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# Predicting delayed graft incompatibility in sweet cherry by peroxidase isoenzyme analysis

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**Abstract.** This study was carried out on one-year old sweet cherry trees of 'Burlat', 'Canada Giant', 'Hedelfinger', 'Summer Sun' and 'Sunburst' grafted on both 'Colt' and 'Gisela 6' in order to determine their compatibility. For this purpose, peroxidase (PRX) isoenzyme bands were identified by polyacrylamide gel electrophoresis (PAGE). Samples of inner bark were taken 12 months after grafting from the following three zones: rootstock, grafting union (which included 5 cm above and below grafting union) and central part of the scion. 'Hybrid' zymogram of PRX from grafting union having all bands from both graft partners might indicate compatible grafting combination between 'Summer Sun' and 'Colt'. In all the other cultivar/rootstock combinations, some symptoms of incompatibility can be expected. The results showed that peroxidase activity could be used as a parameter in the early determination of possible graft incompatibility in sweet cherry.

Keywords: graft incompatibility, peroxidase activity, Prunus avium L., rootstock, grafting

#### Introduction

Fruit trees are usually formed by grafting the scion (upper part) of one plant on the root system (rootstock) of another plant. In order for a combination to be successful, a good union between the scion and the rootstock is necessary. Graft incompatibility in fruit trees is one of the greatest obstacles in rootstock breeding (Davarynejad et al., 2008) and depends on anatomical, physiological and biochemical factors. Hudina et al. (2014) emphasised that incompatibility is a complex of physiological phenomenon occurring in some plants, defined by adjustment of the metabolisms of the grafted union partners (cultivar/rootstock combi-

nation), growth conditions, the presence/absence of viruses, environmental conditions, and the nutritional status of plants, as well as other unpredictables and stress-inducing factors.

Graft incompatibility frequently occurs, especially in inter-specific combinations such as the case of pear grafted on quince, apricot grafted on other *Prunus* species. In many instances, incompatibility is manifested by the breaking of the trees at the point of the union (Ciobotari et al., 2010). Regardless of incompatibility, trees can be grown for some years, without any signs of a mismatch indicating the presence of false functional vascular connections (Errea & Felipe, 1993; Hartmann et al., 1997). However, there are several external signs to detect graft incompatibility in-

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cluding graft union uniformity, lack of lignification, foliage yellowing, vegetative growth decline and vigor and anatomical abnormalities (Hartmann et al., 1997; Gülen et al., 2005). In some cases, it takes several years for symptoms to appear, and sometimes anatomical observation does not correlate with the incompatibility (Andrews & Marquez, 1993). Delayed appearance of the symptoms increases the time required for detection of graft compatibility and slows down selection within rootstock breeding programmes. Early and accurate prediction of graft incompatibility is of great importance because incompatible combinations could be eliminated, while compatible ones could be singled out (Petkou et al., 2004; Gökbayrak et al., 2007).

Literature provides little information on biochemical and molecular mechanisms involved in graft incompatibility (Pina & Errea, 2008; Ciobotari et al., 2010; Hudina et al., 2014). In the prior studies, Gülen et al. (2002, 2005), Musacchi et al. (2002), Fernandez-Garcia et al. (2004) and Güçlü & Koyuncu (2012a,b) reported that increase of indole-3-acetic acid oxidase (IAAox), polyphenol oxidase (PPO) and peroxidase (PDO, PRX) activity can be used as an index of the degree of graft incompatibility. Peroxidases (EC 1.11.1.7), enzymes with numerous biochemical and physiological roles in higher plants, have been reported to parallel hormonally-induced changes in tissue growth and differentiation (Feucht et al., 1983). They participate in plant growth, differentiation and development processes, including auxin catabolism, ethylene biosynthesis, plasma membrane redox systems and generation of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), cell wall edification, lignification and suberization, as well as response to pathogens (Has-Schön et al., 2005). Peroxidase isoenzymes are tissue-specific (Manganaris & Alston, 1992; Zapata et al., 1995; Čolić et al., 2013) and developmentally regulated (Smila et al., 2007).

