AcademicPres

Available online at www.notulaebotanicae.ro

Print ISSN 0255-965X; Electronic 1842-4309

Not Bot Horti Agrobo, 2012, 40(1):243-246

Notulae Botanicae Horti Agrobotanici Cluj-Napoca

A Method for Prediction of Graft Incompatibility in Sweet Cherry

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Abstract

This study was carried out on 1-year old trees of '0900 Ziraat' variety grafted onto 'Kuş kirazi', 'Kara idris', 'Sari idris', 'MaxMa 14', 'MaxMa 60' and 'Gisela 5' in order to determine their compatibility. For this purpose, total peroxidase activity was determined by spectrophotomery assay. Taking ground tissue samples were planned three times as before the grafting (beginning), and then 8 and 12 months after grafting. Total peroxidase activities of rootstocks before grafting varied between 10.80 ΔAg.min. ('Kuş kirazi') and 7.83 ΔAg.min. ('Kara idris') and were found to be statistically important. The peroxidase activity of '0900 Ziraat' was 11.07ΔAg.min. and the closest value occurred in *Prunus avium* rootstock. The most different values occurred in rootstocks of 'Kara idris' and 'Gisela 5'. The results showed that peroxidase activity increased in rootstock and graft scion. This increase had higher values in heterogenetic combinations especially in '0900 Ziraat'/'Gisela 5' and '0900 Ziraat'/'Kara idris' grafts. Peroxidase activity was decreased at the 12th month for all combinations. The highest value was obtained from '0900 Ziraat'/'MaxMa 14' combinaion with 29.17 ΔAg.min. while lowest one was '0900 Ziraat'/'Kuş kirazi' with 17.39. The findings showed that peroxidase activity could be used as a parameter in early determination of graft incompatibility.

Keywords: graft incompatibility, peroxidase activity, *Prunus avium*, rootstock grafting

Introduction

Graft incompatibility is a complex, anatomical, physiological and biochemical process that is not fully understood. Compatible graft is typically comprised of three major events: Cohesion of the rootstock and scion; proliferation of callus cells at the graft interface; and vascular dedifferentiation across the graft interface (Hartmann *et al*., 1997; Moore, 1984). 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 (Gülen *et al*., 2005; Hartman *et al*., 1997). 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 as well as biochemical and the molecular mechanisms involved are not well understood (Pina and Errea, 2008). Analysis of isozymes and peroxidase activity can be used for prediction 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). Peroxidase activities and isozymes patterns have been reported to parallel hormonally-induced changes in tissue growth and differentiation (Feucht *et al*., 1983). Peroxidases (EC 1.11.1.7) are haem proteins and contain iron (III) protoporphyrin IX (ferriprotoporphyrin IX) as the prosthetic group. They have an electro-molecular mass range of 30.000 to 150.000 Da. These are a group of oxidoreductases that catalyze the reduction of peroxides, such as hydrogen peroxide and the oxidation of a variety of organic and inorganic compounds (Hamid and Ur-Rehman, 2009). Peroxidases are enzymes with numerous biochemical and physiological roles in higher plants. They participate in plant growth, differentiation and development processes, including auxin catabolism, ethylene biosynthesis, plasma membrane redox systems and generation of H_2O_2, cell wall edification, lignification and suberization, as well as response to pathogens (Has-Schön *et al*., 2005). Graft incompatibility is not very common as sexual incompatibility in sweet cherry (Sitarek, 2006). Sweet cherry rootstocks have not been investigated to the extent of apple rootstocks and so there are relatively few studies of sweet cherry graft incompatibility. Research works reported that there can be graft incompatibility in sweet cherry if scion and rootstock are from different origin.

The aim of this study was to investigate the possibility of using peroxidase activity analysis for early prediction of graft incompatibility between homogenetic and heterogenetic combinations of '0900 Ziraat' cherry cultivar.

Material and methods

The plant material of this study consisted of 1-year old trees of '0900 Ziraat' variety which was grafted on 'Kuş kirazi' (*Prunus avium* L.), 'Kara idris' (*Prunus mahaleb* L.), 'Sari idris' (*Prunus mahaleb* L.), 'MaxMa 14' (Brokforest, *Prunus avium* x *Prunus mahaleb*), 'MaxMa 60' (*Prunus avium* x *Prunus mahaleb*), 'CAB 6P' (*Prunus cerasus*) and 'Gisela 5' (*Prunus cerasus* x *Prunus canescens*) rootstocks. Saplings were grafted at the beginning of the September 2010. Samples were taken before the grafting (beginning), and then 8 and 12 months after grafting. In addition, ungrafted samples were taken at the same times. Barks were removed by using a razor blade 4 cm above and below the graft union and graft zone. Samples were immediately frozen in liquid N_2 and stored -80°C until analysis.

