We are IntechOpen,
the world's leading publisher of
Open Access books
Built by scientists, for scientists

4,800
Open access books available

122,000
International authors and editors

135M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

CLARIVATE ANALYTICS
BOOK
CITATION
INDEX
INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

4

Gel Electrophoresis of Grapevine (*Vitis vinifera* L.) Isozymes – A Review

Gizella Jahnke, János Májer and János Remete
University of Pannonia Centre of Agricultural Sciences
Research Institute for Viticulture and Enology
Badacsony
Hungary

1. Introduction

Several articles were written from the beginning of the fifties about the presence of plant enzymes in multiple forms. The major discussion was questioning whether these forms are artifacts that rose during the purification or not. To show that these forms are not artifacts in 1952 Jermyn divided his original peroxidase juice into two parts by acidic precipitation. The precipitate contained the A and B, while the supernatant the C and D points. Two components were found in the purified peroxidase solution; one migrated to the anode, the other to the cathode (Jermyn and Thomas, 1954).

The first major step for the starting up of isozyme analysis was the development of starch gel electrophoresis by Smithies (1955). The second major step was the demonstration of the direct visualization of isozymes in the stach gel by specific histochemical stains by Hunter and Markert in 1957 (McMillin in Tanksley and Orton, 1983).

The term isozyme was formed by Market and Moller (1959), using this word for different molecular forms of enzymes with the same substrate specifity.

Proteins - as the primary products of structural genes - are very alluring for the direct genetic studies. Variation in the DNA coding sequences frequently (but not all the cases) causes variation in the primary conformation of the proteins. In un-natural environments the detection of this variation is very difficult, because in such conditions the base of the separation is only the size of the protein (molecular weight). In natural environments the change of a single amino acid can detectably modify the migration. The extraction from a single tissue can contain a lot of proteins, which - in the case of non-specific (e.g. Comassie blue) staining - can result in a complex pattern, that makes it difficult to identify the homolog (allelic) and non-homolog enzymes. This problem can be solved by the application of enzyme-specific staining after the electrophoresis (Shields et al. in Tanksley and Orton, 1983).

The analysis of isozymes and their functions is the subject of functional genomics. The study of the gene expression in the level of RNA and proteins can give answers to a lot of open questions (Bernardi, 2004).
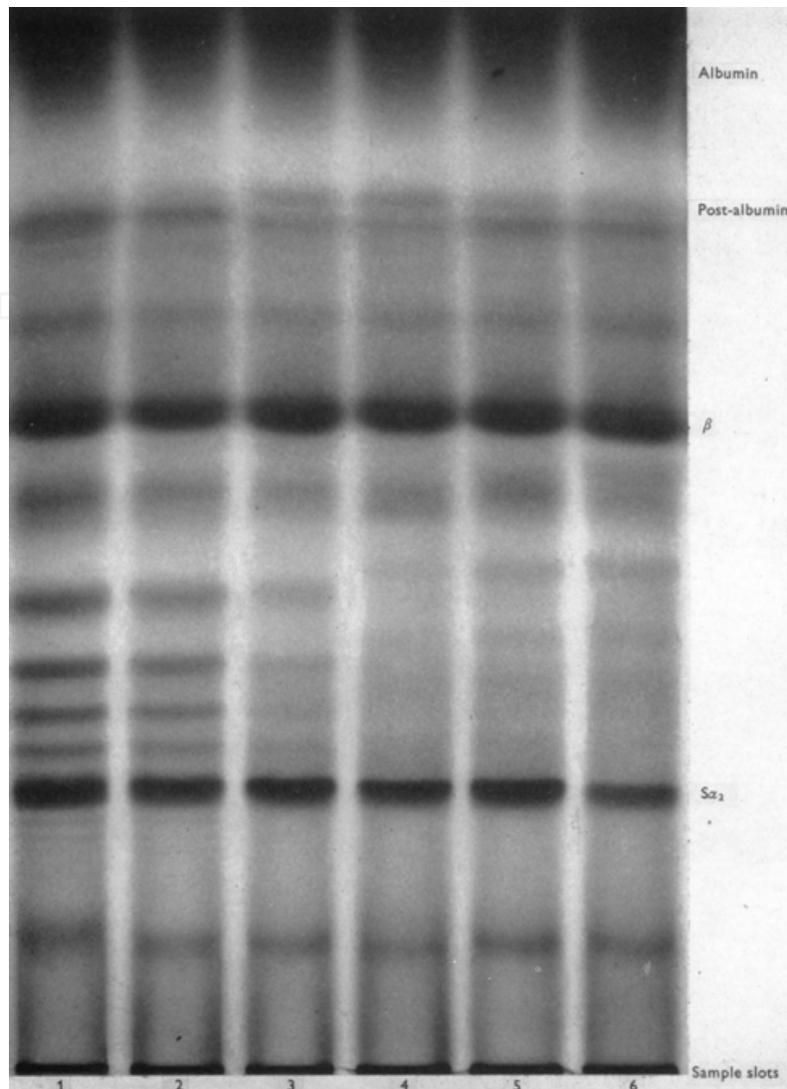
Fig. 1. A photograph of the result obtained by vertical starch-gel electrophoresis (approx. 19 hr. at 5v/cm.) with serum samples from six healthy individuals. Only the section of gel from the sample slots to the albumin is included in the photograph. Samples 1 and 2 are from female identical twins, 45 years old. Samples 3-5 are from 9-year-old female non-identical quadruplets and sample 6 is from their mother. (Smithies, 1958)

2. Grouping of isozymes

Isozymes are divided into three categories depending on the way they are biosynthesized:

1. isoenzymes (multilocus isozymes) arise from multiple gene loci, which code for structurally distinct polypeptide chains of the enzyme;
2. allozymes (or alleloenzymes), are structurally distinct variants of a particular polypeptide chain, coded by multiple alleles at a single locus;
3. secondary isozymes result from post-translational modifications of the enzyme structure;

The distinction between multiple alleles and multiple gene loci as causes of isozyme formation is that multiple alleles are the result of differences between individual members

of a certain species, whereas multiple loci are common to all members of a species (Markert, 1975.).

The most probable reason for the presence of multilocus enzyme forms is the gene duplication. The gene duplication — the multiplication of genes in the genom — can come into existence by, for example, not equal crossing over. The frequency of mutation of various structural genes can be different, as a result of which some genes only rarely present in different allelic variant, as more alleles present in the population of other isoforms. This difference can be accepted as the evidence of a separate locus.

For the formation of multilocus isoforms a different evolutionary way can be imagined. It is probable, that the variation of the structural genes of originally different enzymes can cause the formation of similar catalytic functions. (H. Nagy, 1999).

Enzymes with variable substrates generally show higher variability itseves (catechol oxidase, acid phosphatase, peroxidase, esterase), but the amount of allozymic polymorphism is an increasing function of environmental variation. "Observations on natural pupulatians are cited which substantiate the claim that allozymic polymorphism is primarily due to selection acting on environmental variation in gene function. …a large portion of the observed allozymic variation is due to a rather specific type of phenomenon: substrate variability" (Gillespie and Langley, 1974).

