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DEHYDROGENASE ISOENZYME POLYMORPHISM IN SELECTED ALMOND GENOTYPES (*PRUNUS AMYGDALUS* BATSCH.)

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Abstract

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Isoenzyme polymorphism was studied in 20 almond (*Prunus amygdalus* Batsch.) genotypes, selected in the region of northern Serbia (Vojvodina). Five enzyme systems: alcohol dehydrogenase (ADH), formate dehydrogenase (FDH), glutamate dehydrogenase (GDH), malate dehydrogenase (MDH) and shikimate dehydrogenase (SDH) were studied using the method of polyacrilamide gel electrophoresis. Three systems (FDH, GDH and SDH) were found to be polymorphic. This polymorphism allowed unique identification of five genotypes, while the remaining 15 genotypes were divided into five groups. Cluster analysis (UPGA method) was used to construct a dendrogram on which two clusters with different number of genotypes could be separated. Obtained results indicate that isoenzyme polymorphism in almond is higher than previously found. Studied enzyme systems, FDH, GDH and SDH, can be used to identify genetic variability in almond.

Key words: Prunus amygdalus, electrophoresis, dehydrogenase, polymorphism, cluster analysis
Abbreviations: aspartate aminotransferase (AAT), aconitase (ACO), acid phosphatase (ACP), alcohol dehydrogenase (ADH), formate dehydrogenase (FDH), glutamate dehydrogenase (GDH), glucose phosphate isomerase (GPI), isocitrate dehydrogenase (IDH), leucine aminopeptidase (LAP), malate dehydrogenase (MDH), 6-phosphogluconate dehydrogenase (PGD), phosphoglucomutase (PGM), shikimate dehydrogenase (SDH), polyacrilamide gel electrophoresis (PAGE)

Introduction

Isoenzymes are multiple molecular forms of enzymes that have the common substrate and differ based on their physical characteristics such as molecular mass, electrical charge, shape and protein structures. Thanks to their difference in electrophoretic mobility they can be separated and analyzed.

Isoenzymes have been used in plant genetic and breeding due to their characteristics such as simple inheritance, codominant expression, lack of gene interactions, polymorphism present in many plant species and absence of environmental effects. They can be identified in different tissues and this process is relatively simple and inexpensive (Weeden and Wendel, 1989). Isoenzyme's variability is rich source of ge-

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netic markers that can be used for identification of cultivars and hybrids, early selection, detection of genetic diversity, quantification of genetic relationships between populations (Byrne, 1989).

Arulsekar et al. (1986) studied almond isoenzyme polymorphism. By studying six enzyme systems – AAT, GPI, LAP, MDH, PGD and PGM the authors have found polymorphism in 9 of 12 enzyme loci while peach polymorphism was found only in one. Byrne (1990) also reported that almond had greater isoenzyme polymorphism compared to apricot and peach. Origin of Californian almond germplasm was determined by Hauagge et al. (1987) who studied four enzyme systems (AAT, GPI, LAP and PGM). Based on variability of nine enzyme systems in pollen, Cereso et al. (1989) concluded that the most useful enzyme systems for cultivar identification were PGM, CAT, ACP and LAP. According to Jackson and Clarke (1991) IDH, 6PGD and SDH in almond are polymorphic systems with dimeric structure. Polymorphism of GPI, LAP, PGM, SDH, IDH, AAT, ACO, and MDH in almond was reported by Arus et al. (1992). Iranian almond cultivars, as well as species, showed new alleles for IDH, AAT, PGM, GPI and SDH, which indicated this area as an important gene pool for almond (Vezvaei, 2003).

We studied dehydrogenase isoenzyme polymorphism of 20 almond genotypes selected in the region of Slamkamen hill with a goal to establish usability of studied isoenzymes in identification of genetic variability in almond.

Materials and Methods

Twenty almond genotypes selected in the region of northern Serbia (Vojvodina) were studied. Five enzyme systems - alcohol dehydrogenase (ADH), formate dehydrogenase (FDH), glutamate dehydrogenase (GDH), malate dehydrogenase (MDH) and shikimate dehydrogenase (SDH) - were analyzed.

Inner bark of one-year-old shoots was used for enzyme extraction. Preparation of samples was done in accordance with the protocol given by Boskovich et al. (1994) for stone fruit species. Vertical PAGE was used for isoenzyme analysis. Polyacrylamide gel containing 8% acrylamide was used for separation.

