ 7.5–8.0), neutral (pH ≅ 6.0–7.0) and the acidic (pH ≅ 4.5–5.5) pH ranges, while in the acidic one an extra fraction, pH ≅ 5.1 was detected in Kékfrankos relative to Zweigelt.

Our findings indicate that esterase isozyme patterns obtained at after blooming phenological phase are suitable for differentiation among *Vitis vinifera* L. even in case of hybrids.

One of our previous studies showed that in grapevine no peroxidase activity could be found at sprouting phenological phase. However, analyses of peroxidase isozyme patterns obtained in the dormant phanological phase was applicable for differentiation among certain grapevine *(Stefanovits-Bányai* et. al 1998).

Figs. 6–9 show our recent data obtained for various grapevine varieties when samples were collected after bloomig and fruit set phenological phases.

Peroxidase isozyme patterns of Pinot gris, Chardonnay, Riesling Theses and Chasselas are shown in *Fig. 6*, both qualitative and quantitative differences can be detected in the whole pH range (pH ≅ 3–9) for these variety but Chasselas samples.

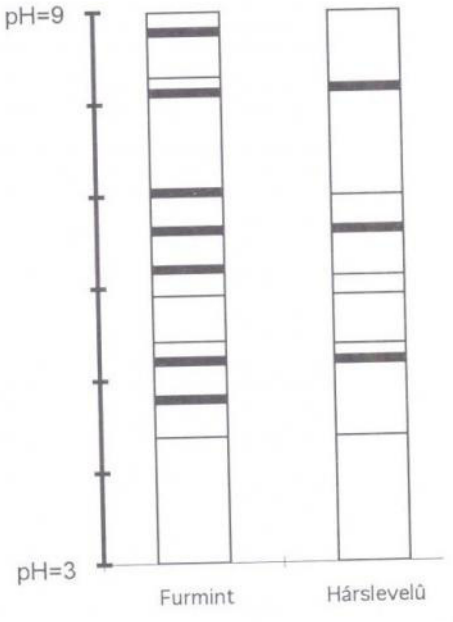

Figure 4 Characteristic esterase isozyme patterns of Furmint and Hárslevelű samples collected after blooming in Tokaj

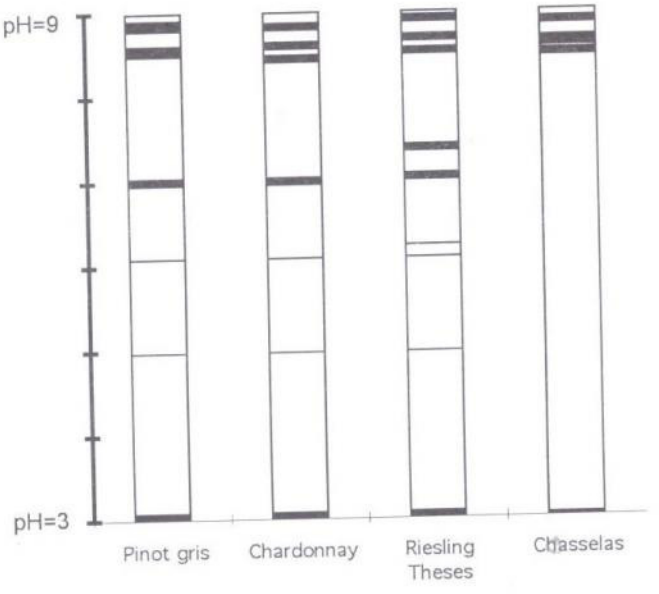

Figure 6 Characteristic peroxidase isozyme patterns of Pinot gris, Chardonnay, Riesling Theses and Chasselas samples collected after blooming in Szigetcsép