Enzymes with a single, special substrate show lower variability (glucose phosphate isomerase, phosphoglucomutase, glutamate-oxalacetate transaminase, glucose-6-phosphate transaminase etc.), but the banding patterns are less affected by the environment (Gillespie and Kojima, 1968; Gillespie and Langley, 1974).

3. Separation of isozymes by gel electrophoresis

Isoenzymes can be separated by electrophoresis or isoelectric focusing. The isozymes — under given proper circumstances — show peculiar patterns in the gel, which are called zymogramm (Hunter and Markert 1957).

Electrophoresis is a type of chromathography. The power for the separation of proteins is the difference in voltage between the two ends of the gel. The movement of proteins in the electric field is effected by their weight, shape and charge (Smith, 1960; Bálint and Bíró in Bíró, 1989).

The gel for the separation can be made from starch, agarose or acrilamide (Fig 2.). A standardized method of starch gel electrophoresis is used by UPOV (1996) for the analysis of identity of plant cultivars by isozyme analysis (Baum, 1986).

Advantages of the starch gel are that it is non-toxic, and more isozymes can be analysed by the slicing of a thick gel. More recently polyacrylamide gels are used because of their larger resolution. The porous structure of poliacrilamide gel is formed through a process of polymerization of acrylamide ($CH_2=CH-CO-NH_2$) and bis-acrylamide ($CH_2=CH-CO-NH-CH_2-NH-CO-CH=CH_2$). As a result of polimerisation a colourless, diaphanous, flexible, and consistent gel arises, which is resistant to scalding or chilling. The density, viscosity and size of poles are determined by the concentration of acrylamide and bis-acrylamide. (Hajósné Novák és Stefanovitsné Bányai in Hajósné Novák, 1999).
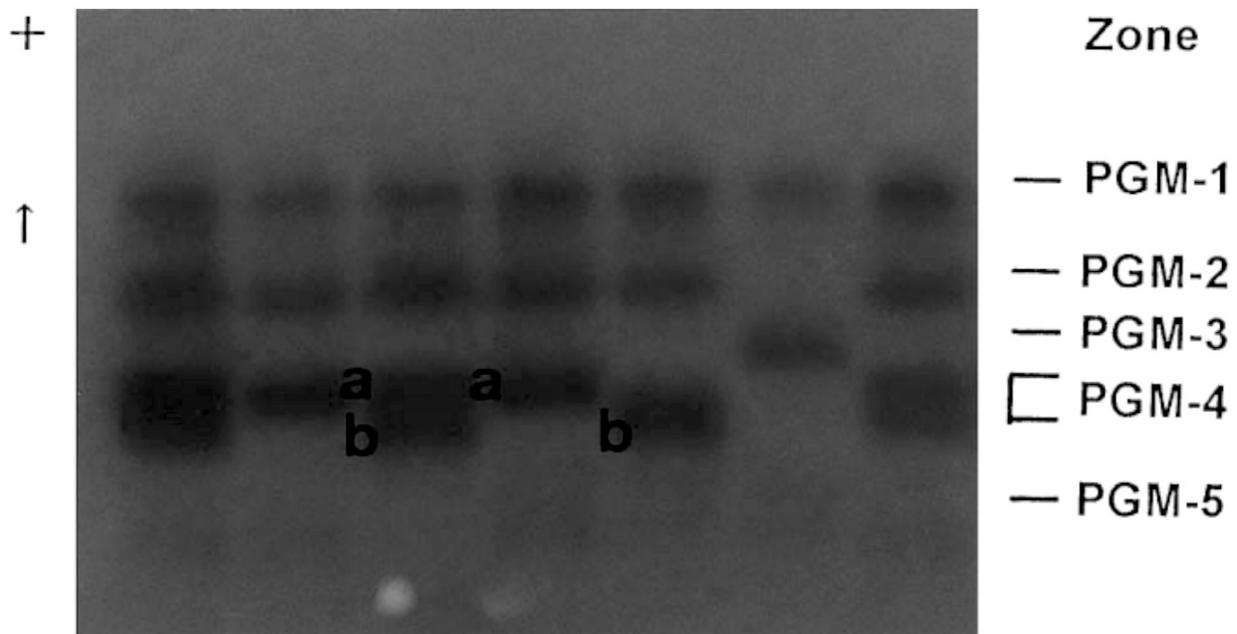
Fig. 2. Segregation of isozyme bands in PGM zone 4. Each column shows representative individuals of a selfed population of Cherry Bell-4. (Nomura et al., 1999.)

4. Separation of isozymes by isoelectric focusing

The charge of proteins is determined by the ratio of acidous and alkaline molecule parts and the rate of their dissociation. The rate of dissociation is determined by the pH of the surroundings of the molecule. The isoelectric point (pI) of the protein is the pH at which the acidous and alkaline molecule parts equally dissociate, the protein's net charge being zero. In a surrounding where the pH is lower than the isoelectric point, the net charge of the protein will be positive, in turn when the pH is higher than the IP, the net charge will be negative (Hames, 1990).

Isoelectric focusing of proteins can be carried out in a gel, in which a pH gradient is generated. Under voltage the proteins migrate to the point of the gel, where their net charge is equal to zero (pI). For this method thin poliacrilamide or agarose gels are used (Fig. 3).

The advantages of isoelectric focusing in opposition of gel electrophoresis are that the isozyme variants can be identified based on their isoelectric points, which results more accurate determination of isoforms, than the identification based on Rf values. On the other hand, the used gels in isoelectric focusing are thinner, so the separation is faster (Patterson and Payne, 1989).