The most of the new dwarfing rootstocks for sweet cherry trees are interspecific hybrids and their introduction into production should be preceded by detailed investigations of the compatibility between the two graft components (Sitarek, 2006). Therefore, the aim of this study was to determine the possibility of using peroxidase activity analysis for early prediction of graft incompatibility between cultivar and rootstock, through the assessment of one-year old sweet cherry trees of five cultivars grafted on two rootstocks.

#### **Material and Methods**

Plant material for this study was one-year-old sweet cherry trees. Grafting was done in the spring 2013. The two following rootstocks for sweet cherry, both interspecific hybrids, 'Colt' (*Prunus avium* L. sel.  $F2/2 \times P$ . pseudocerasus Lindl., 2n = 3x = 24) and 'Gisela 6' (*Prunus cerasus* L.  $\times$  *Prunus canescens* Bois, 2n = 3x = 24), were used. The sweet cherry cultivars 'Burlat', 'Canada Giant', 'Hedelfinger', 'Summer Sun' and 'Sunburst' were grafted on abovementioned rootstocks. The cultivar/rootstock combination was represented with three trees each. The trial was conducted at 'Radmilovac' facility of the Faculty of Agriculture, University of Belgrade. During investigations, all necessary agro-technical measurements were done in the trial.

For isozyme analysis, tissue samples of the inner bark of one-year-old trees were taken in spring 2014, 12 months after grafting, and used for the extraction and evaluation of peroxidase activity. Samples were taken from the following three zones: rootstock, grafting union which included 5 cm above and below grafting union, and central part of the scion. Vertical polyacrylamide gel electrophoresis (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.

Electrophoresis was performed at +4 °C and consisted of three phases. Prior to sample loading, preelectrophoresis was done for 45 min at 100 V. After that samples of 25  $\mu$ l of enzyme extracts were loaded. The second phase lasted 45 min at 100 V. The third stage was carried out at 400 V for 3 h.

Gels were visually observed and bands that represent isoenzyme patterns were analyzed. Isoenzyme loci and alleles were labeled in accordance with recommendations given by Weeden (1988) and Tobutt (1993).

#### **Results and Discussion**

The first studies of the role of peroxidase in the grafting compatibility were conducted in *Quercus* and *Castanea* by Santamour (1988a, 1988b, 1989), who re-

ported that if cambial isoperoxidase profiles of rootstock and scion are similar, a compatible union will occur when they grafted. Contrary, incompatible graft partners lacked similarity of isozyme patterns. On the basis of that assumption Gülen et al. (2002) determined 'Beurre Hardy' as compatible and 'Barlett' as incompatible with 'Quince A'.

In sweet cherry, graft incompatibility is not very common as sexual incompatibility. Usually, there are no problems with the growth or cropping of sweet cherry trees on the Mazzard rootstock. It may be different when the rootstock is a hybrid of the *Prunus* species or a selection of other than P. avium (Sitarek, 2006). In addition, Sitarek and Grzyb (2007) found that cultivar 'Heidegger' grafted on dwarf rootstocks 'P-HL A' and 'P-HL B' (Prunus avium L. × Prunus cerasus L.), as well as on 'Gisela 5' (Prunus cerasus L. × Prunus canescens Bois) showed visible symptoms of incompatibility: leaf yellowing and growth inhibition in the middle of the growing season and high mortality of trees during the first year in the orchard. In order to predict graft incompatibility in sweet cherry, Güçlü and Koyuncu (2012b) measured total peroxidase activity by spectrophotometry. They found highest peroxidase activity at the heterogenetic graft that suggested that the lignification finished earlier than in homogenetic combination.