Electrophoresis was carried out on +4°C through three phases. The gels were pre-run for 45 min at 100 V after which 25 µl of enzyme extract was loaded per gel. Electrophoresis was carried out initially for 45 minutes at 100 V, and then at 400 V for variable time (3 to 4 hours), depending on the mobility of particular enzyme. Staining procedures were essentially based on the protocol for isoenzymes given by Boskovich et al. (1994).

Genetic interpretations for regions attributed to polymorphic loci were proposed. Alleles and loci were labeled in accordance with suggestions given by Weeden (1988) and Tobutt (1993). 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 conducted with the program 'Statistica' (StatSoft, Inc., Tulsa, Oklahoma, USA).

Results and Discussion

Out of five analyzed enzyme systems, three (FDH, GDH and SDH) were polymorphic. Each of those three systems was polymorphic in one locus that had two alleles. Due to the absence of hybridization and segregation data, genetic interpretations for polymorphic loci should be considered tentative.

Alcohol dehydrogenase. ADH analysis resulted in zymograms with three regions of activity. However, polymorphism was not detected in any of them. These results confirmed findings by Friend and Carter (1989), Cerezo et al. (1989) and Mowrey et al. (1990) that ADH in almond is monomorphic system. Contrary, ADH polymorphism was observed in the other stone fruit species: peach (Gasich et al., 2001) and apricot (Milatovich et al., 2009).

Formate dehydrogenase. Literature indicates that, so far, this system was not studied in almond.

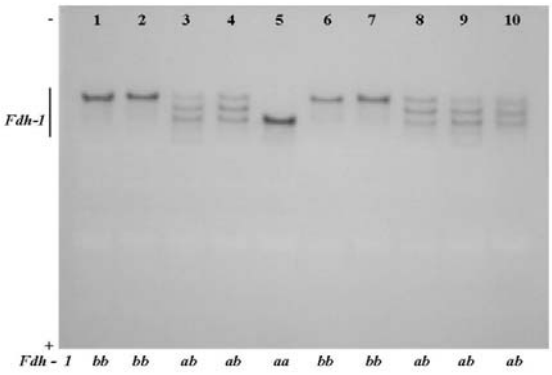

Fig. 1. FDH (formate dehydrogenase) zymograms of analyzed almond genotypes with proposed genetic interpretation: 12/03 (1), 23/03 (2), 29/03 (3), 11/03 (4), 14/03 (5), 27/03 (6), 19/03 (7), B/04 (8), 22/03 (9), 18/03 (10)

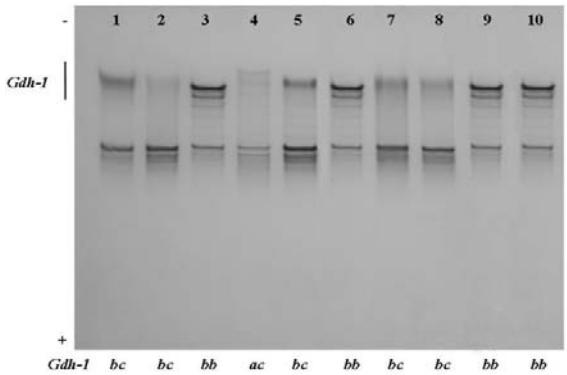

Fig. 2. GDH (glutamate dehydrogenase) zymograms of analyzed almond genotypes with proposed genetic interpretation: 12/03 (1), 23/03 (2), 1/03 (3), 10/03 (4), 3/03 (5), 27/03(6), 27/03 (7), 29/03(8), 16/03 (9), 22/03 (10)

This system was characterized with good activity and variability in one locus. This locus, marked as *Fdh-1*, had two alleles and three genotypes (Figure 1). Heterozygous phenotype *ab* was presented most frequently (10 genotypes). Homozygous genotype *bb* was found in nine, while homozygote phenotype *aa* was presented only in genotype 14/03. Zymograms showed three bends of the heterozygote phenotype, which confirmed Weeden and Wendel's (1989) findings about dimeric structure of this enzyme.

Glutamate dehidrogenase. On GDH zymograms two regions of activity was observed of which the region closer to the cathode was highly polymorphic (Figure 2). Three phenotypes were proposed for the loci marked as *Gdh -1: bb* (14 genotypes), *ac* (genotypes 10/03) and *bc* (5 genotypes). Presence of seven bands in heterozygote phenotypes *ac* and *bc* is in accordance with reports by Weeden and Wendel (1989) about its hexameric structure. This is the first report of GDH polymorphism in almond. Mowrey et al. (1990) analyzed isoenzymes in almond pollen and noted lack of variability for GDH.