Enzyme extraction was conducted according to Gülen *et al*. (2002) and Güçlü and Koyuncu (2012). Ground tissues were homogenized by using buffer phosphate extraction solution (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 EDTA and 4% (w/v) PVP-40, and final pH was adjusted to 7.5. Sixty milliliter of extraction solution was added to 0.6 g samples and homoginized at 10.000 rpm for 30 min at 4°C. Supernatant was used for electrophoresis.

Peroxidase activity was determined by spectrophotometric method according to Eryılmaz (2007). The reaction mixture (consisted of 0.25% (v/v) guaiacol in 1 ml 0.1 M sodium phosphate buffer, pH 7.0, containing 0.1% hydrogen peroxide 100 µl) was added to initiate the reaction and followed spectrophotometrically at 470 nm. The absorbance increase at 470 nm due to the guaiacol oxidation was recorded for 2 minutes. The study used a randomized block design with three replicate and 3 saplings were used each replicate. Statistical analyses were performed with GLM models (General Linear Model) using SPSS (V.10; Statistical software, SPPS. Inc., USA). Tukey's test was used to determine significant differences between group means.

Results

Peroxidase activities of ungrafted rootstocks and '0900 Ziraat' are listed in Tab. 1. The mean values were compared among the rootstocks, mean peroxidase activity ranged from 10.94 ΔA/g.min. ('Kuş kirazi') to 8.01 ΔA/g.min. ('Kara idris') (p<0.01). According to sampling periods, peroxidase activity values was increased in general at the 12th month (p<0.05). Similarly '0900 Ziraat' peroxidase activity was increased month by month until reached 11.17 ΔA/g.min.

After the grafting period peroxidase activity below the graft zone (rootstock) was analyzed and rootstock*period interaction was found to be statistically significant (p<0.01). At 8 months the highest peroxidase activity was obtained from 'Kuş kirazi' (12.81 ΔA/g.min.) while the lowest was 'Kara idris' (7.94 ΔA/g.min.) (p<0.01). Peroxidase activity was found fluctatuate after the 12th months after the grafting. Except for 'Sari idris' and 'CAB 6P' peroxidase activity was decreased (Tab. 2).

The effect of rootstocks and sampling period on the peroxidase activity was found to be statistically significant (p<0.01). It was found that'0900 Ziraat'/'CAB 6P' was the best combination, in terms of peroxidase activity with a value of 21.07 ΔA/g.min. while '0900 Ziraat'/'Gisela 5' combination had 12.21 ΔA/g.min. Contrary to 8th month results, at the 12th month, '0900 'Ziraat'/'Gisela 5' combination had the highest peroxidase activity (Tab. 3). According to taking sample periods, peroxidase activity was increased 12 months after grafting for all combinations.

As it can be observed from Tab. 4, rootstock*period interaction was found to be statistically significant different (p<0.01). At the 8th month, the highest peroxidase activity value was determined in '0900 Ziraat'/'Gisela 5' (39.92 ΔA/g.min.) combination while the lowest value was '0900 Ziraat'/'Kuş kirazi' (21.45 ΔA/g.min.). Peroxidase activity was decreased at the 12th month for all combinations.

Tab. 1. Peroxidase activities of ungrafted rootstocks and scion (ΔA/g.min.)

	Periods			
	Beginning	8 month	12 month	Mean
'Kuş kirazi'	10.80	11.00	11.03	10.94 a[x]
'Kara idris'	7.83	8.10	8.12	8.01 f
'Sari idris'	8.53	8.71	8.84	8.69 e
'MaxMa 14'	9.75	9.79	9.80	9.78 b
'MaxMa 60'	9.33	9.41	9.54	9.43 c
'Gisela 5'	8.52	8.71	8.89	8.71 de
'CAB 6P'	8.78	8.83	8.90	8.84 d
'0900 Ziraat'	11.07 B[y]	11.13 AB	11.17 A	11.12
Mean	9.07 C[y]	9.22 B	9.30 A	

[x]Different small letters in the same column indicate significant differences
[y]Different capital letters in the same column indicate significant differences according to Tukey multiple range test. p (rootstocks) <0.01, p (scion)<0.05

Tab. 2. Peroxidase activities of belove the graft union (ΔA/g. min.)