Diagrams of PAGE profiles of cultivar, rootstock and graft union isoperoxidases of assessed sweet cherry cultivar/rootstock combinations are shown on Figures 1–5. In all, four regions of PRX (*Prx-1* to *Prx-4*) activity were observed. Slow migrating region *Prx-1* was monomorphic in the assessed cultivars, rootstocks and graft unions, which is in accordance with results of Güçlü and Koyuncu (2012a). Activity in the region *Prx-2* and phenotype *aa* was determined for all cultivars and graft union 'Summer Sun'/'Colt' (Fig. 1). while activity in region *Prx-3* was unique for 'Canada Giant' and 'Canada Giant'/'Gisela 6' graft union and presented as phenotype *aa* (Fig. 2).

The highest polymorphism was found in fast migrating Prx-4 region, represented with three phenotypes. All sweet cherry cultivars were monomorphic (bb), while 'Colt' and 'Gisela 6' showed unique patterns (ab and bc), respectively) and can be easily distinguished from each other and cultivars. This fact may contribute to the expression of graft incompatibility. This is in accordance with results of Petkou et al.

(2004), who detected differences among the electrophoretic patterns of the anionic peroxidase in horticulturally compatible and incompatible pear/quince grafts.

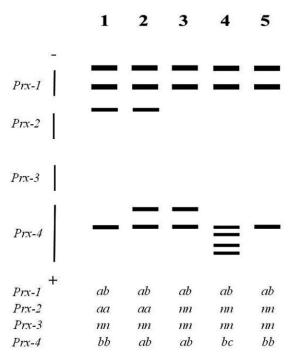


Fig. 1. Diagrams of zymograms of 'Summer Sun', 'Colt' and 'Gisela 6' for the PRX enzyme system: 1 – 'Summer Sun'; 2 – Graft union 'Summer Sun','Colt'; 3 – 'Colt'; 4 – 'Gisela 6'; 5 Graft union 'Sumer Sun','Gisela 6'

Sl. 1. Diagrami PAGE zimograma sorte Summer Sun i podloga Colt i Gisela 6 za PRX enzimski sistem: 1. Summer Sun; 2. Spojno mesto Summer Sun/Colt; 3. Colt; 4. Gisela 6; 5. Spojno mesto Sumer Sun/Gisela 6

Experimental results revealed differences in the isoperoxidases banding patterns for the graft union between the variants of cultivar/rootstock. Regarding the graft union 'Summer Sun'/'Colt' (Fig. 1, phenotype 2), the pattern was consisted of both cultivar and rootstock banding patterns (phenotypes 1 and 3) and might indicate compatible graft. In commercial production, 'Summer Sun'/'Colt' graft gives a very productive tree with good fruit size, which supports our result. Contrary, 'Summer Sun'/'Gisela 6' graft union (Fig. 1, phenotype 5) lacked bands from 'Summer Sun' in *Prx-2* region and from 'Gisela 6' in *Prx-4* region that indicate possible graft incompatibility.

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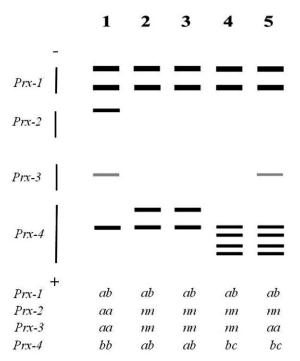
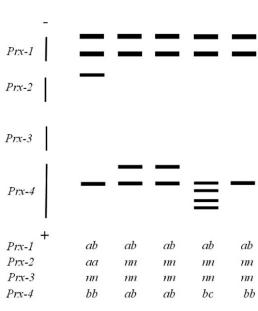


Fig. 2. Diagrams of zymograms of 'Canada Giant', 'Colt' and 'Gisela 6' for the PRX enzyme system: 1 – 'Canada Giant'; 2 – Graft union 'Canada Giant'/'Colt'; 3 – 'Colt'; 4 – 'Gisela 6'; 5 – Graft union 'Canada Giant'/'Gisela 6'

