

*Full Length Research Paper*

# Peroxidase isozyme profiles in some sweet cherry rootstocks and '0900 Ziraat' cherry variety

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Accepted 13 December, 2011

**This study was carried out on one- year old trees of '0900 Ziraat' variety grafted onto Kuş kirazı, Kara idris, Sarı idris, MaxMa 14, MaxMa 60 and Gisela 5 in order to determine their compatibility. For this purpose, peroxidase isoenzyme bands were determined with polyacrylamide gel electrophoresis (PAGE). Saplings were grafted at the beginning of September with 'T' budding. Bark samples were taken before and 12 months after the grafting. Barks were removed by using a razor blade 4 cm above and below the graft union and graft zone. Analysis of profiles revealed isoperoxidases bands Rf = 0.39 band A and Rf =0.42 band B that were both on scion and rootstocks. Peroxidase profiles are found similar in scion and rootstocks.**

**Key words:** Graft compatibility, peroxidase, cherry, PAGE.

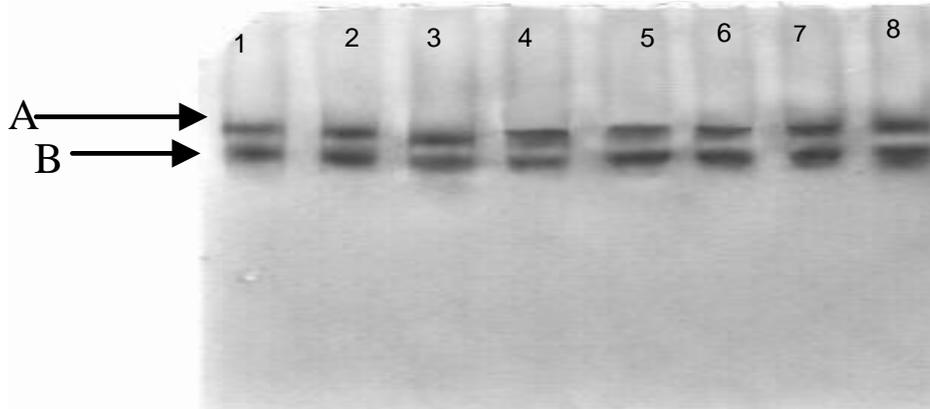
## INTRODUCTION

Peroxidases (EC 1.11.1.7) are part of large group of enzymes known collectively as oxidoreductases (Clemente, 1998). Peroxidases have been implicated in a wide range of cellular reactions such as phenolic compound oxidation, indole-3-acetic acid oxidation, lignification and polysaccharide cross-linking (Lee et al., 2001). Also they are known as stress enzymes toward pathogens, salt, metal ions (Bakardjieva et al., 1996). There are several external symptoms to detect graft incompatibility including graft union uniformity, lack of lignification, yellowing of foliage, decline in vegetative growth and vigor and anatomical abnormalities (Hartmann et al., 1997; Gülen et al., 2005). Appearance of these symptoms could take several years. Graft incompatibility in fruit trees is one of the greatest obstacles in rootstock breeding (Davarynejad et al., 2008). Although an increasing number of studies have tested for graft incompatibility in herbaceous and woody

plants, there is limited information available and biochemical and the molecular mechanisms involved are not well understood (Pina and Errea, 2008). Analysis of isozymes and peroxidase activity can be used for predicting graft incompatibility. Early and accurate prediction of graft incompatibility is of great importance because incompatible combinations could be avoided, while compatible ones could be selected (Gökbayrak et al., 2007; Petkou et al., 2004). Some researchers demonstrated that enzyme banding patterns could be related to graft compatibility (Feucht et al., 1983; Fernandez-Garcia et al., 2004; Gülen et al., 2005). Santamour (1980) defined role of peroxidase in graft compatibility as; 1) lignification is essential for a strong and permanent graft union; 2) peroxidase isoenzymes mediate the polymerization of cinnamic alcohols to lignin and also the bonding of lignin to carbohydrates and 3) the greater the similarity of isoperoxidase bands between stock and scion, the greater the chances of long term graft compatibility. An early and accurate prediction of graft incompatibility has great importance because incompatible combinations could be avoided while compatible one could be selected (Petkou et al., 2004; Gökbayrak et al., 2007). Whereas an increasing number of studies detect for graft incompatibility in herbaceous and woody plants, there is limited information especially in cherry, on

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**Abbreviations:** PAGE, polyacrylamide gel electrophoresis; PVP-40, polyvinylpyrrolidone; QA, quince A; S.Ö, Sebahattin Özbek Quince clones.