Previously isoelectric focusing had disadvantages, as it required practice and the staining of the gels, because of the wide pH gradient, was difficult (Patterson és Payne, 1989), but nowadays these cause no problem. With the use of ampholites, the preparation of pH gradient gels needs even less practice, and you can even purchase ones. Neither causes problems with the staining of this so-called immobilized pH gradient gels, because the pH gradient can be removed by the washing of the gel.
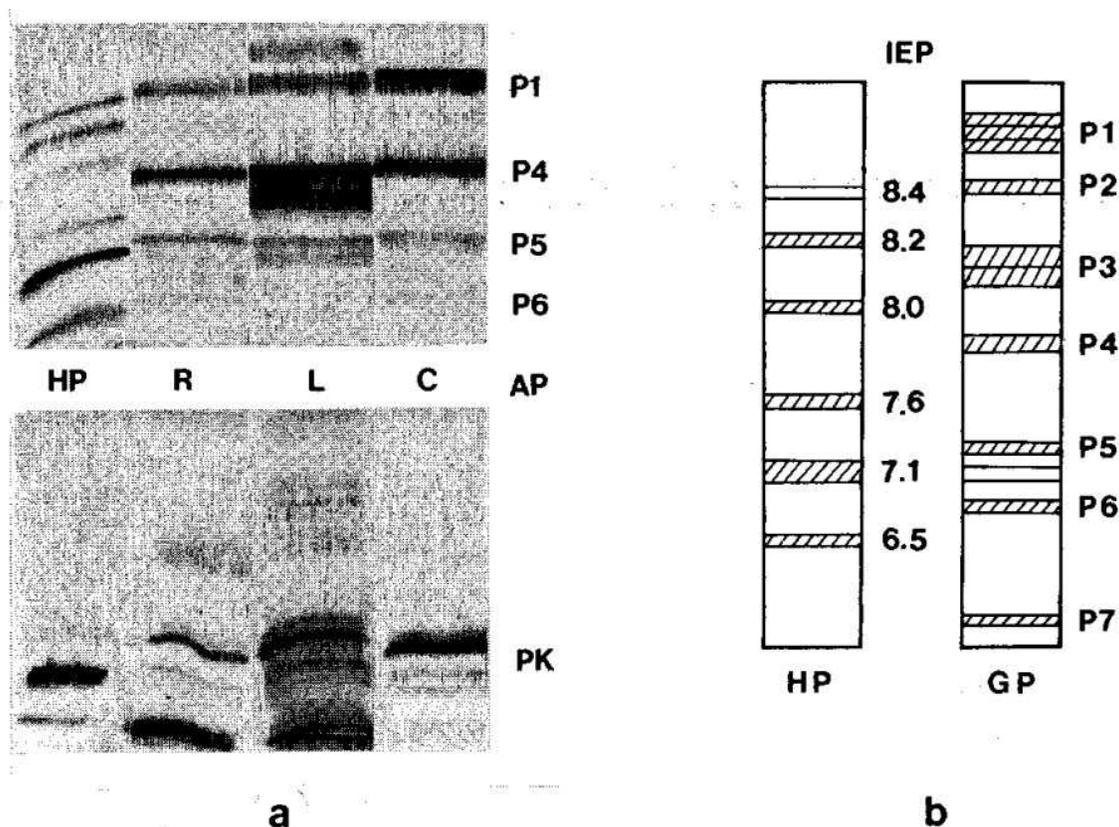
Fig. 3. Isoelectric focusing patterns of horseradish (HP), and grapevine (GP) peroxidases. IEP isoelectric points, P1-P7 names of isoenzyme bands, AP application zone, PK cathodic peroxidase. -a) anodic and cathodic pattern of peroxidases obtained from different organs of *Vitis riparia*. L Leaf, R root, C callus. The bands of horseradish peroxidases are distorted due to a margin effect on the slab. -b) Shematic diagram of the anodic part of peroxidase patterns. (Bachmann and Blaich, 1988)

5. Advantages and disadvantages of isozyme analyses

"The utility of isozymes as genetic markers is generally attributed to their frequent polymorphism, codominance, single gene-Mendelian inheritance, rapid, simple and relatively inexpensive assay and their ubiquity in plant tissues and organs (even in embrios and pollen). Although the selective neutrality of isozymes has been debated, it seems highly probable that they are adaptive under certain circumstances." (Bretting and Widrlechner 1995a).

Other advantages of isozyme analysis are the rapid analyses of samples, a small amount of plant material is sufficient. Young plants can be tested and selected based on their genotypes for features, which morphologically appear later. These can mean significant temporal and financial savings in the case of the breeding of annual plants. Now the best cost-efficient markers are the isozymes (Bretting and Widrlechner, 1995b).

Disadvantages of isozyme analysis as against the DNA markers, that they are organ-, tissue- and developmental stage-specific (Fig 4.). They often go through post-transcriptional modifications, which limit their usage (Staub et al., 1996).
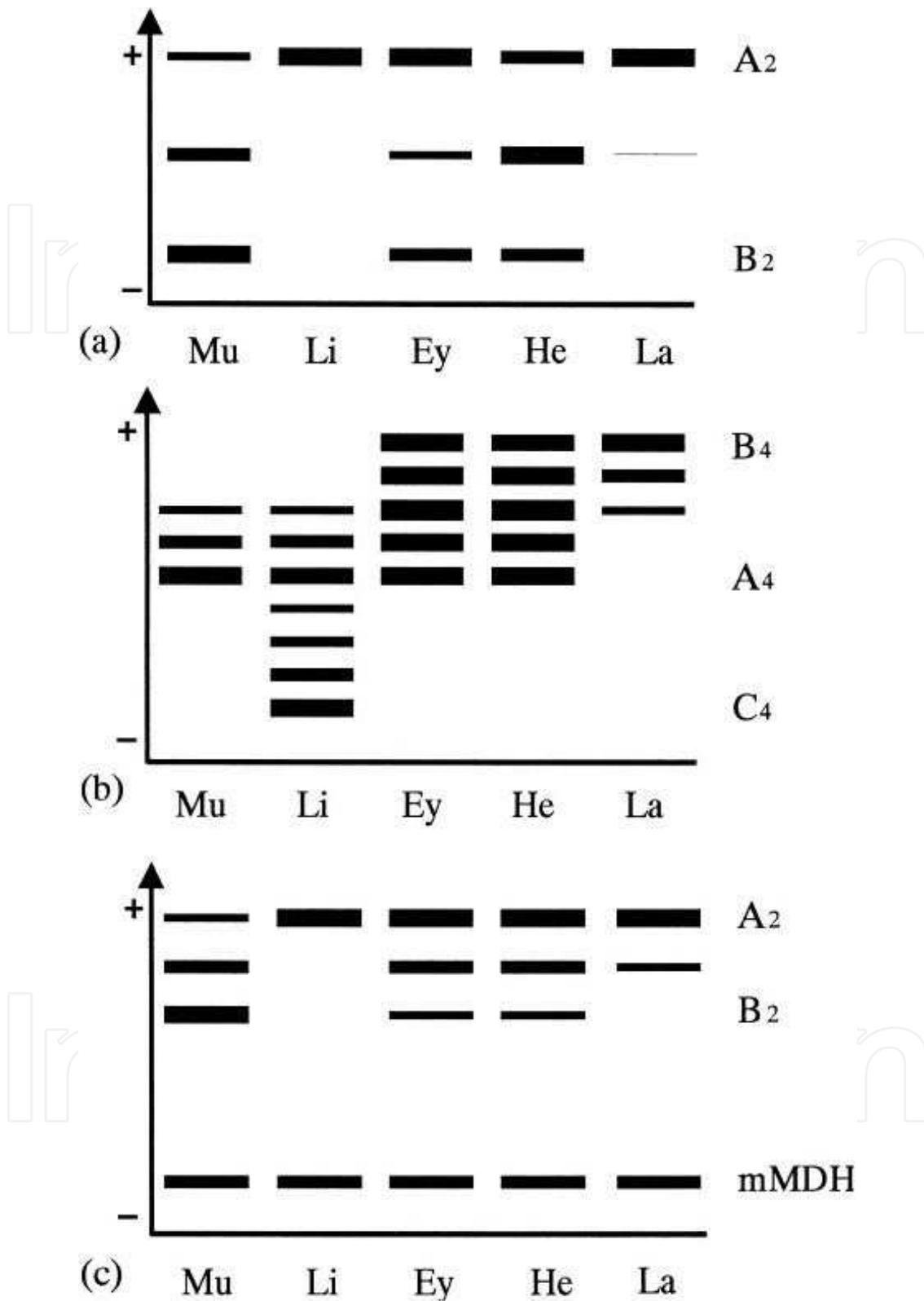
Fig. 4. Schematic representation of the differential expression of GPI (a), LDH (b) and MDH (c) isozymes in adult tissues (Mu, white muscle; Li, liver; Ey, eye; He, heart) and larvae (La) of L. cephalus. The egg pattern is identical to the larval one. Differences in line thickness refer to different staining intensities. (Manaresi et al. 1998)

