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Isoenzyme Polymorphism in Apricot Cultivars

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Abstract

Eighteen enzyme systems were studied in 50 apricot (*Prunus armeniaca* L.) cultivars of diverse geographical origin using polyacrylamide gel electrophoresis. Thirteen systems were polymorphic at 20 loci. This polymorphism allowed unique identification of 31 cultivars, while the remaining 19 cultivars were divided into six groups. The most useful enzymes in apricot cultivar identification were esterase, amylase, alanine (or leucine) aminopeptidase and glutamate dehydrogenase. Isoenzyme variability was highest in the cultivar groups originating from Central Asia and from the former USSR countries. European and North American cultivars showed a medium level of variability, while polymorphism was lowest in genotypes from Serbia.

Apricot cultivars have been classified into six ecogeographical groups: European, Central Asian, Irano-Caucasian, Dzungar-Zailij, East Chinese, and North Chinese (9). The European group is the most economically important and includes cultivars commercially grown in Europe, North America, South Africa and Australia. This group is the youngest in origin and the least variable (9).

Isoenzymes are different molecular forms of proteins that have the same enzymatic specificity. They are single-gene characters that are expressed codominantly. Due to these features and to the relatively simple analytical procedure used to detect them, they have been used extensively in plant genetics and breeding. In *Prunus* breeding isoenzymes have been used for identification of cultivars and interspecific hybrids, detection of phylogenetic relationships among species, marking genes controlling economically important traits, and creating genetic maps (12). Even though DNA markers, especially SSRs (simple sequence repeats), are becoming the overwhelming tool for genetic fingerprinting, they are not expressed characters and require expensive equipment, still not available in many countries. For these reasons, isoenzymes are still useful expressed markers for cultivar identification.

Byrne and Littleton (5) were the first to study isoenzyme variability in apricot. Out of seven enzymes, three [malate dehydrogenase, phosphogluconate dehydrogenase (PGD) and phosphoglucomutase (PGM)] were polymorphic at five loci. Additional enzymes and loci were described later by other authors: Battistini and Sansavini (2) for acid phosphatase (ACP) and esterase (EST), Badenes et al. (1) for glutamate oxaloacetate transaminase (GOT), glutamate dehydrogenase (GDH) and superoxide dehydrogenase, Manganaris et al. (11) for aconitase, alcohol dehydrogenase (ADH), diaphorase, formate dehydrogenase, leucine aminopeptidase (LAP), malic enzyme (ME) and peroxidase (PRX).

The objective of this paper was to study isoenzyme polymorphism of these and additional enzymes in apricot cultivars of diverse geographical origin to identify and characterize the genotypes and establish their genetic relationship.

Materials and Methods

Fifty apricot cultivars from Europe, Central Asia and North America were analyzed. They were sampled from the apricot varietal collection of the Faculty of Agriculture, Belgrade, Serbia. In the experimental orchard each cultivar was represented by five trees. Inner bark of one-year-old shoots was used for enzyme extraction. Preparation of samples was done in accordance with the protocol given by Bošković et al. (4) for stone fruit species. In the case of monomorphic isoenzymes only one extraction was done, but if the isoenzyme

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proved to be polymorphic at least two extractions were made.

Eighteen enzyme systems were studied: acid phosphatase (ACP, EC 3.1.3.2), alanine aminopeptidase (AAP, EC 3.4.11.2), alcohol dehydrogenase (ADH, EC 1.1.1.1), alkaline phosphatase (AKP, EC 3.1.3.1), amylase (AMY, EC 3.2.1.1), endopeptidase (ENP, EC 3.4.22.-), esterase (EST, EC 3.1.1.1), glucose-6-phosphate dehydrogenase (G6PDH, EC 1.1.1.49), glucose phosphate isomerase (GPI, EC 5.3.1.9), glutamate dehydrogenase (GDH, EC 1.4.1.2), glutamate oxaloacetate transaminase (GOT, EC 2.6.1.1), leucine aminopeptidase (LAP, EC 3.4.11.1), malic enzyme (ME, EC 1.1.1.40), menadione reductase (MNR, EC 1.6.99.2), peroxidase (PRX, EC 1.11.1.7), phosphoglucomutase (PGM, EC 5.4.2.2), phosphogluconate dehydrogenase (PGD, EC 1.1.1.44) and shikimate dehydrogenase (SDH, EC 1.1.1.25).

