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PEROXIDASE ISOENZYME POLYMORPHISM IN THE GENUS *PRUNUS*, SUBGENUS *CERASUS**

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SUMMARY: The polymorphism of peroxidase was studied in 31 cherry accessions, representing six following species Prunus cerasus, Prunus avium, Prunus fruticosa, Prunus mahaleb, Prunus serrulata, Prunus gondouinii and two widely-used standard cherry rootstocks 'Gisela 5' and 'Colt'. Six 'Oblačinska'sour cherries, four wild sweet cherries, five ground and one mahaleb genotypes were selected from the natural populations of Serbia. Inner barks from one-year-old shoots and young actively growing leaves were used for protein extraction. The polymorphism of peroxidase was obtained both for leaf and inner bark tissues. The analysis of the leaf material showed the unique zymograms for all six species and two interspecies hybrids. Higher numbers of polymorphic loci and banding patterns were detected when protein was extracted from the leaves, than from inner bark. Obtained results indicate that the polymorphism determination of genus Prunus, subgenus Cerasus can be done on the basis of peroxidase, but it would not be useful for discrimination of different genotypes and clones.

Key words: *peroxidase, electrophoresis, polymorphism, cherry, Prunus spp.*

INTRODUCTION

Conservation of genetic resources, essential for future breeding programs, requires a good characterization of the genetic diversity of germplasm and a proper assignment of individual genotypes to species (Tavaud et al., 2004). A description of morphological

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characteristics is the usual method for preliminary evaluations of genetic diversity because it is fast and simple, but it can be used only among morphologically distinguishable accessions. The morphological variation, a product of the genotype-environment interaction, is an important parameter, but much diversity that remains unexpressed morphologically can be revealed by biochemical and molecular markers. Isoenzymes were among the first markers applied in horticultural science. They allow the identification of plants in early stages of development and are not affected by environmental conditions. They can be separated and analyzed due to their differences in electrophoretic mobility. Isoenzymes are used as genetic markers of the genus *Prunus* because of their stability, co-dominant expression and reproducibility (Martinez-Gomez et al., 2003). For these reasons, isoenzymes are useful for the identification of genetic polymorphism (Daeil, 2004). Over the past decade, different types of molecular markers, such as AFLPs (Tavaud al., 2004), RFLPs (Bouhadida et al., 2007), SSRs (Ercisli et al., 2011) and RAPDs (Zamani et al., 2012), have been used for the genetic characterization of the *Prunus* germplasm and the establishment of genetic relationships between cultivars and species, but the data obtaining through isozymes is relatively inexpensive compared to DNA which analysis require sophisticated instruments.

The isoenzyme analysis is used in genetics and breeding of the genus *Prunus* for identification of cultivars (Milatović et al., 2009, Nikolić et al., 2010), phylogenetic relationships among species (Daeil, 2004) and for analyzing the genetic variability of native populations (Čolić et al., 2010, 2012). Beaver et al. (1995) conducted a research on characterizations of the genus *Prunus* subgenus *Cerasus*, based on isoenzymes. They studied seven isoenzyme systems in sweet, sour and ground cherries, verifying that this technique was efficient in detecting polymorphism among them. Moreover, Corts *et al.* (2008) pointed out that problems with synonymies and homonymies frequently present in the characterization of cultivars can be solved on the basis of isoenzyme genotypes. Čolić et al. (2012) reported that ADH, IDH and SDH were the most polymorphic and most useful to identify genetic variability in the genus *Prunus* subgenus *Cerasus*.

Peroxidases are enzymes with numerous biochemical and physiological roles in higher plants. They participate in plant growth as well as in differentiation and development processes, including auxin catabolism, ethylene biosynthesis, plasma membrane redox system and generation of H_2O_2, cell wall edification, lignifications and suberization, and response to pathogen (Has-Schön et al., 2005). Peroxidase isoenzymes are tissue-specific (Manganaris and Alston, 1991, Zapata et al., 1995) and developmentally regulated (Smila et al., 2007). A study of peroxidase isoenzyme profiles in some sweet cherry rootstocks and cherry varieties conducted by Güçlü and Koyuncu (2012) showed that peroxidase profiles were similar in scions and rootstocks.

Based on the above background, this study was undertaken to compare the peroxidase variation revealed from two tissues (leaf and inner bark tissue), and to establish the usability of peroxidase for evaluating genetic diversity of the genus *Prunus* subgenus *Cerasus*, in order to make germplasm evaluation more efficient.