6. Isozyme analysis in grape

Enzyme banding patterns for over 60 varieties of wine and table grapes were determined by gel electrophoresis by Wolfe (1976). Enzymes were extracted from ripe berries of each variety and separated by electrophoresis in a starch gel. Enzyme bands were detected by developing the gels in a buffered solution that produced an insoluble dye at the site of enzyme activity. The varieties were assayed for leucine aminopeptidase, indophenol oxidase, acid phosphatase, catechol oxidase, alcohol dehydrogenase, esterase, and peroxidase. The first four enzymes listed were found the most useful for distinguishing varieties.

Enzyme-banding patterns of catechol oxidase, acid phosphatase, esterase, alcohol dehydrogenase, indophenol oxidase, and leucine aminopeptidase obtained by enzyme staining of starch gel electropherograms allow the distinction of berries of the grape cultivars Perlette, Thompson Seedless, Superior Seedless and an early ripening sport of Superior Seedless (Schwennesen et al., 1982).

Twenty-seven cultivars and feral accessions from four Vitis species were examined by SUBDEN et al. (1987) for 12 isozyme systems exhibiting polymorphism. Using extracts from woody tissue and a protocol to avoid isozyme inactivation by polyphenolics and other materials, 27 of 29 strains exhibited unique isozyme banding patterns for glucose-6-phosphate isomerase, peptidase, and acid phosphatase. Implications for genetic homogeneity screening of nursery stock or identifying unknown samples are discussed.

German researchers analysed the isozymes of peroxidase by isoelectric focusing. Purified internodal phloem extracts from dormant wood were used. In the 6-11 pH range 8 bands were found, 71 Vitis species and varieties were identified (Bachmann and Blaich, 1988).

Genetic analysis of 11 allozyme polymorphisms was performed by Weeden (1988) on the progeny of 'Cayuga White' x 'Aurore', two complex interspecific grape (*Vitis*) hybrids. Segregation for most of the polymorphisms closely approximated monogenic Mendelian ratios, and eight new isozyme loci were defined for grape. Joint segregation analysis among the isozyme loci revealed three multilocus linkage groups (ACP-1—PGM-c; ACP-2—AAT-c; GPI-c—LAP-1). These results demonstrate that sufficient allozyme polymorphism exists in grape to establish many multilocus linkage groups and that this genetic analysis can be accomplished using extant progeny or progeny readily produced from highly heterozygous clones.

The pattern of the systems PER and ACP from 8 vines of *Vitis vinifera* L. has been studied in 1988 by Royo et al. (1989). A method to differentiate and characterize 6 clones of *Vitis vinifera* L. has been established by gaining the variability of the PER pattern from the band pattern constantly present in any vine, and the total band pattern from another vine (not only amongst the vines but also along the vegetative cycle). In the vines investigated no difference has been found for the ACP system.

Three enzymes in 5 cultivars of *Vitis vinifera* L. are analyzed by PAGE in young leaves. With acid phosphatase, arylesterase and glutamat-oxalat transaminase more or less different isoenzyme patterns of the different cultivars were obtained. There were no interclonal differences. The most polymorph enzyme was the arylesterase. The best results were obtained with young leaves from sprouting buds (Eiras-Dias et al., 1989).

Starch-gel electrophoresis was used by Walters et al (1989) for the analysis of *Vitis vinifera* L. cultivars, interspecific *Vitis* hybrids and wild individuals of *Vitis riparia* Michx. They suggest a simple and inexpensive procedure for the extraction of active enzymes from grape, which is rapid and efficient. Starch-gel electrophoresis with different optimized gel-electrode buffer systems is used for 40 different isoenzymes, 14 of which were consistently resolvable and showed variation among different cultivars.

Isozyme analysis is one of the means suitable to characterize clonally propagated cultivars. Isoelectric focusing was used to reveal differences in isozyme patterns between tissue-cultured plants and mother plants, for the cultivars Barbera, Queen of the Vineyards, Dolcetto and Delight. In cultivar Barbera both 2n and 4n plants were considered. Leaf samples were collected from shoots grown on cuttings under controlled environmental conditions and from plants obtained by tissue culture. The buds used for tissue culture were taken from the same shooted cuttings. Leaf extracts were analyzed by isoelectric focusing considering the following isozymes: AcPH (acid phosphatase), GPI (glucose phosphate isomerase) and PGM (phosphoglucomutase). The banding patterns of GPI and PGM showed differences among the cultivars, while for AcPH there seemed to be no differences among them in the pH range considered. There were no differences between isozyme patterns of the Barbera 2n and Barbera 4n. The main difference between in vitro plants and mother plants was the amount of isozyme evaluated by densitometric measurements. In all the cultivars, the amount of isozymes for AcPH was higher in mother plants than in in vitro ones, while for PGM and GPI it was the opposite. This can be due to the different environmental conditions affecting cellular metabolism (Botta et al., 1990).

The idea of using woody stems during the resting period instead of leaves for the isozyme analysis arose in 1990. Kozma et al. (1990) analysed the esterase isozymes of varieties from different convarietas and interspecific hybrid families by poliacrilamide gel electrophoresis and isoelectric focusing. Based on their results they established, that the phloem extracts from woody stems collected in the resting period or shoots colleting in spring give good reproducible patterns, but the leaf extracts give irreproducible patterns.

Tests were carried out on different types of calli and somatic embryos of V. rupestris using 2-D electrophoresis. The investigation carried out by Martinelli et al. (1993) is focused on the isozyme patterns of AcP (acid phosphatase), ADH (alcohol dehydrogenase), EST (esterase), G6PDH (gluconate-6-phosphate dehydrogenase) and PGM (phosphoglucomutase). A typical variation of isozyme pattern could be observed during the different steps of somatic embryogenesis. De-differentiated callus showed other types of isoenzyme pattern compared to those obtained during the development of somatic embryos.

Similarly to Kozma et al. Bachmann (1994) used extracts from phloem of dormant canes for the isozyme analysis. This comprehensive study has analysed the peroxidase isozyme banding patterns of 313 different cultivars and species of *Vitis* using isoelectric focusing on polyacrylamid gels. The author reports that acidic peroxidases were characteristic for *Vitis vinifera* L. cultivars with only a 5 % frequency of occurrence in other *Vitis* species. Variation in neutral to basic peroxidases could be used to group together similar cultivars independent of berry colour, e.g. Pinot noir, Pinot gris, Pinot blanc and Pinot meunier. However, other examples of colour variants, e.g. Merlot noir and blanc were clearly different using peroxidase banding.