Polyacrylamide gel with Tris-boric acid buffer was used for all enzymes except for amylase, where the gels were made with Tris-glycine buffer. The gels contained 8% acrylamide for separating all enzymes except for detection of GOT, GDH and ME where 6% acrylamide gels were used. Gels were cast in glass tubes that were 70 mm long and 6 mm in diameter. They were allowed to polymerize for at least one hour. The gels were pre-run for 45 minutes at 80 V and 20 µl of enzyme extract was loaded per gel. Electrophoresis was carried out initially for 30 minutes at 80 V, and then for 80 to 240 minutes (depending on the mobility of particular enzyme) at 160 V.

Staining procedures were essentially based on the protocol for isoenzymes given by Bošković et al. (4). Staining for AMY and GPI was done in accordance with Vallejos (17), and for GOT, ME, MNR and PGM in accordance with Wendel and Weeden (19). Amylase and peroxidase staining were performed at room temperature, while the others were done at 35 - 37°C. After staining, the gels were fixed with 7% acetic acid, except for peroxidase and kept in distilled water in the refrigerator at 4°C.

Genetic interpretations for regions atributed to polymorphic loci were proposed. However, in the absence of segregation data, these interpretations should be considered tentative. Alleles and loci were labeled in accordance with suggestions given by Weeden (18) and Tobutt (16).

Data obtained by analysis of polymorphic loci were transformed to a binary system [0/1 code for band (allele) presence or absence]. The unweighted pair group method with arithmetic mean (UPGMA) was used to construct a dendrogram. Statistical analysis was performed using the Statistica (StatSoft, Inc., Tulsa, Oklahoma, USA) program.

Results

Isoenzyme banding patterns. Of 18 analyzed enzymes, three were monomorphic: G6PDH, GPI and PGD. Battistini and Sansavini (2) have also reported that G6PDH and GPI are monomorphic in apricot. However, Byrne and Littleton (5) and Manganaris et al. (11) have observed polymorphism for PGD. This discrepancy is most likely due to differences in the electrophoretic procedure. The remaining enzymes were polymorphic. Sample zymograms of the enzymes not illustrated below are available on request from the authors.

Acid phosphatase (ACP). Only the fast zone showed polymorphism with two bands – *a* and *b* and three genotypes: *aa*, *bb* and *ab* (Figure 1). Battistini and Sansavini (2) and Manganaris et al. (11) found also one polymorphic region with three and two genotypes respectively.

Alanine aminopeptidase (AAP). One polymorphic region was detected and two loci were proposed (Figure 2). The locus closer to the anode was marked as *Aap-1* and had four genotypes: presence of fast band (*a*-), presence of slow band (*b*-), presence of both bands (*ab*) and lack of activity (*nn*). The locus closer to the cathode was marked as *Aap-2* and had four bands or alleles (*a*, *b*, *c*, *d*) and four genotypes (*aa*, *bb*, *bd*, *cd*).

Alcohol dehydrogenase (ADH). Although three polymorphic zones of activity were observed, only the most anodal region could be reliably scored. It was marked as locus *Adh-1* with two genotypes (*bb* and *ab*).

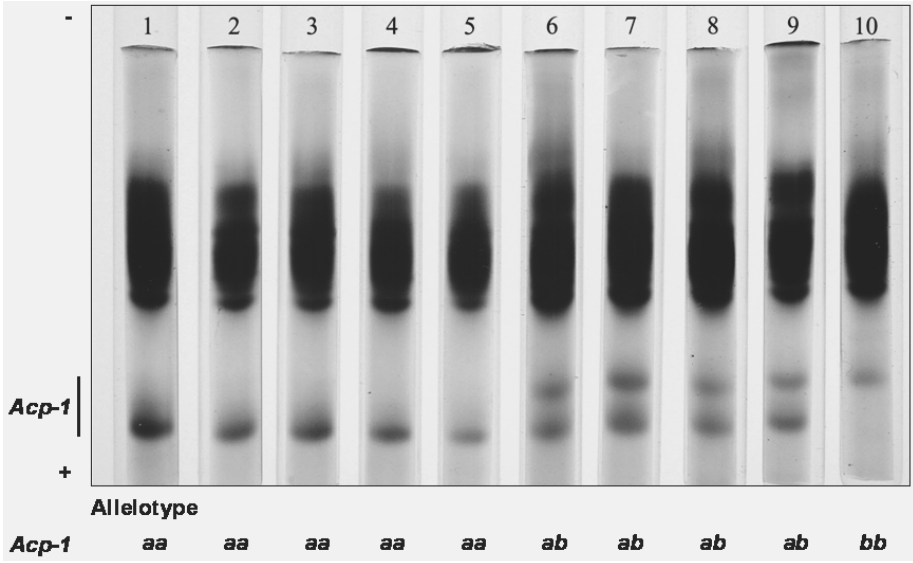

Figure 1. ACP (acid phosphatase) genotypes of analyzed apricot cultivars with proposed genetic interpretation on the x-axis. The lanes are: 'Vera' (1), 'Hungarian Best' (2), 'Kostjuzhenzkyi' (3), 'Cegledi Orijas' (4), 'Kecskemeter Rose' (5), 'Nugget' (6), 'Frühe Kittse' (7), 'Stark Early Orange' (8), 'Karola' (9), 'Stella' (10).