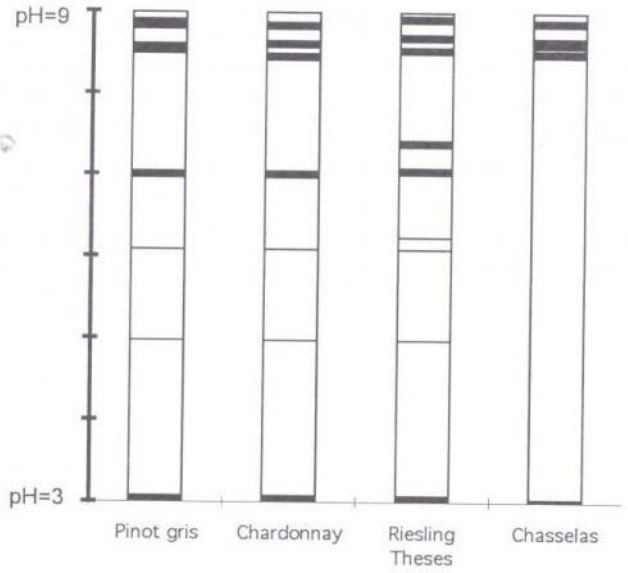

Figure 7 Characteristic peroxidase isozyme patterns of Pinot gris, Chardonnay, Riesling Theses and Chasselas samples collected at fruit set in Szigetcsép

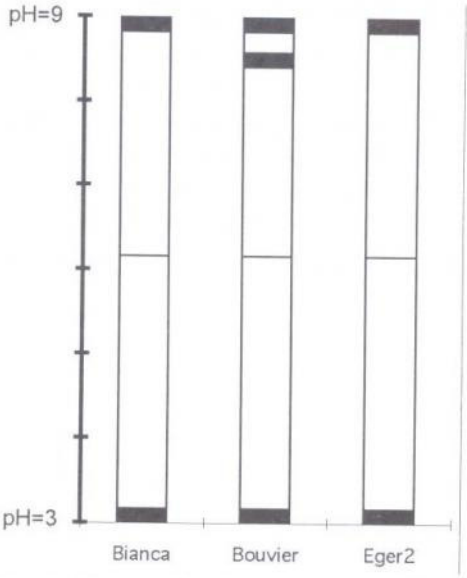

Figure 9 Characteristic peroxidase isozyme patterns of Bianca and his parents Eger 2 and Bouvier samples collected at fruit set in Szigetcsép

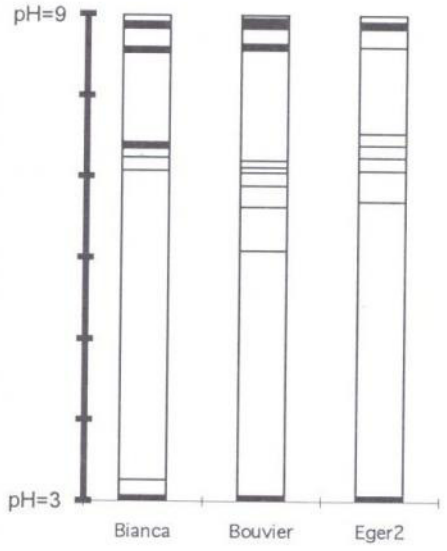

Figure 8 Characteristic peroxidase isozyme patterns of Bianca and his parents Eger2 and Bouvier samples collected after bloming in Szigetcsép

Peroxidase isozyme patterns of the same species at the fruit set are shown in *Fig. 7*. In the upper basic and in the lower acidic pH ranges partically the same fractions with increasing activities can be found. Fractions from the neutral pH range can not be detected any more. These findings indicate, that for sample collection after blooming phenological phase is suitable for differentiation among different grapevine varieties.

Similar conclusions can be drawn from the peroxidase isozyme patterns of interspecific hybrid Bianca and his parents Eger2 and Bouvier using different sampling times as it is shown in *Figs. 8* and *9*, where samples were collected at after blooming and at fruit set.

In one of our previous works *(Stefanovits-Bányai* et al. 1998) also the after blooming phase was found appropiate for differentiation among various varieties according to their esterase isozyme patterns.

Our recent findings indicate that not only esterase but peroxidase isozyme patterns are suitable for identification of grapevine varieties when obtained at after blooming sampling time.

Summarizing our data we conclude that evaluating differences among the isozyme patterns by constructing schemes as they are shown in the figures a clear-cut picture can be obtained. That kind of drawing could be useful for later evaluation and/or identification of any new samples.

Acknowledgement

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