Shiraishi et al. (1994) used GPI and PGM isozyme banding patterns for the detection of hybrid origin of seedlings during their triploide grape breeding. First they analysed 99 diploide cultivars, 20 diploide plants from 8 wild *Vitis* species and populations from the crosses between them . In the GPI-2 locus 13 in the GPM locus 11 allels was found. Data showd high genetical differences between Vitis species . After that, the GPI-2 and PGM-2 genotype of 6 diploide and 4 tetraploide cultivars (used for the crosses) were determined. 15 diploide x tertraploide crosses were made. Trisomy gene expression was detected in 92 out of 98 seedlings, as 6 showed diploide patterns.

Seed proteins and enzymes (AcP, ADH, EST, G-6-PDH, MDH, PGM, POD) from several cultivars and wild ecotypes of *Vitis vinifera* L. have been used to evaluate taxonomic differences between *V. vinifera* sspp. sativa and sylvestris (Scienza et al., 1994). Only total proteins in the pH range of 4.0-5.5 and AcP, EST and G-6-PDH were useful for genotype differentiation. The cluster analysis (UPGMA), based on Jaccard genetic distance and determined on the presence/absence of electrophoretic profiles, reveals 2 distinct groups, supporting the hypothesis of the authors that *V. sativa* and *V. silvestris* should be regarded as 2 separate taxa.

Studies on the induction characteristics and the fine structure of grapevine cells cultured in vitro were undertaken with cultivar Monastrell berry samples of different developmental stages between fruit set and veraison (Zapata et al., 1996). Medium composition, electron microscopy application and protoplast isolation procedures are explained. It could be shown that the intensity of cell development and callus induction percentage depended on the berry growth stage; the de-differentiation process is mainly located in meso-carp tissues. Cultured cells showed to be highly vacuolated with their cytoplasm reduced to a very thin peripheral layer (containing golgi sacks).

Ros Barceló et al. (1996) studied the gene expression of isozymes of providase in downy mildew resistant (*Vitis vinifera* x *Vitis rupestris*) x *Vitis riparia* hybrids and in the susceptible *Vitis vinifera* parent. The peroxidase isoenzyme type B3 (PI=8,9) expressed in the phloem and leaves of resistant hybrids was completely absent in the susceptible parent.

To test whether the basic peroxidase isoenzyme B3 may be considered as a molecular marker of disease resistance in *Vitis* species, suspension cell cultures derived from the downy mildew susceptible *V. vinifera* parent species were treated with an elicitor (cellulase Onoztika R-10) from the soil fungus *Trichoderma viride*, a specific and well-known elicitor of disease resistance reactions in grapevines. The results showed that treatment with the elicitor induces, simultaneously with the activation of the disease resistance mechanism, the appearance of B3 in the cell cultures. These results suggest that the basic peroxidase isoenzyme B3 may be considered as a marker of disease resistance in Vitis species.

Isoenzymes from grapevine woody stems and shoots were evaluated for their use in identification of varieties and clones by Royo et al. (1997). Plant extracts were separated by polyacrylamide gel electrophoresis. Isoenzyme analysis was carried out for esterases, peroxidases, catechol oxidase, glutamate oxalacetate transaminase and acid phosphatase. The plant material was grown and sampled at two localities in Spain, with different climatic conditions. Sampling was carried out bimonthly for two consecutive years in order to find out the influence of the environment and time of the year. Each isozyme system had a

pattern defined by 'fixed' bands that were always present at both localities and during the resting period of the plant (autumn — winter).

An evaluation of the genetic diversity of 'Albariño' (*Vitis vinifera* L.) was carried out by Vidal et al. (1998). The 73 isozyme and 308 RAPD markers were common in the samples tested. The results show the existence of a genetic homogeneity within 'Albariño'cultivated in Galicia. Minor ampelographic differences among samples could be due to external factors rather than to genetic differences.

DNA and isoenzyme analyses were used to characterize 20 table grape cultivars including Moscato d'Amburgo, Italia, Sultanina, Bicane and some recently released new cultivars (Crespan et al., 1999). GPI and PGM isoenzyme systems were able to separate the cultivars into 9 groups whereas the 8 microsatellite loci that were analysed revealed a higher discriminating power. Parentage analysis confirmed that the cultivar Italia was obtained from the crossing Bicane x Moscato d'Amburgo.

Hungarian researchers used isoelectric focusing for the peroxidase and esterase isozymes of some grapevine cultivars. Samples were gathered at different times of the year. The leaf samples after blooming were found the best for the identification of varieties, but they found the phloem extracts of woody stems also suitable for cultivar identification (Stefanovits-Bányai et al., 1999; Stefanovits-Bányai et al, 2002).

Sixty-four Muscat flavoured grapevine accessions were analysed in the work of Crespan and Milani (2001). An analysis was performed at two isozymes and 25 microsatellite loci. The 64 accessions were reduced to 20, which were easily distinguishable from each other at the molecular level by as few as two microsatellite loci. The remaining 44 were found to be synonyms. Three mutants with red and pink coloured berries were identified in the Moscato bianco group. Moscato nero encompasses at least two, Moscato rosa three different varieties. It seems that only two of the analysed Muscats are the main progenitors of the Muscat family: Moscato bianco and Muscat of Alexandria, which in turn are joined by a direct parent-offspring link.

Sànchez-Escribano et al. (1998) analysed 43 table grape varieties by 6 isoenzyme systems (PER, CO, GOT SOD, EST, AcP). The last 2 enzymes were found unsuitable for identification, by the combination of the zymogram of the other 4 enzymes, they were able to identify 31 cultivars, as the remaining 12 were clustered to 5 groups.

Protein and esterase isozyme patterns of authentic grapes and wines of 13 white wine cultivars were determined by means of isoelectric focussing (range of pI: 2.5-10) by Paar et al (1999). Esterase staining with grapes showed active zones mainly in the alkaline pI-range, with most of the cultivars, however indicating no qualitative, but only quantitative differences. Staining of the protein patterns of grapes and wines with Coomassie Brilliant Blue proved to be well suitable for the differentiation of cultivars. With grapes as well as wines the most predicative bandings focussed in the acid pI-range of 4. With the cultivars Grüner Veltliner, Rotgipfler and Riesling Italico the protein banding patterns were so characteristic, that these cultivars were easily identified, whereas with the other cultivars detailed comparisons of the phenogrammes were necessary.

Isozyme and RAPD markers were used for the characterization of Hungarian grapevine varieties and their parents (HAJÓS-NOVÁK And HAJDÚ 2003). The cathecol-oxidase system was found the most suitable for identification proposes.