MATERIAL AND METHODS

The plant material (Table 1) consists of 31 cherry genotypes representing six following species, *Prunus cerasus* (12), *Prunus avium* (11), *Prunus fruticosa (5)*, *Prunus mahaleb (1)*, *Prunus serrulata (1), P. gondouinii(1)* and two interspecies hybrids (Gisela 5 and Colt). Six 'Oblačinska' (autochthonous and heterogeneous cultivar), four wild sweet cherry, five ground cherry genotypes and one mahaleb genotype were selected from natural populations in different parts of Serbia. The selection of genotypes was done according to the observed diversity of phenological and morphological traits of trees and fruits.

Two types of the plant material, inner barks from one-year-old shoots in dormant stage and young actively growing leaves, were used for the extraction and evaluation of peroxidase (PRX) activity. Vertical PAGE was used for the isoenzyme analysis. Polyacrylamide gel containing 8% acrylamide was used for separation. Sample preparation and staining procedures were done in accordance with the protocols given by Bošković et al. (1994) for stone fruit species. The loci of the same enzyme system were numbered progressively, beginning with locus 1 at the most anodal position. Gels were visually observed and bands that represent isoenzyme patterns were analyzed.

RESULTS AND DISCUSSION

The polymorphism of peroxidase (PRX) was established both for the leaf and inner bark tissues. As expected, the two types of tissues revealed different patterns of variation. Our results are agreeable with the findings of Smila et al. (2007) who observed a number of tissue specific isoforms (present in the root and leaf tissues of various pearl millet varieties) for both esterases and peroxidases at each stage of development, where different isozyme banding patterns were obtained. That implies differential activation of genes involved in synthesis of these enzymes at diverse development stages. Greater polymorphism was obtained for the leaf tissues having 12 types of zymograms, while the inner bark tissues showed eight banding patterns. Also, the number of polymorphic loci was higher for the leaf (Figure 1) than for the inner bark (Figure 2) tissues.

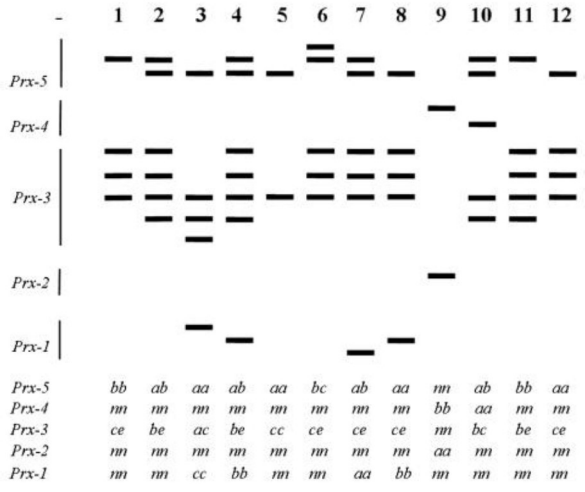

Figure 1. Types of peroxydase (PRX) zymograms obtained from leaves

The results of the electrophoresis were presented in Table 1. The analysis of the leaf material showed the unique zymograms of all six species and two interspecies hybrids. Further, unique activity in locus *Prx-2* was detected only in Colt. Obtained polymorphism for PRX is higher than Čolić et al. (2012) established for dehydrogenase. That makes leaves PRX very useful as potential marker for discrimination of species in subgenus *Cerasus*.

All the *P. avium* (Figure 1, zymogrames 1, 11) and *P. fruticosa* genotypes (Figure 1, zymogrames 8, 12) had zymograms that lacked in *″a″* or *″b″* bands at the locus *Prx -5*, respectively, while both bands were found in the sour cherry genotypes (Figure 1, zymogrames 2, 4,10,). Moreover, the existence of the homozygous locus *Prx 1* that had a ″b″ band both in sour and ground cherries supports the fact that sour cherries arose from hybridization between ground and sweet cherries.

P. avium showed two types of zymograms and among eleven genotypes only KK 6/10 showed pattern 11 (Table 1), with an extra ″b″ band at the locus *Prx-3* (Figure 1). The position of the locus *Prx-3* and three alleles of the cultivated genotypes correspond to the results of Granger et al. (1993), but our results indicate that the number of alleles of wild forms is higher.

Three banding patterns (Table 1) were observed in the sour cherry genotypes. On the basis of activity for the locus *Prx-1*, the commercial *P. cerasus* cultivars can be distinguish from the ‘Oblačinska’, an autochthonous and heterogeneous cultivar. The ornamental genotype BNS lacked *″d″* and *″e″* bands at the locus *Prx-3*, and it showed activity only in the locus *Prx-4*.