Sl. 2. Diagrami PAGE zimograma sorte Canada Giant i podloga Colt i Gisela 6 za PRX enzimski sistem: 1. Canada Giant; 2. Spojno mesto Canada Giant/Colt; 3. Colt; 4. Gisela 6; 5. Spojno mesto Canada Giant/Gisela 6

Our data for 'Canada Giant'/'Gisela 6' graft union showed common bands with 'Canada Giant' in *Prx-3* and with 'Gisela 6' in *Prx-4* (Fig. 2, phenotype 5), while the graft union with 'Colt' (Fig. 2, phenotype 2) lacked band in the region *Prx-4* found in 'Canada Giant'. For both cultivar/rootstock grafts, band *a* missed in the *Prx-2*. Those results indicate potential incompatibility with 'Colt' and possible delayed graft incompatibility with 'Gisela 6'.

We found that isoperoxidase profiles of 'Burlat' and 'Hedelfinger' grafted on 'Colt' and 'Gisela 6' were the same (Fig. 3–4). Samples from the graft union with 'Colt' (phenotype 2) lacked band in region *Prx-2*. Based on the results of Grzyb et al. (1998), who emphasized that 'Burlat' and 'Hedelfinger' grafted on 'Colt' are good examples of delayed incompatibility, we assume that absence of band in *Prx-2* region is might be related to incompatibility graft. Additionally, zymograms of 'Burlat'/'Gisela 6' and 'Hedelfin-



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Fig. 3. Diagrams of zymograms of 'Burlat', 'Colt' and 'Gisela 6' for the PRX enzyme system: 1 - 'Burlat'; 2 - Graft union 'Burlat'/'Colt'; 3 - 'Colt'; 4 - 'Gisela 6'; 5 - Graft union 'Burlat'/'Gisela 6'

Sl. 3. Diagrami PAGE zimograma sorte Burlat i podloga Colt i Gisela 6 za PRX enzimski sistem: 1. Burlat; 2. Spojno mesto Burlat/Colt; 3. Colt; 4. Gisela 6; 5. Spojno mesto Burlat/Gisela 6

ger'/'Gisela 6' graft unions lacked *c* band in *Prx-4* region. For those grafts with 'Gisela 6', we expect that incompatibility would occur earlier. Isoperoxidase zymograms of graft unions 'Sunburst'/'Colt' and 'Sunburst'/'Gisela 6' showed the same bands in *Prx-4* as rootstocks 'Colt' and 'Gisela 6', respectively (Fig. 5; phenotypes 2 and 5, respectively). Since band *a* in *Prx-2* region was not detected in both graft unions, delayed incompatibility can occur.

On the basis of our results, we assume that the absence of band a in Prx-2 region of the graft union might be related to incompatibility. Incompatibility may manifest itself already in the nursery as a low percentage of buds and grafts taken, weak growth of maidens, maidens breaking in the graft zone during strong winds or yellowish leaves (Sitarek, 2006). In case that all necessary agro-technical measurements were done in the orchard, stressful conditions such as too wet or too dry soil can cause delayed incompatibility.

Altough in our study graft partners did not have common patterns in all regions of peroxidase activity

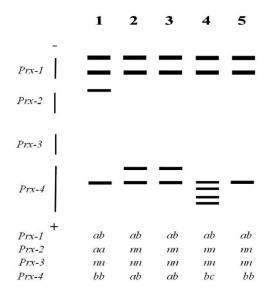


Fig. 4. Diagrams of zymograms of 'Hedelfinger', 'Colt' and 'Gisela 6' for the PRX enzyme system: 1 – 'Hedelfinger'; 2 – Graft union 'Hedelfinger'/'Colt'; 3 – 'Colt'; 4 – 'Gisela 6'; 5 – Graft union 'Hedelfinger'/'Gisela 6'

Sl. 4. Diagrami PAGE zimograma sorte Hedelfinger i podloga Colt i Gisela 6 za PRX enzimski sistem: 1. Hedelfinger; 2. Spojno mesto Hedelfinger/Colt; 3. Colt; 4. Gisela 6; 5. Spojno mesto Hedelfinger/Gisela 6