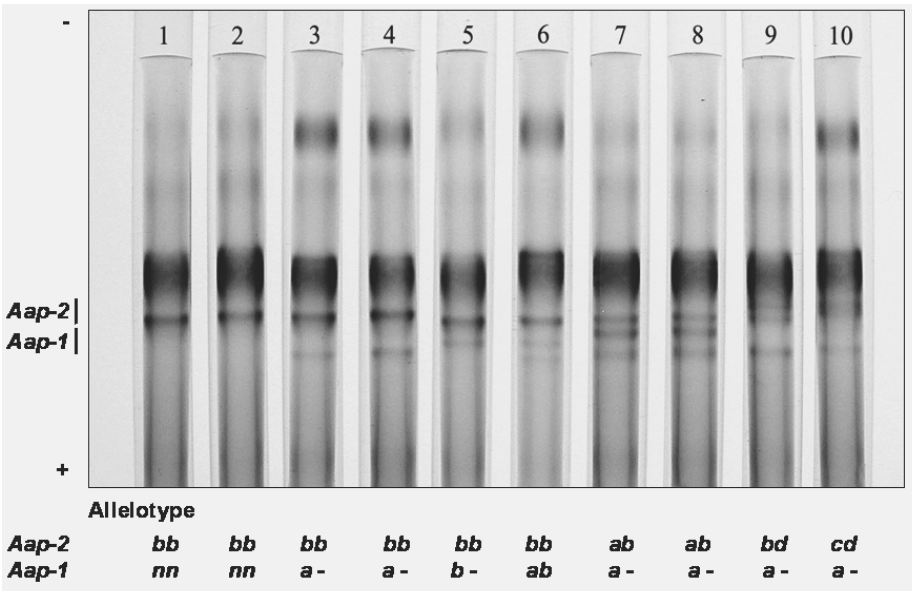

Figure 2. AAP (alanine aminopeptidase) genotypes of analyzed apricot cultivars with proposed genetic interpretation on the x-axis. The lanes are: 'Melitopol Early' (1), 'Arzami Aromatic' (2), 'Hungarian Best' (3), 'Detskyi' (4), 'Sulmona' (5), 'Harcot' (6), 'Fruhe Kittse' (7), 'Karola' (8), 'Oranzhevokrasnyi' (9), 'Nectarine – Apricot' (10).

Alkaline phosphatase (AKP). One region of activity was detected and it was identical to locus *Acp-1* of acid phosphatase. Identity of *Acp-1* and *Akp-1* loci was also described in cherry (3) and peach (7).

Amylase (AMY). Two zones of activity were explained as three polymorphic loci (Figure 3). The locus closest to the anode, *Amy-1*, had two alleles (*a, b*) and three genotypes (*aa*, *bb*, *ab*). Locus *Amy-2* showed the greatest variability with four alleles (*a*, *b*, *c*, *d*) and nine different genotypes (*ab*, *ac*, *ad*, *bb*, *bc*, *bd*, *cc*, *cd* and *dd*). Locus *Amy-3* had only two genotypes: presence of band (*a*-) and absence of activity (*nn*).

Endopeptidase (ENP). There was one polymorphic region. Three alleles (*a*, *b*, *c*) and three genotypes (*bb*, *ab*, *ac*) were found.

Esterase (EST). Among the studied enzymes, esterase gave the most complex zymograms (Figure 4). Five polymorphic loci were proposed: *Est-1* with four genotypes (*aa*, *ac*, *bc*, *cc*), *Est-2* with two genotypes (*bb*, *ab*), *Est-3* with five different genotypes (*nn*, *b-* , *c-*, *ac*, *bc*), *Est-4* with either present or absent band (*a-*, *nn*), and *Est-5* with four genotypes (*nn*, *a-*, *b -*, *ab*).

Glutamate dehydrogenase (GDH). One highly polymorphic region with three alleles (*a*, *b*, *c*) and six different genotypes (*aa*, *bb*, *cc*, *ab*, *ac*, *bc*) were detected (Figure 5). This enzyme is a hexamer. If alleles are in the homozygous state only one band can be observed on the zymogram, and if they are in the heterozygous state seven bands can be observed. Of these seven bands, the central one has the strongest activity, while the more anodal and cathodal bands become weaker and weaker. Due to its high polymorphism and good resolution, GDH proved to be one of the most useful enzymes for the identification of apricot cultivars.