Isozyme and SSR analysis were carried out for the differentiation of the grapevine cultivars Kéknyelű and Picolit. The name of the grapevine cultivar 'Kéknyelű' has become inseparable from the name of the Badacsony vine region (Hungary), whose fame is also well known beyond the Hungarian frontier. In the Vitis International Variety Catalogue (http://www.genres.de/idb/vitis/) 'Kéknyelű' is reported, as the synonym of the Italian grapevine cultivar 'Picolit'. Vertical poliacrylamide-gel electrophoresis was used for the investigation isoenzymes of catechol-oxidase (CO) and acid phosphatase (AcP). Microsatellite analyses were carried out at 6 loci (VVS2, VVS16, VrZag79, VVMD7, VMC4A1, VMC4G6). The results of the isoenzymatic and microsatellite analyses confirmed, that this two cultivars are different (Jahnke et al., 2007).

Jahnke et al. (2009) investigated the genetic diversity of Hungarian grapevine cultivars with biochemical and molecular markers (isoenzyme and SSR). The isoenzyme patterns of 4 enzyme systems (catechol-oxidase, glutamate-oxalacetate-transaminase, acid phosphatase and peroxidase) and the microsatellite profile in 6 loci (VVS2, VVS16, VVMD7, VMC4A1, VMC4G6, VrZag79) of 48 grapevine varieties were analysed.

The results with CO, GOT, AcP and PER enzymes were reproducible and the zymograms obtained from the woody stems were independent from the time of sampling during the dormant period of the grape (Fig 5.).

Based on the isoenzyme patterns of these 4 enzymes most of the investigated varieties (40/48) were identified. A correlation was found between the isoenzyme patterns and the classification to convarietas of the varieties.
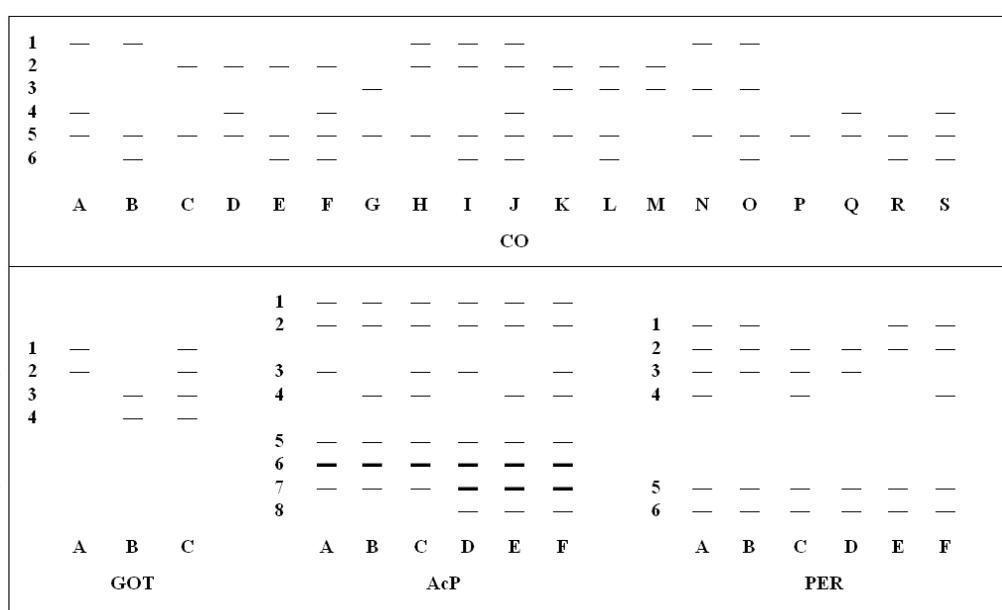

Fig. 5. Characteristic interpretative zymograms observed for CO, GOT, AcP and PER enzymes. The letters mark the different types of isoenzyme patterns, while numbers refer to the number of different isoenzyme bands (Jahnke et al. 2009.)

It was established, that while the varieties of the convarietas pontica differed from those of the convarietas orientalis and occidentalis, the two latter groups could have not been differentiated from each other. Based on the SSR (simple sequence repeat) analyses 46 of the

48 investigated varieties were identified. Even 'Pinot blanc' and 'Pinot gris' cultivars belonging to the same conculta (Pinot) could be differentiated in their VMC4A1 locus.
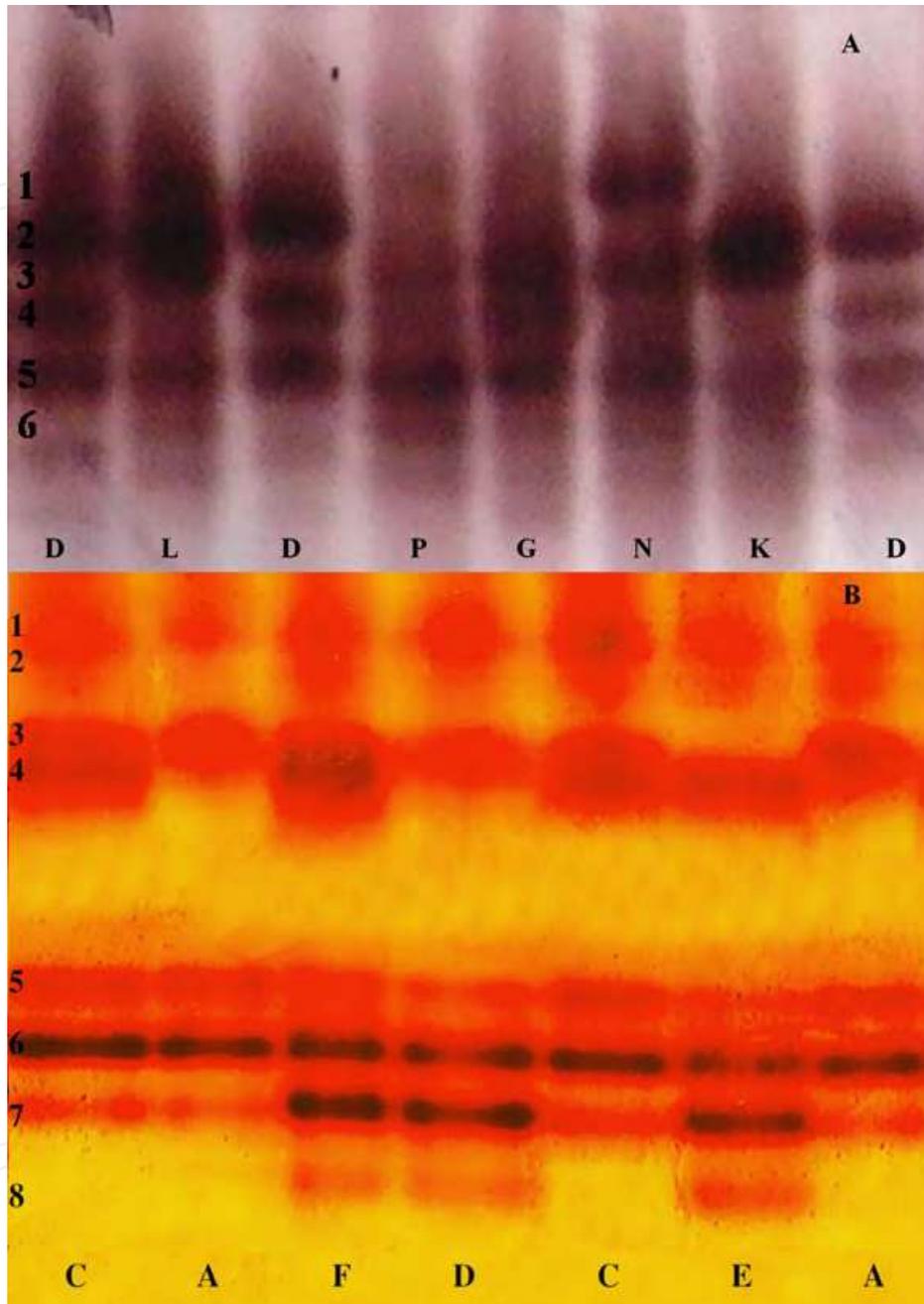