The *Prunus fruticosa* genotypes showed two types of zymograms (8 and 12). Activities were recorded at *Prx-3* and *Prx-5* – the loci with the same alleles. The two genotypes had an additional zone of activity - *Prx-1*.

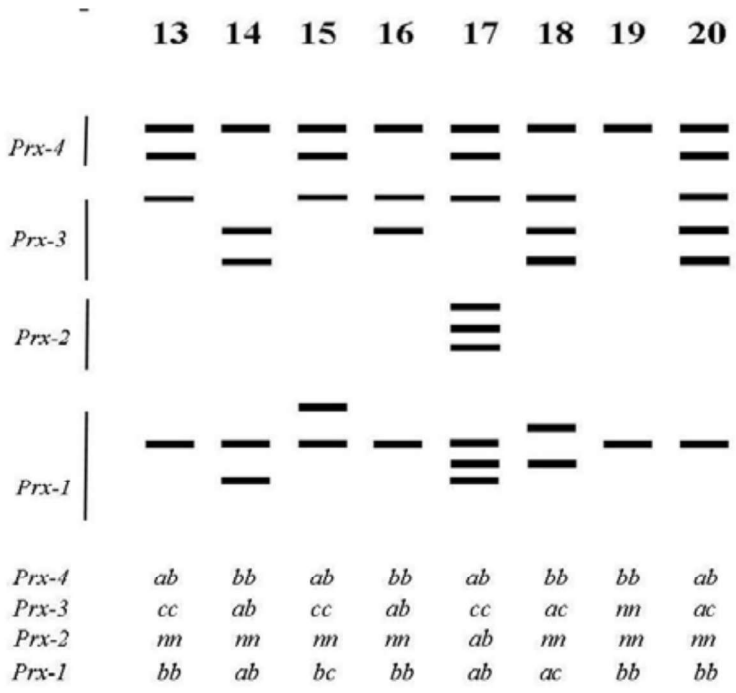

Figure 2. Types of peroxydase (PRX) zymogrames obtained from inner bark

The analysis of inner bark tissues resulted in three zones of activity, except for *P. serrulata* that showed additional activity only at the locus *Prx-2*. As with leaves, the following species could be determined according to their characteristic PRX phenotype: *P. avium, P. serrulata,* Colt, NS KK 6/10. Also, Maynard (*P. cerasus*) can be distinguishing from Oblačinska and commercial sour cherry cultivars on the basis of additional bands in loci *Prx-1* and *Prx-3*.

Greatest polymorphism and three banding patterns were found in *P. avium* (Figure 2, zymogrames 13, 15 and 19). This is not in accordance with the results of Güçlü and Koyuncu (2012), who evaluated a local Turkish rootstock based on *P. avium* and *P. mahaleb*, MaxMa 14, MaxMa 60, CAB 6P and Gisela 5 rootstocks, as well as a cherry variety "0900 Ziraat" and reported that peroxidase system was monomorphic. Also, for *P. avium* and *P. mahaleb*, the obtained polymorphism of PRX was higher than reported by Şeker (2008), who detected a high activity only in one zone.

The number of polymorphic loci varied in depends of a species and tissue used for the analysis, as presented in Table 2. Ten out of the eleven *P. avium* genotypes were monomorphic at the locus *Prx-3,* while an additional allele ″b″ was found for NS KK 6/10 in the leaves samples. When barks were used, variability was detected for the presence or absence of the allele ″b″ at the locus *Prx-1*, activity at the locus *Prx-3* and an allele ″a″ at the locus *Prx-4*.

Table 1. Zymogram patterns of peroxydase (PRX) of the investigated cultivars and genotypes