Glutamate oxaloacetate transaminase (GOT). This enzyme has a dimeric structure. One polymorphic region was revealed, with two homozygous genotypes (*aa* and *bb*), which gave one band each and one heterozygous genotype (*ab*), resulting in three bands

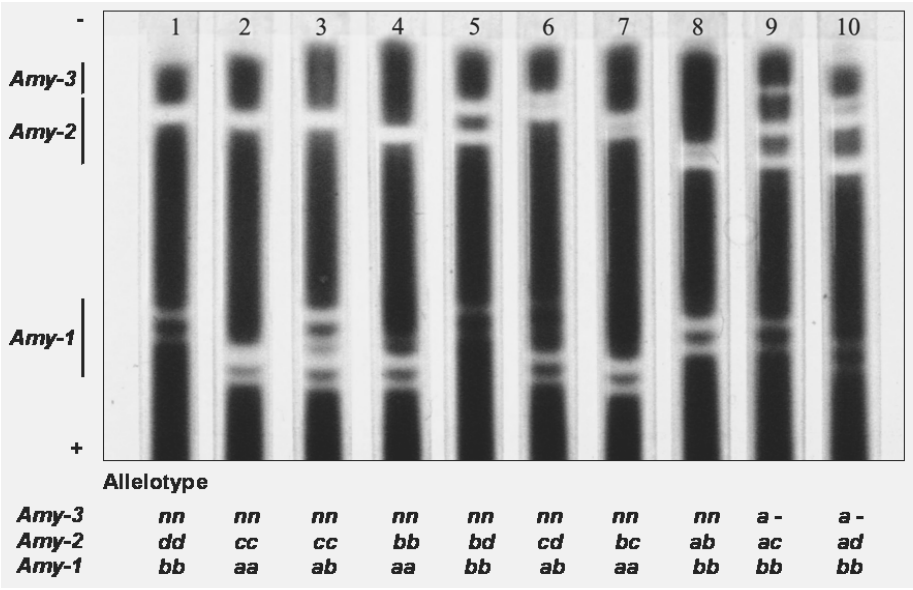

Figure 3. AMY (amylase) genotypes of analyzed apricot cultivars with proposed genetic interpretation on the x-axis. The lanes are: 'Detskyi' (1), 'NS-4' (2), 'Vynoslivyi' (3), 'Drjanovska Kasna' (4), 'Silistrenska Kompotna' (5), 'Bergeron' (6), 'Cegledi Biborkajszi' (7), 'Hindu-kush' (8), 'Nectarine – Apricot' (9), 'Oranzhevokrasnyi' (10).

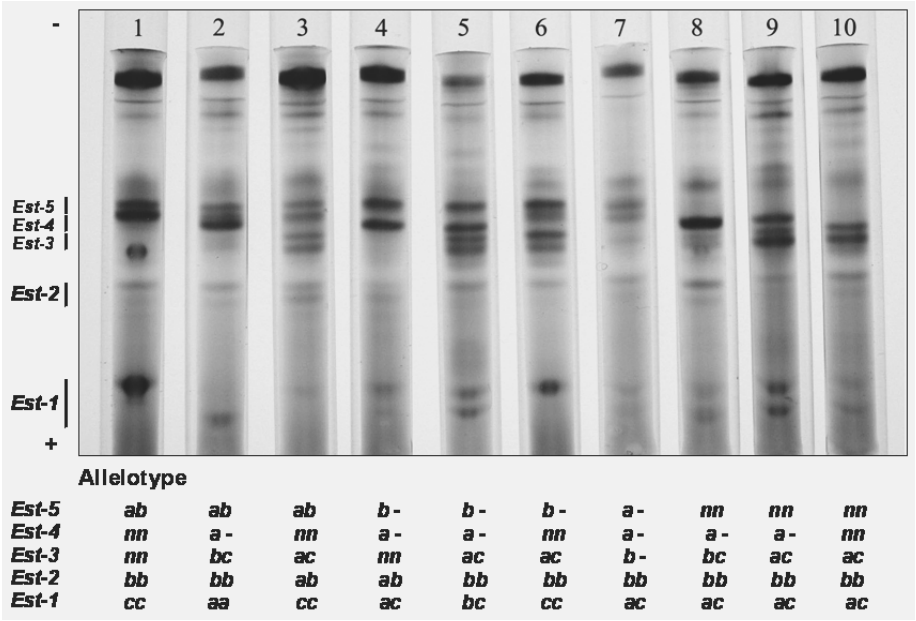

Figure 4. EST (esterase) genotypes of analyzed apricot cultivars with proposed genetic interpretation on the x-axis. The lanes are: 'Arzami Aromatic' (1), 'Cacak's Flat' (2), 'Jubileinyi' (3), 'Nectarine – Apricot' (4), 'Nugget' (5), 'Harcot' (6), 'Bergeron' (7), 'Hungarian Best' (8), 'Hindu-kush' (9), 'Stella' (10).