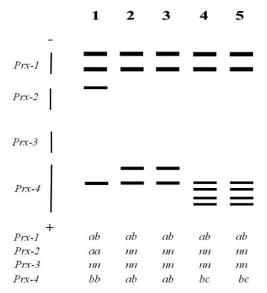


Fig. 5. Diagrams of zymograms of 'Sunburst', 'Colt' and 'Gisela 6' for the PRX enzyme system: 1 – 'Sunburst'; 2 – Graft union 'Sunburst'/'Colt'; 3 – 'Colt'; 4 – 'Gisela 6'; 5 – Graft union 'Sunburst'/'Gisela 6'

SI. 5. Diagrami PAGE zimograma sorte Sunburst i podloga Colt i Gisela 6 za PRX enzimski sistem: 1. Sunburst; 2. Spojno mesto Sunburst/Colt; 3. Colt; 4. Gisela 6; 5. Spojno mesto Sunburst/Gisela 6 except for 'Summer Sun'/'Colt', we observed that 'hybrid' zymogram with all bands from both graft partners may indicate compatible graft.

#### Conclusion

Many factors can cause incompatibility between cultivar and rootstock. For sweet cherry tree, this mainly occurs when the rootstock is a interspecific hybrid of the *Prunus* species. Graft union formation may progress through the entire sequence of the graft union formation process – more or less normal formation of xylem, phloem and periderm (outer bark), before degeneration of the graft union occurs as much as years later. We found the polymorphism of PRX, as biochemical markers, can be used for predicting delayed graft incompatibility in cherry. On the basis of the results, we recommend that samples for analysis of the peroxidase activity should be taken from cultivar and rootstock, as well as from grafting union in order to avoid wrong prediction of compatibility.

Our results indicate compatibility graft 'Summer Sun'/'Colt'. Early incompatibility can be expected for grafts 'Summer Sun'/'Gisela 6', 'Canada Giant'/'Colt', 'Hedelfinger'/'Gisela 6', 'Burlat/'Colt', 'Burlat'/'Gisela 6', 'Sunburst'/'Colt' and 'Sunburst'/'Gisela 6'. Delayed incompatibility can occur for graft 'Canada Giant'/'Gisela 6'.

#### Acknowledgement

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# MOGUĆNOST PREDVIĐANJA POZNE INKOMPATIBILNOSTI KOD TREŠNJE ANALIZOM PEROKSIDAZNE AKTIVNOSTI

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#### Rezime

Istraživanja su sprovedena na jednogodišnjim sadnicama trešnje sorti Burlat, Canada Giant, Hedelfinger, Summer Sun i Sunburst, kalemljenih na podloge Colt i Gisela 6. Cilj istraživanja je bio da se utvrdi kompatibilnost ispitivanih kombinacija sorta/podloga. Kompatibilnost je utvrđena na osnovu peroksidazne (PRX) aktivnosti, za čiju je analizu korićen metod poliakrilamidne gel elektroforeze (PAGE). Uzorci unutrašnje kore su uzeti 12 meseci posle kalemljenja, iz sledeće tri zone: podloga, spojno mesto (dužine 5 cm iznad i 5 cm ispod spojnog mesta) i sorta. "Hibridni" PRX zimogram dobijen analizom spojnog mesta, koji je sadr-

žao sve trake sorte Summer Sun i podloge Colt, ukazuje na kompatibilnost ove kombinacije. U svim ostalim kobinacijama sorta/podloga mogu se očekivati neki od simptoma inkompatibilnosti. Rezultati ukazuju da se peroksidazna aktivnost može koristiti kao parametar za utvrđivanje pozne inkompatibilnosti sorti trešnje na vegetativnim podlogama koje su dobijene međuvrsnom hibridizacijom.

**Ključne reči:** inkompatibilnost sorta-podloga, peroksidazna aktivnost, *Prunus avium L*:, podloga, kalemljenje

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