	Cultivar/genotype	Species/Interspecies hybrid	Type of zymogram	
			Leaves	Inner bark
1	Drogan's yellow	*P. avium*	1	13
2	Celeste	*P. avium*	1	13
3	Victoria	*P. avium*	1	13
4	Early Star	*P. avium*	1	15
5	Vera	*P. avium*	1	13
6	Sara	*P. avium*	1	13
7	DT X9-wild cherry	*P. avium*	1	13
8	DT X3-wild cherry	*P. avium*	1	13
9	DT X7-wild cherry	*P. avium*	1	13
10	DT K9-wild cherry	*P. avium*	1	13
11	NS KK 6/10-dwarf	*P. avium*	11	19
12	Lara	*P. cerasus*	2	16
13	Montmorency	*P. cerasus*	2	16
14	Rexelle	*P. cerasus*	2	16
15	Keleris 16	*P. cerasus*	2	16
16	Oblačinska UD 6	*P. cerasus*	4	16
17	Oblačinska UD 8	*P. cerasus*	4	16
18	Oblačinska D1 R	*P. cerasus*	4	16
19	Oblačinska D4 R	*P. cerasus*	4	16
20	Oblačinska II/10 R	*P. cerasus*	4	16
21	Oblačinska XI/3 R	*P. cerasus*	4	16
22	Maynard-dwarf	*P. cerasus*	4	18
23	BNS-ornamental	*P. cerasus*	10	16
24	SV 1	*P. fruticosa*	8	16
25	SV 2	*P. fruticosa*	12	16
26	SV 3	*P. fruticosa*	12	20
27	SV 5	*P. fruticosa*	8	20
28	SV 7	*P. fruticosa*	12	20
29	Radmilovac	*P. gondouinii*	5	16
30	Amonagawa	*P. serrulata*	6	17
31	TT	*P. mahaleb*	3	14
32	Colt	*P. avium x P. pseudocerasus*	9	15
33	Gisela 5	*P.cerasus x P. canescens*	7	16

The *P. cerasus* genotypes were monomorphic at the locus *Prx-5*, polymorphic at *Prx-3* and they varied for presence or absence of activity at Prx-1 and Prx-4 when leaves were used. Bark samples showed polymorphism for *Prx-1* and *Prx-3*. Our results indicate that a considerable variability of pomological and technological properties established by Nikolić et al. (2005) and Rakonjac et al. (2010) for the 'Oblačinska' sour cherry are not followed by PRX polymorphism.

The *P. fruticosa* genotypes were separated into two groups according to their *Prx-1* activity in leaves. The bark samples were polymorphic at *Prx-3* and *Prx-4*.

Table 2. Polymorphic loci of peroxidase (PRX) in genus *Prunus*, subgenus *Cerasus*

Leaves		Inner bark of one-year-old shoots	
Species	Polymorphic loci	Species	Polymorphic loci
P. avium	*Prx-3*	*P. avium*	*Prx-1, Prx-3, Prx-4*
P. cerasus	*Prx-1, Prx-3, Prx-4*	*P. cerasus*	*Prx-1, Prx-3*
P. fruticosa	*Prx -1*	*P. fruticosa*	*Prx-3, Prx-4*

CONCLUSION

The results of our study confirmed the organ specificity of PRX isoenzyme pattern *P. mahaleb* and *P. serrulata* were distinguished from other species for their PRX polymorphism. A unique PRX profile was also determined for NS KK 6/10. Morphological differences among *P. avium, P. fruticosa* and *P. cerasus* were followed by PRX polymorphism. We found the polymorphism of PRX, as biochemical markers, sufficient for identifying the genetic diversity in the genus *Prunus* subgenus *Cerasus*, but not useful for further discrimination of different genotypes and clones within the same species.

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POLIMORFIZAM PEROKSIDAZA RODA *PRUNUS*, PODROD *CERASUS*

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Izvod

Polimorfizam peroksidaza proučavan je kod 31 genotipa roda Prunus u okviru šest vrsta podroda Cerasus: Prunus cerasus, Prunus avium, Prunus fruticosa, Prunus mahaleb, Prunus serrulata i Prunus gondouinii, kao i kod dva interspecies hibrida koji se koriste kao standardne podloge za trešnju: Gisela 5 i Colt. Šest genotipova Oblačinske višnje, četiri divlje trešnje, pet genotipova stepske višnje i jedan genotip magrive su selekcionisani iz prirodnih populacija u Srbiji. Za ekstrakciju proteina korišćena je unutrašnja kora jednogodišnjih grančica i mlado lišće. Polimorfizam peroksidaza je uočen u oba tkiva. Analizom lista dobijeni su jedinstveni peroksidazni zimogrami za svaku od ispitivanih vrsta i oba interspecijes hibrida. Veći broj polimorfnih lokusa i tipova zimograma utvrđen je kada je za proteinski ekstrakt korišćeno lišće, u poređenju sa unutrašnjom korom jednogodišnjih grančica. Dobijeni rezultati ukazuju da je na osnovu polimorfizma peroksidaza moguće determinisati vrste roda *Prunus*, podrod *Cerasus*, ali ne i utvrditi razlike među genotipovima i klonovima.

Ključne reči: peroksidaze, elektroforeza, polimorfizam, trešnja, *Prunus* spp.

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