**Figure 1.** Peroxidase profiles of '0900 Ziraat' scion and rootstocks before the grafting. From left to right: 1, '0900 Ziraat'; 2, Kuş kirazı; 3, Kara idris; 4, Sarı idris; 5, MaxMa 14; 6, MaxMa 60; 7, Gisela 5; 8, CAB 6P.

the bio-chemical and the molecular mechanism involved (Pina and Errea, 2008).

Sweet cherry rootstocks are not investigated as apple rootstocks. Hence, sweet cherry graft studies are inadequate for rootstocks and also scion. The aim of this study was to investigate peroxidase bands of six sweet cherry rootstocks and '0900 Ziraat' cherry variety, which is very important for Turkey.

## MATERIALS AND METHODS

Plant material of this study are one-year old trees of '0900 Ziraat' variety, which was grafted on Kuş kirazı (*Prunus avium* L.), kara idris (*Prunus mahaleb* L.), sarı idris (*P. mahaleb* L.), MaxMa 14 (Brokforest, *P. avium* × *P. mahaleb*), MaxMa 60 (*P. avium* × *P. mahaleb*), CAB 6P (*Prunus cerasus*) and Gisela 5 (*P. cerasus* × *Prunus canescens*) rootstocks. Saplings were grafted at the beginning of September. Samples were taken before and 12 months after grafting. Barks were removed by using a razor blade 4 cm above and below the graft union and graft zone and these were frozen right away in liquid N<sub>2</sub> and stored -80°C until used.

### Enzyme extraction

Enzyme extraction was conducted according to Gülen, (2002). Ground tissues were homogenized in extraction buffer (0.1 M potassium phosphate (pH 7.5); 30 mM boric acid; 50 mM L-ascorbic acid; 17 mM sodium metabisulfite; 16 mM dithiocarbamic acid; 1 mM ethylenediaminetetraacetic acid (EDTA) and 4% (w/v) PVP-40, and final pH was adjusted to 7.5), then 60 ml extraction buffer was added to 0.6 g samples and homogenized at 10.000 rpm for 30 min at 4°C. Supernatant was used for electrophoresis.

### Polyacrylamid gel electrophoresis

Polyacrylamid gel electrophoresis (PAGE) was performed with a mini Protean II electrophoresis unit (Bio-Rad). 10% separation gel and 5% stacking gel were used according to Gülen (2000). 20 µL sample was loaded and electrophoresis was run at 150 V for about 3 h and at 4°C. Gels were stained for peroxidase using the method

of Wendel and Weeden (1989) as described by Gülen et al., (2005). Gels were rinsed with distilled water, fixed and stored with 10% glycerol. Manganaris and Alston (1992) method was used for the relative distance (Rf value) of bands; Rf = 1.0, the distance to the fastest band, and Rf = the starting point of the running.

## RESULTS AND DISCUSSION

Peroxidase bands (Figure 1) were taken before the graftings scion and rootstock bark samples. Analysis of profiles revealed isoperoxidases bands Rf = 0.39 band A and Rf = 0.42 band B that were on both scion and rootstocks. Peroxidase profiles are found similar on scion and rootstocks. After 12 months, the grafting bark samples were taken 4 cm above (scion) and below (rootstock) the graft union and graft zone. Peroxidase bands which were determined beginning of the study (Rf=0.39 A and Rf=0.42 B) were observed clearly 12 months later (Figure 2). Rootstock samples taken from combinations of '0900 Ziraat'/ Kara idris and '0900 Ziraat'/Gisela 5 band profiles are more lighter than others. Peroxidase profiles darkness is increased for all combinations at graft zone. This increase is observed especially in combination of '0900 Ziraat'/Gisela 5' and '0900 Ziraat'/MaxMa 14.