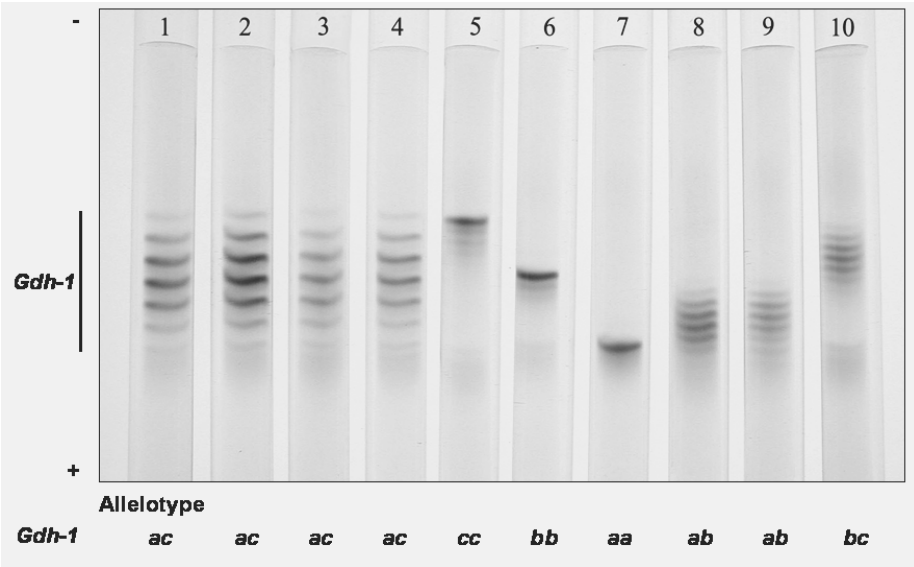

Figure 5. GDH (glutamate dehydrogenase) genotypes of analyzed apricot cultivars with proposed genetic interpretation on the x-axis. The lanes are: 'Hungarian Best' (1), 'Cegledi Orijas' (2), 'Biljana' (3), 'Alfred' (4), 'Selena' (5), 'Arzami Aromatic' (6), 'Harcot' (7), 'Stella' (8), 'Senetate' (9), 'Hindu-kush' (10).

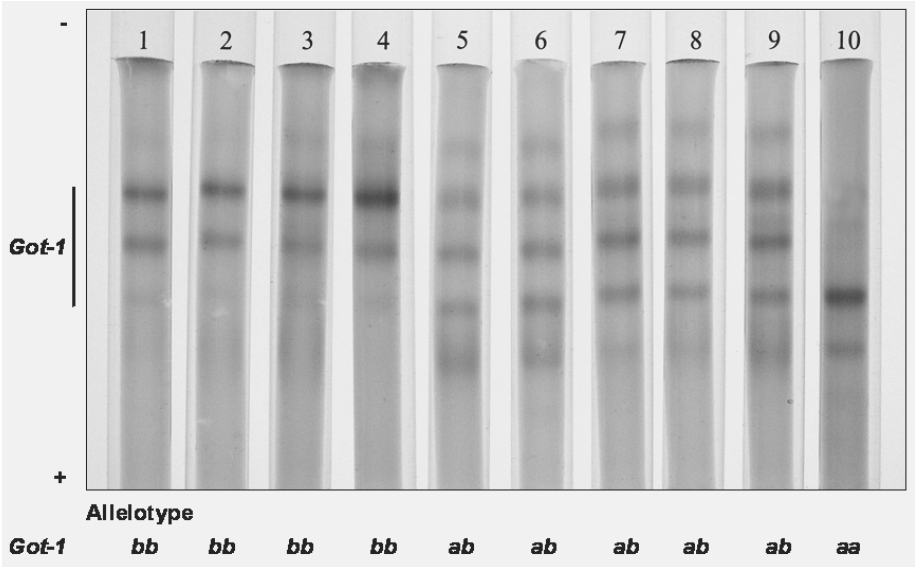

Figure 6. GOT (glutamate oxaloacetate transaminase) genotypes of analyzed apricot cultivars with proposed genetic interpretation on the x-axis. The lanes are: 'Krasnyi Partizan' (1), 'Roxana' (2), 'Drjanovska Kasna' (3), 'Bergeron' (4), 'Cacak's Gold' (5), 'Hungarian Best' (6), 'Ambrosia' (7), 'Cegledi Orijas' (8), 'Kishiniev Early' (9), 'Detskyi' (10).

on the zymogram (Figure 6).