Fig. 6. Isoenzyme gel photos for CO (A) and AcP (B) respectively. The numbers show the band numbers, and the capital letters the banding pattern types shown in Figure 5. (Jahnke et al. 2009)

7. References

Bachmann O. (1994): Peroxidase isoenzyme patterns in Vitaceae. Vitis 33: 151-153.

Bachmann O., Blaich R. (1988): Isoelectric focusing of grapevine peroxidases as a tool for ampelography. Vitis 27: 147-155.

Bálint M., Bíró E. (1989): A fehérjekutatás fizikai-kémiai, preparatív és analitikai módszerei. 129-166. p. In: Bíró E. (Eds.) Biokémia I. Budapest: Tankönyv K.

Baum B. R. (1986): International Registration of Cultivars with Emphasis on Barley: Procedures and Methods of Producing a Register. Acta Horticulturae (182):237-250.

Bernardi G. (2004): Structural and Evolutionary Genomics. Natural Selection in Genome Evolution. Elsevier, Amsterdam

Botta R., Vallania R., Miaja M. L., Vergano G., Me G. (1990): Isozyme pattern comparison between tissue-cultured grapevines and mother plants. Vitis Special Issue: 88-92.

Bretting P. K., Widrechner M. P. (1995b): Genetic Markers and Horticultural Germplasm Management. HortScience 30(7) 1349-1356.

BrettingP. K., Widrlechner M. P (1995a): Genetic markers and plant genetic resource management. Plant Breeding Reviews (13): 11-86.

Crespan M., Botta R., Milani N. (1999): Molecular characterisation of twenty seedless table grape cultivars (Vitis vinifera L.). Vitis 38: 87-92.

Crespan M., Milani N. (2001): The Muscats: A molecular analysis of synonyms, homonyms and genetic relationship within a large family of grapevine cultivars. Vitis 40: 23-30.

Eiras-Dias J. E. J., Sousa B., Cabral F., Carcalho I. (1989): Isozymatic characterisation of portuguese vine varieties of Vitis vinifera L. Rivista di Viticoltura e di Enologia 1: 23-26.

Gillespie J. H., Kojima K. (1968): The degree of polymorphisms in enzymes involved in energy production compared to that in nonspecific enzymes in 2 Drosophila-ananassae populations. Proceedings of the National Academy of Science of the USA 61: 582-585.

Gillespie J. H., Langleyc. H. (1974): A general model to account of enzyme variation of natural populations. Genetics 76: 837-846.

H. Nagy A. (1999): A genetikai variabilitás vizsgálata izoenzimekkel. 25-39 p. In: HAJÓSNÉ NOVÁK M. (Eds.): Genetikai variabilitás a növénynemesítésben. Budapest: Mezőgazda Kiadó, 142 p.

Hajósné Novák M., Stefanovitsné Bányai É. (1999): Az izoenzimek elválasztása gélelektroforézissel. 99-104 p. In: HAJÓSNÉ NOVÁK M. (Szerk.): Genetikai variabilitás a növénynemesítésben. Budapest: Mezőgazda Kiadó, 142 p.

Hajós-Novák M., Hajdu E. (2003): Isozyme and DNA fingerprinting characterization of two hungarian wine grape hybrids and their parents. Acta Horticulturae 603: 211-216.

Hames B. D. (1990): One-dimensional polyacrylamide gel electrophoresis. 1.-139. p. In: Hames B.D., Rickwood D. (Eds.): Gel electrophoresis of proteins. ILR press at Oxford University press, Oxford- New York- Tokyo, Sec. Ed.

Hunter R. L., Markert C. L. (1957): Histochemical Demonstration of Enzymes Separated by Zone Electrophoresis in Starch Gels. *Science* (125): 1294-1295

Hunter R. L., Markert C. L. (1957): Histochemical demonstration of enzymes separated by zone electrophoresis in starch gels. Science 125: 124-1295.

Jahnke G., Korbuly J., Májer J., Györffyné Molnár Júlia (2007): Discrimination of the grapevine cultivars 'Picolit' and 'Kéknyelű ' with molecular markers. Scientia Horticulturae (114): 71-73.

Jahnke G., Májer J., Lakatos A., Györffyné Molnár J., Deák E., Stevanovits-Bányai É., Varga P. (2009): Isoenzyme and microsatellite analysis of Vitis vinifera L. varieties from the Hungarian grape germplasm. Scientia Horticulturae (120): 213–221.

Jermyn M. A., Thomas R. (1954): Multiple components in horse-radish peroxidase. Biochemical Journal. 56: 631-639.

Kozma P., H. Nagy A., Juhász O. (1990): Inheritence of isoenzymes and soluable proteins in grape vareties and F1 hybrids. Proceedings of the 5th International Symposium on Grape Breeding ; Vitis Special Issue: 134-141

Li, B.; Xu, Y. & Choi, J. (1996). Applying Machine Learning Techniques, *Proceedings of ASME 2010 4th International Conference on Energy Sustainability*, pp. 14-17, ISBN 842-6508-23-3, Phoenix, Arizona, USA, May 17-22, 2010

Lima, P.; Bonarini, A. & Mataric, M. (2004). *Application of Machine Learning,* InTech, ISBN 978-953-7619-34-3, Vienna, Austria

Manaresi S., Mantovani B., Zaccanti F. (1998): Comparative Analysis of Glucosephosphate Isomerase, Lactate Dehydrogenase and Malate Dehydrogenase Isozymes in 9 Cyprinid Species from Italy. Zoological Science 15:461-467.

Markert C., Moller L. F. (1959): Multiple forms of enzymes: tissue, ontogenetic, and species specific patterns. Proceedings of the National Academy of Sciences of the USA. 45: 753-763.

Markert C. (Ed.). (1975): Isozymes. Vol 1–4, Academic Press, New York.

Martinelli L., Scienza A., Villa P., De Ponti P., Gianazza E. (1993): Enzyme Markers for Somatic Embriogenezis in Vitis. Journal of Plant Phisiology 141: 476-481. p.

Mcmillin D. E. (1983): Plant isozymes: a historical perspective. 3-13. p. In: TANKSLEY S. D., ORTON T. J. (Szerk.): Isozymes in Plant Genetics and Breeding. Amsterdam: Elsevier Science Publishers B.V.