The first studies about this subject were made by Santamour et al. (1983, 1986) in Quercus and Castanea. Santamour (1988) proposed that when cambial isoperoxidase profiles of stock and scion are similar, a compatible union would occur when they are grafted. Gülen et al., (2005) determined compatible station Beurre Hardy (compatible) and Barlett (incompatible) with clones of "S.Ö." and Quince A. Many isoenzyme bands were observed as common in the two scions. However, one anodal peroxidase band A (Rf = 0.88) detected in Beurre Hardy was not in Barlett samples. Moreover, band B (Rf = 0.68) was detected in Beurre Hardy and not in Barlett or any of rootstocks. Same bands were found by another

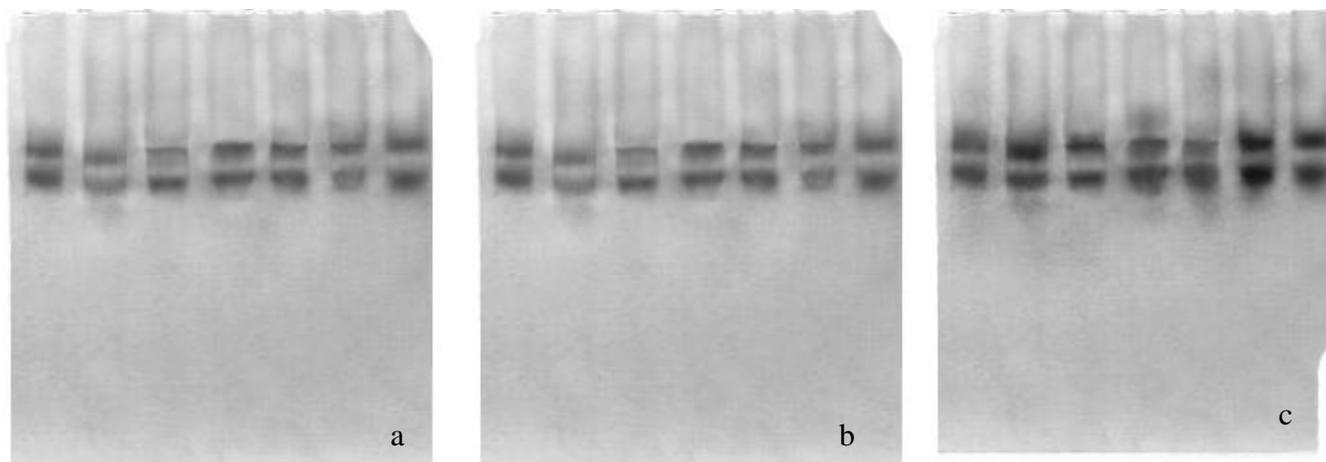


Figure 2. Twelve months after (a) Below the graft zone; (b) graft zone; (c) above the graft zone.

study in pear (Davarynejad et al., 2008). They proposed that presence of A band in the graft union was an indication of compatibility of Beurre Hardy and Passa with QA, whereas the absence of this band was related to incompatibility of Dargazi, Shahmivah and Torsh pear cultivars. Similarly a peroxidase band was observed in Tsakoniki/EM A (compatible) pear combination, but not in Williams/EM A (incompatible) combination (Petkou, 2004). In our study, two peroxidase bands were observed both before and after the grafting. So according to this data kuş kirazı, kara idris, sarı idris, MaxMa 14, MaxMa 60, CAB 6P and Gisela 5 rootstocks are used to be for "0900 Ziraat". Their peroxidase profiles are also similar. However, considering the graft zone samples, some differences were seen in terms of band darkness. Some researchers suggested that homogenetic and heterogenetic grafts give different reaction to grafting (Feucht et al., 1983; Olmstead, 2004). Parallel to this argument homogenetic combination '0900 Ziraat'/Kuş kirazi combination samples were more lighter than '0900 Ziraat'/Gisela 5 and '0900 Ziraat'/MaxMa 14. It was suggested that these combinations had lower peroxidase activity (Güçlü, 2010).