Leucine aminopeptidase (LAP). One polymorphic region with two loci was observed. It was identical to that of AAP. The same case was reported in cherry (3) and in peach (7).

Malic enzyme (ME). This enzyme is tetramer. There was one polymorphic region with two homozygous genotypes which gave one band, and one heterozygous genotype which gave five bands.

Menadione reductase (MNR). There were two zones of activity (Figure 7). One of them was polymorphic with two genotypes (*aa* and *ab*).

Peroxidase (PRX). Two zones of strong activity and one of very weak activity were detected. One zone showed polymorphism with two alleles (*a*, *b*) and three genotypes (*aa*, *bb*, *ab*).

Phosphoglucomutase (PGM). One polymorphic region was revealed. There were two aleles (*a* and *b*) and two genotypes (*aa* and *ab*).

Shikimate dehydrogenase (SDH). Only one polymorphic region with two genotypes (*bb* and *ab*) was detected.

Cultivar identification. Out of 50 studied cultivars, 31 were uniquely identified. The remaining 19 cultivars fell into the following six groups: 1) 'Hungarian Best' group: 'Hungarian Best', 'Gönci Magyar Kajszi', 'Cacak's Gold', 'Biljana', 'Vera' and 'NS–5' 2) 'Cegledi Orias' group: 'Cegledi Orias', 'Ligeti Orias', 'Szegedi Mammut' and 'Kostjuzhenskyi' 3) 'Ambrosia' group: 'Ambrosia', 'Festivalna' and 'NS – 3' 4) 'Stark Early Orange' and 'Nugget' 5) 'Frühe Kittse' and 'Karola' 6) 'Frushka Gora Early' and 'Novi Sad Early'.

Among the studied enzymes, EST, AMY, AAP (LAP) and GDH exhibited the highest efficiency in cultivar characterization, revealing 20, 17, 7 and 6 phenotypes respectively. These four enzymes were sufficient for unique identification of 31 apricot cultivars.

Variability in cultivar groups by geographical origin. The analyzed apricot cultivars were divided into six groups according to their geographical origin (Table 1). The cultivar group of Central Asian origin consisted of

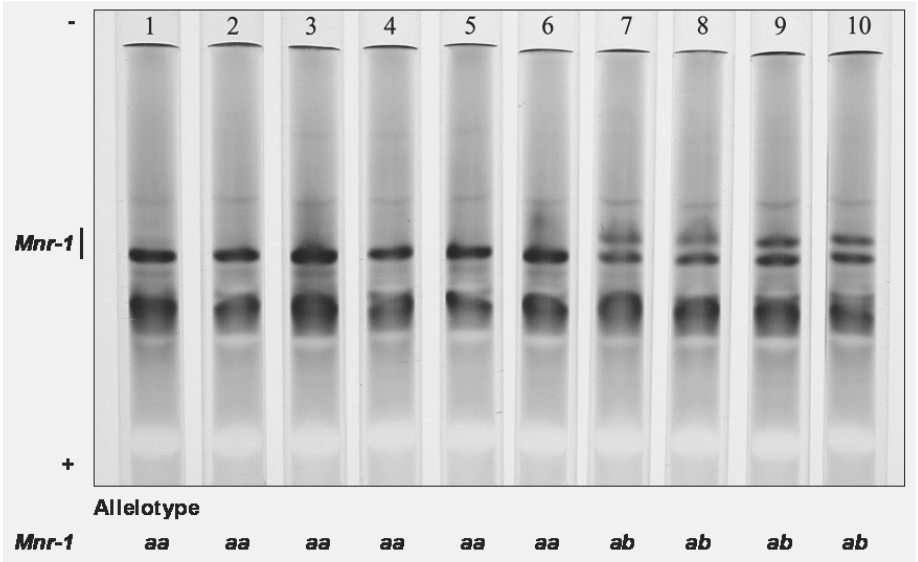

Figure 7. MNR (menadione reductase) genotypes of analyzed apricot cultivars with proposed genetic interpretation on the x-axis. The lanes are: 'Tyrinthos' (1), 'Cacak's Gold' (2), 'Polonais' (3), 'Ligeti Orias' (4), 'Kecskemeter Rose' (5), 'Kecskemeter Rose' (6), 'Nectarine – Apricot' (7), 'Jubileinyi' (8), 'Vinoslivyi' (9), 'Hindu-kush' (10).

four cultivars and had the highest number of polymorphic loci – 16 (out of 20 proposed). The group of nine cultivars from the former USSR countries had 15 polymorphic loci. The East European group of 16 cultivars, and the West European group of six cultivars were both polymorphic for 12 loci. The North American group consisted of six cultivars and was polymorphic for 11 loci. The lowest variability was observed in a group of nine cultivars and selections from Serbia, with only eight polymorphic loci.