Nomura K., Yoneda K., Inoue H., Tateishi A., Kanehira T., Isobe K. (1999): Identification and linkage analysis of isozymes loci in open-pollinated varieties of radish (*Raphanus sativus* L.). Scientia Horticulturae 82: 1-8.

Paar E., Doubek S., Eder R. (1999): Differenzierung von Wiessweinsorten mittels isoelektischer Fokussierung. Mitteilungen Klosterneuburg 49:176-185.

Patterson B. A., Payne L. A. (1989): Zymograms of plant extracts using isoelectric focusing on ultra thin layers. Acta Horticulturae 247:163-169.

Ros Barceló A., Zapata J. M., Calderón A. A. (1996): A Basic Peroxidase Isoenzyme, Marker of Resistance Against Plasmopara viticola in Grapevines, is Induced by an Elicitor from Trichoderma viride in Susceptible Grapevines. Journal of Phytopathology 144: 309-313

Royo B., Gonzalez J., Laquidain M. J., Larumbe M. P. (1989): Caracterization mediante analisi isoenzimatico de clones de la vinifera "Garnacha" (Vitis vinifera L.). Investigación agraria. Producción y protección vegetales. 4: 343-354.

Royo J. B., Cabello F., Miranda S., Gogorcena Y., Gonzalez J., Moreno S., Itoiz R., Ortiz J. M. (1997): The use of isoenzymes in characterisation of grapevines (Vitis vinifera L.). Influence of the environment and time of sampling. Scientia Horticulturae 69: 145-155.

Sànchez-Escribano E., Ortiz J. M., Cenis J. L. (1998): Identification of table grape cultivars (Vitis vinifera L.) by the isozymes from the woody stems. Genetic Resources and Crop Evolution 45: 173-179.

Schwennesen J., Mielke E. A., Wolfe W. H. (1982): Identification if Seedless Table Grape Cultivars and Bud Sport with Berry Isozymes. HortScience 17: 366-368.

Scienza A., Villa P., Tedesco G., Parini L., Ettort C., Magenes S., Gianazza E. (1994): A chemotaxonomic investigation on Vitis vinifera L. II. Comparison among ssp. sativa traditional cultivars and wild biotypes of ssp. silvestris from various Italian regions. Vitis 33: 217-224.

Scienza A., Villa P., Tedesco G., Parini L., Ettort C., Magenes S., Gianazza E. (1994): A chemotaxonomic investigation on Vitis vinifera L. II. Comparison among ssp. sativa traditional cultivars and wild biotypes of ssp. silvestris from various Italian regions. Vitis 33: 217-224.

Shields C. R., Orton T. J., Stuber W. (1983): An outline of general resource needs and procedures for the electrophoretic separation of active enzymes from plant tissue. 443-467 p. In: Tanksley S. D., Orton T. J. (Eds.): Isozymes in Plant Genetics and Breeding. Amsterdam: Elsevier Science Publishers

Shiraishi S, Ohmia Wakama C., Hiramatsu M. (1994): Variation of Gluceosephosphate Isomerase and Phosphoglucomutase Isozymes in Vitis and Their use in Grape Breeding. J. Fac. Agr., Kyushu Univ., 38 (3.4), 255-272.

Siegwart, R. (2001). Indirect Manipulation of a Sphere on a Flat Disk Using Force Information. *International Journal of Advanced Robotic Systems,* Vol.6, No.4, (December 2009), pp. 12-16, ISSN 1729-8806

Smith I. (1960). Chromatographic and electrophoretic techniques. Vol. II Zone electrophoresis. Heinemann, London.

Smithies O. (1955): Zone Electrophoresis in Starch Gels.: Group Variations in the Serum Proteins of Normal Human Adults. Biochemical Journal 61: 629-641.

Smithies O. (1958): An Improved Procedure for Starch-gel Electrophoresis: Further Variations in the Serum Proteins of Normal Individuals. Biochemical Journal 71: 585-587.

Staub J. E., Serquen F. C., Gupta M. (1996): Genetic Markers, Map Construction, and Their Application in Plant Breeeding. HortScience 31: 729-740.

Stefanovits-Bányai É, Lakatos Zs., Kerepesi I., Balogh I. (1999): Esterase and peroxidase isozyme patterns of some Vitis vinifera L. species. Publicationes Uniersitate Horticulturae Alimentariae LIX: 42-46.

Stefanovits-Bányai É., Lakatos S., Hajós-Novák M., Hajdu E., Balogh I. (2002): Recent developments in biochemical characterisation of Vitis vinifera L. varieties in Hungary. International Journal of Horticultural Science 8: 57-61.

Subden R. E., Krizus A., Lougheed S. C., Carey K. (1987): Isozyme Characterisation of Vitis Species and Some Cultivars. American Journal of Enology and Viticulture 38:176-181.

UPOV (1996): Guidelines for the conduct of tests for distinctness, uniformity and stability. Rape seed (Brassica napus L. oleifera).

UPOV, Geneva, Switzerland TG/36/6

Vidal J. R., Moreno S., Masa A., Ortiz J. M. (1998): Study of the genetic homogeneity of Albariño (Vitis vinifera L.) growing in Galicia (Spain) using isozyme and RAPD markers. Vitis 37: 145-146.

Walters T. W., Posluszny U., Kevan P. G. (1989): Isozyme analysis of the grape (Vitis). I. A practical solution. Canadian Journal of Botany 67: 2894-2899.

Weeden N. F., Reisch B. I., Martens M. H. E. (1988): Genetic Analysis of Isozyme Polimorohism in Grape. Journal of the American Society for Horticultural Sciences 113 (5): 765-769.

Wolfe W. H (1976): Identification of grape varieties by isozyme banding patterns. American Journal of Enology and Viticulture 27: 68-73.
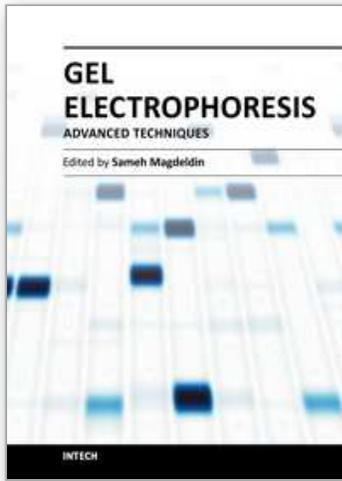
Gel Electrophoresis - Advanced Techniques

Edited by Dr. Sameh Magdeldin

As a basic concept, gel electrophoresis is a biotechnology technique in which macromolecules such as DNA, RNA or protein are fractionated according to their physical properties such as molecular weight or charge. These molecules are forced through a porous gel matrix under electric field enabling uncounted applications and uses. Delivered between your hands, a second book of this Gel electrophoresis series (Gel Electrophoresis- Advanced Techniques) covers a part, but not all, applications of this versatile technique in both medical and life science fields. We try to keep the contents of the book crisp and comprehensive, and hope that it will receive overwhelming interest and deliver benefits and valuable information to the readers.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

INTECH
open science | open minds