Isoperoxidase bands and graft compatibility relationships studies were concentrated on some fruit trees (pear), but there are few studies about cherry. This study is new for these cherry combinations. Our results can therefore be used for the grafting in cherry.

## ACKNOWLEDGEMENT

This study was supported by the Süleyman Demirel University Research Fund, Project No. 1609-D-08.

## REFERENCES

Bakardjieva NT, Cristova NV, Cristov K (1996). Reaction of peroxidase

from different plant species to increased temperatures and the effect of calcium and zinc ions. In: Obinger C, Burner U, Ebermann R, Penel C, Greppin H, eds. Proceedings of the IV International Symposium on Plant Peroxidases: Biochemistry and Physiology. University of Vienna, Austria.

Clemente E (1998). Purification and thermostability of isoperoxidase from oranges. *Phytochemistry*, 49(1): 29-36.

Davarynejad GH, Shahriari F, Hamid H (2008). Identification of graft incompatibility of pear cultivars on Quince rootstock by using isozymes banding pattern and starch. *Asian J. Plant Sci.* 7(1): 109-112.

Fernandez-Garcia N, Carvajal M, Olmos E (2004). Graft union formation in tomato plants: peroxidase and catalase involvement. *Ann. Bot.* 93: 53-60.

Feucht W, Schmid PPS, Christ E (1983). Compatibility in *Prunus avium/Prunus cerasus* grafts during initial phase. II. reduction of cell number and peroxidases in the rootstock cambium. *Sci. Hortic.* 21: 225-231.

Gökbayrak Z, Söylemezoğlu G, Akkurt M, Çelik H (2007). Determination of grafting compatibility of grapevine with electrophoretic methods. *Sci. Hortic.* 113: 343-352.

Gülen Sertli H (2000). Investigation of rootstock/scion relationships in quince and pear by isozyme analysis. Çukurova University Grad. Sch. Appl. Nat. Sci. Dep. Horticult. P.HD thesis Adana. p. 136.

Gülen H, Çelik M, Polat M, Eriş A (2005). Cambial isoperoxidases related to graft compatibility in pear-quince graft combinations. *Turk. J. Agr. Forest.* 29(1): 83-89.

Güçlü F (2010). Investigation of rootstock/scion relationships in sweet cherries by biochemical methods. Süleyman Demirel University. Graduate School of Applied and Natural Sciences Department of Horticulture PhD thesis p. 103, Isparta.

Hartmann HT, Kester DE, Davies Jr. F, Geneve RL (1997). *Plant Propagation Principles and Practices*. Sixth Edition, Prentice Hall, New Jersey.

Lee HS, Kim SE, Lee YM (2001). Purification and characterization of cationic isoperoxidase from scented-geranium. *Phytochemistry*, 58: 859-864.

Manganaris AG, Alston FH (1992). Inheritance and linkage relationships of peroxidase isoenzymes in apple. *Theor. Appl. Genet.* 83: 392-399.

Olmstead MA (2004). Vaskuler and Metabolic Impact of Dwarfing Rootstocks on Sweet Cherry (*Prunus avium*, L.), Michigan State University, P.HD Thesis. p. 208.

Petkou D, Diamantidis G, Vassilakakis M (2004). Anionic peroxidase isoform profiles from calli and barks of pear cultivars and of the quince rootstock EM A. *J. Biol. Res.* 2: 51-55.

Pina A, Errea P (2008). Influence of graft incompatibility on gene expression and enzymatic activity of UDP-glucose pyrophosphorylase. *Plant Sci.* 174: 502-509.

Santamour Jr. FS (1983). Cambial peroxidase patterns in *Quercus* related to taxonomics classification and graft compatibility. Bull. Torrey Bot. Club. 110(3): 280-286.

Santamour Jr. FS, McArdle AJ, Jaynes RA (1986). Cambial isoperoxidase patterns in *Castanea*, JEH. 4(1): 14-16.

Santamour Jr. FS (1988). Graft compatibility in woody plants: an expanded perspective. JEH. 6(1): 27-32.