Genetic relationship among cultivars. A dendrogram, based on UPGMA cluster analysis indicates four clusters with different numbers of cultivars (Figure 8). Cluster A is the biggest and contained 39 cultivars of mostly European origin and 'Alfred', an American cultivar. The 'Hungarian Best' group (six cultivars) is connected to the 'Ambrosia' group (three cultivars), while together they are connected to the 'Cegledi Orias' group (four cultivars) at a small distance. This indicates that these cultivars are closely related.

Cluster B consisted of five cultivars originating from North America. These cultivars were put in three groups that were genetically fairly distant from each other. 'Stella' was the only cultivar in the first group. The second group consisted of two cultivars ('Stark Early Orange' and 'Nugget') that had the same isoenzyme profile. The third group was composed of two cultivars: 'Harcot' and 'NJ A-1' that were connected at a small linkage distance. Their grouping agrees with their pedigree, since 'NJ A-1' is a parent of 'Harcot'.

Cluster C had four cultivars. Two of them are of Ukrainian origin ('Vynoslivyi' and 'Jubileinyi') and two originated from Central Asia ('Arzami Aromatic' and 'Nectarine – Apricot'). Though in the same cluster, these cultivars are genetically rather distant, because they are connected at a long linkage distance.

Cluster D consisted of two Central Asian cultivars ('Oranzhevokrasnyi' and 'Hindu-kush') which were distant from each other, suggesting significant genetic differences.

Table 1. Polymorphic loci in apricot cultivar groups of diverse geographical origin.

Geographical orgin of cultivar groups	Cultivars	No. of cultivars	No. of polymorphic loci	Polymorphic loci
East and Central Europe (Hungary, Romania, Bulgaria, Austria, Czech Republic)	Hungarian Best, Gonci Magyar Kajszi, Cegledi Orias, Ligeti Orias, Szegedi Mammut, Cegledi Biborkajszi, Kecskemeter Rose, Sulmona, Selena, Marculesti 22/6, Festivalna, Silistrenska Kompotna, Drjanovska Kasna, Roxana, Frühe Kittse, Karola	16	12	*Aap (Lap)-1, 2; Acp (Akp)-1; Amy-1, 2, 3; Enp-1; Est-1, 5; Gdh-1; Got-1; Pgm-1*
Former USSR (Russia, Ukraine, Moldavia)	Kostjuzhenskyi, Vynoslivyi, Jubilejnyi, Detskyi, Senetate, Kishiniev Early, Melitopol Early, Priusadebnyi, Krasnyi Partizan	9	15	*Aap (Lap)-1; Amy-1, 2, 3; Enp-1; Est-1, 2, 3, 4, 5; Gdh-1; Got-1; Mnr-1; Pgm-1; Sdh-1*
Serbia	Cacak's Gold, Cacak's Flat, Biljana Vera, NS-3, NS-4, NS-5, Frushka Gora Early, Novi Sad Early	9	8	*Aap (Lap)-1; Amy, 1, 2; Enp-1; Est-1, 5; Gdh-1; Got-1*
Western and Southern Europe (France, Italy, Greece)	Bergeron, Polonais, San Castrese, Baracca, Ambroisia, Tyrinthos	6	12	*Aap (Lap)-1; Amy-1, 2, 3,; Enp-1; Est-1, 3, 4, 5; Gdh-1; Got-1; Me-1*
North America (USA, Canada)	Stark Early Orange, Nugget, Stella, Alfred, NJ A-1, Harcot	6	11	*Aap (Lap)-1; Acp (Akp)-1; Amy-2; Est-1, 3, 4, 5; Gdh-1; Got-1; Me-1; Pgm-1*
Central Asia	Oranzhevokrasnyi, Hindu-kush, Arzami aromatic, Nectarine-Apricot	4	16	*Aap (Lap)-1, 2; Adh-1; Amy-1, 2, 3; Est-1, 2, 3, 4, 5; Gdh-1; Me-1, Mnr-1; Prx-1; Sdh-1*

Discussion

The electrophoretic procedure provided good separation and clear activity for all attempted enzymes allowing detection of 20 loci and 54 alleles. Three of these loci, *Enp-1*, *Mnr-1* and *Sdh-1*, have not been previously reported in apricot.