Malate dehidrogenase. MDH analysis resulted with a simple zymogram with one region of activity but without observable variability. In contrast to these results, Byrne (1989) and Cerezo and Socias I Company (1992) noted variability in locus closer to cathode. These differences in results can be attributed to different tissue used for extraction (leaf) and different type of gel (starch) used by these authors.

Shikimate dehydrogenase. SDH analysis produced zymograms that showed one polymorphic region of activity marked as *Sdh - 1* (Figure 3). Three phenotypes were identified: *aa* (genotype 27/03), *bb* (eight genotypes) and *ab* (11 genotypes). Cerezo and Socias I Company (1992), as well as, Vezvaei (2003) have also found one polymorphic locus with three phenotypes in almond. Polymorphism of SDH was also identified in other species of genus *Prunus* - apricot (Zhebentyayeva and Sivolap, 2000; Milatovich et al., 2009), peach (Gasich et al., 2001) and cherry (Granger, 1996; Corts et al., 2008).

Of 20 studied almond genotypes, five (10/03, 12/03, 14/03, 23/03 and 27/03) showed greater genetic variability. These genotypes had unique zymograms, while the remaining 15 genotypes were divided into five groups.

Application of cluster analysis on all polymorphic loci resulted in a dendrogram shown on Figure 4. Analyzed almond genotypes are connected on different hierarchical levels. Two related groups are identi-

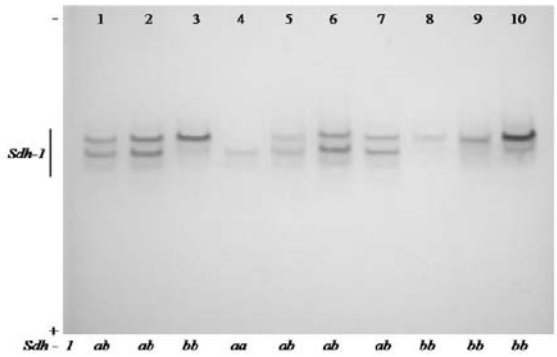

Fig. 3. SDH (shikimate dehydrogenase) zymograms of analyzed almond genotypes with proposed genetic interpretation: 28/03 (1), 29/03 (2), 12/03 (3), 27/03 (4), 23/07 (5), 14/03 (6), 22/03 (7), 25/03 (8), 11/03 (9), 1/03 (10)

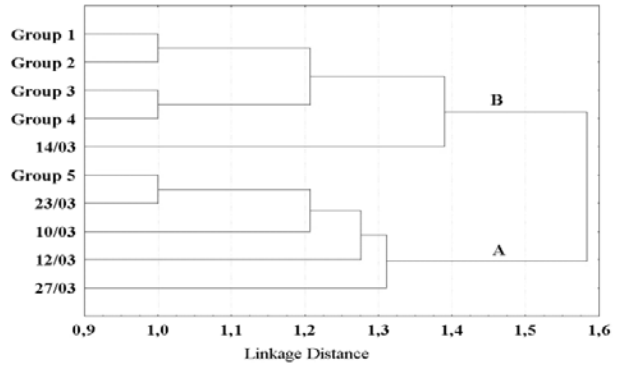

Fig. 4. Dendrogram of 20 analyzed almond genotypes generated from the isoenzyme data by UPGMA cluster analysis: Group 1 - 1/03, 24/03, 25/03; Group 2 - 11/03, 15/03, 18/03; Group 3 - 16/03, 17/03, 22/03, 28/03, A/04; Group 4 -19/03, B/04; Group 5 - 3/03, 29/03

fied: Cluster A which includes six genotypes and cluster B with 14 genotypes. In cluster A, group 5 is closely related to genotypes 23/03 (d=1.0). In cluster B, groups 1, 2, 3 and 4 together make a subgroup which is the most dissimilar (d=1.4) to genotype 14/03. Polymorphism of GDH was the most significant of all studied isoenzymes for cluster groupings.

Conclusions

Polymorphism was observed in three of five studied enzyme systems (FDH, GDH and SDH). One polymorphic locus was identified for each of these three systems. Polymorphism of FDH, GDH and SDH equally affected genotype separation because all three systems had two alleles and three genotypes.

Five of 20 examined genotypes had distinctive izoenzyme profiles. The remaining genotypes were separated into five groups.

Dendrogram shows two clusters with different number of cultivars. Cluster A includes six and cluster B 14 genotypes. Results show that enzyme systems FDH, GDH and SDH can be useful for identification of genetic variability in almond.

Acknowledgements

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