Out of 50 apricot cultivars, 31 had unique isoenzyme profiles, while the other 19 were divided into six groups. The Hungarian Best group is the most numerous and consisted of two cultivars originating from Hungary and four from Serbia. These cultivars could be clones or open-pollinated seedlings of 'Hungarian Best', which is an old cultivar that is widely planted in countries of Central and East Europe, including Serbia.

The highest isoenzyme variability was recorded in the cultivars originating from Central Asia. These cultivars were mutually distant on the dendrogram, a fact which points to their genetic differences. This agrees with earlier reports that this cultivar group is the most variable (9). The cultivars from the former USSR countries also showed high polymorphism. It is not surprising as some of them (e.g. 'Vynoslivyi', 'Senetate', 'Priusadebnyi', 'Melitopol Early' and 'Kishiniev Early') originated from apricot cultivars of the Central Asian ecogeographical group (14). The European and North American cultivars expressed a medium level of variability. Polymorphism was lowest in cultivars and selections from Serbia, indicating their narrow genetic base.

Most of the Serbian cultivars are genetically close to Hungarian cultivars, which indicates their common origin. 'Nectarine – Apricot' was a genotype of unknown origin with glabrous fruit skin. It was genetically much closer to Central Asian than to European cultivars. Sychov (15) reported that in Central Asia, there are glabrous apricot cultivars known as lyuchaks or nectarine – apricots.

The relationships of some cultivars obtained with isoenzyme analysis are supported by data obtained with SSR markers by Maghuly et al. (10). They found that 15 cultivars from the 'Hungarian Best' group were closely related and five of them could even be synonyms. Also, three cultivars from the 'Cegledi Orias' group could not be distinguished, as in our study. The UPGMA dendrogram illustrated a decrease in genetic diversity of cultivars from Central Asia to the former USSR and further to Eastern and Western Europe. This is coherent with historical dissemination of apricot from its center of origin in Central Asia (6). The position of North American cultivars between the European and the Central Asian cultivars indicates that they also have Asian germplasm in their pedigrees in addition to the European one, which agrees with previous studies (8, 10). Based on our results, subgroups within the European group could not be established. This is most likely due to the existence of cultivars derived from crosses between these subgroups (6, 13).

The results of this study confirm that the Central Asian group of apricot cultivars is characterized by higher variability than cultivars from the European eco-geographical group and that isoenzymes are useful expressed markers that can be used in apricot cultivar identification.

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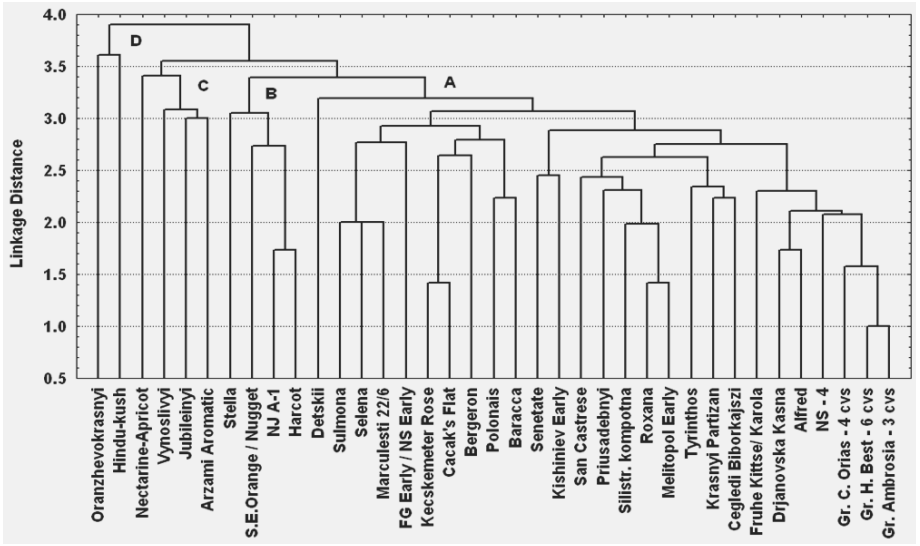

Figure 8. Dendrogram of 50 apricot cultivars generated from the isoenzyme data by UPGMA cluster analysis. Abbreviations used for cultivar names are: S. E. Orange is 'Stark Early Orange', FG Early is 'Frushka Gora Early', NS Early is 'Novi Sad Early', Silistr. kompotna is 'Silistrenska kompotna', C. Orias is 'Cegledi Orias', and H. Best is 'Hungarian Best'. Abbreviation Gr. is for group and cvs is for cultivars.

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