	Periods			
	Beginning	8 month	12 month	Mean
'Kuş kirazi'	10.80 a[x] C[y]	12.81 a A	11.13 a B	11.58
'Kara idris'	7.83 e A	7.94 f A	7.73 f B	7.83
'Sari idris'	8.53 d B	8.71 e A	8.74 e A	8.66
'MaxMa 14'	9.75 b A	9.76 c A	9.01 d B	9.51
'MaxMa 60'	9.33 c B	9.72 c A	9.37 c B	9.47
'Gisela 5'	8.52 d C	10.31 b A	9.44 b B	9.42
'CAB 6P'	8.78 d B	8.92 d B	9.02 d A	8.91
Mean	9.07	9.74	9.21	

[x]Different small letters in the same column indicate significant differences
[y]Different capital letters in the same column indicate significant differences according to Tukey multiple range test (p<0.01)

Tab. 3. Peroxidase activities of above the graft union (ΔA/g. min.)

	Periods		
	8 month	12 month	Mean
'0900 Ziraat'/'Kuş kirazi'	14.53 d[x] B[y]	17.47 d A	14.39
'0900 Ziraat'/'Kara idris'	17.71 c B	19.03 d A	15.92
'0900 Ziraat'/'Sari idris'	13.48 e B	15.30 e A	13.25
'0900 Ziraat'/'MaxMa 14'	19.84 b B	24.51 b A	18.49
'0900 Ziraat'/'MaxMa 60'	15.20 d B	20.05 d A	15.51
'0900 Ziraat'/'Gisela 5'	12.21 f B	28.56 a A	17.22
'0900 Ziraat'/'CAB 6P'	21.07 a B	23.38 c A	18.52
Mean	16.28	21.19	

[x]Different small letters in the same column indicate significant differences
[y]Different capital letters in the same column indicate significant differences according to Tukey multiple range test (p<0.01)

Tab. 4. Peroxidase activities of graft union (ΔA/g.min.)

	Periods		
	8 month	12 month	Mean
'0900 Ziraat'/'Kuş kirazi'	21.45 f[x] A[y]	17.39 d B	19.42
'0900 Ziraat'/'Kara idris'	30.41 c A	19.87 c B	25.14
'0900 Ziraat'/'Sari idris'	29.32 d A	22.89 b B	26.11
'0900 Ziraat'/'MaxMa 14'	35.11 b A	29.17 a B	32.14
'0900 Ziraat'/'MaxMa 60'	24.32 e A	17.79 d B	21.06
'0900 Ziraat'/'Gisela 5'	39.92 a A	29.13 a B	34.53
'0900 Ziraat'/'CAB 6P'	30.41 c A	24.10 b B	27.26
Mean	30.13	22.91	

[x]Different small letters in the same column indicate significant differences
[y]Different capital letters in the same column indicate significant differences according to Tukey multiple range test (p<0.01)

29.17 was the highest value (0900 Ziraat/'MaxMa 14') while 17.39 was lowest (0900 'Ziraat'/'Kuş kirazi').

Discussion

Peroxidases play very important role in higher plants including functions such as oxidation of phenolics, IAA oxidation, lignification, fragmentation of polysaccharides (Clemente, 1998; Hiraga *et al*., 2001). Because of their role in lignification, there has been increasing attention into graft incompatibility in recent years. Some research works were previously conducted on graft incompatibility in fruit trees in terms of peroxidase isoenzyme activity. Before grafting 'Kuş kirazi' had the closed peroxidase activity '0900 Ziraat' with highest value. 'Kara idris', which has known graft incompatibility problems with '0900 Ziraat' had the lowest peroxidase activity. There was a large difference between the values of '0900 Ziraat' and 'Kara idris'. The relationship between high peroxidase activity and graft compatibility was previously reported by many research works (Donaldson, 2001; Fernandez-Garcia *et al*., 2004; Feucht *et al*., 1983). The results of the present study support those of earlier research. Peroxidase activity was highest in 'Kuş kirazi' which is the best compatible with '0900 Ziraat' while the lowest peroxidase activity was obtained from 'Kara idris' which is insufficient compatible with '0900 Ziraat'. There is an augmentation in both rootstocks and scion peroxidase activity after the grafting. Peroxidase is reported as s stress enzyme by previous researchers (Has-Schön *et al*., 2005; Lee *et al*., 2001; Rajeswari *et al*., 2008). If grafting is accepted as a stress factor for trees, increasing peroxidase activity following grafting may explain with these ideas. In this study, peroxidase activity below the graft union after grafting was found to be lower than that above the graft union and graft union. It was suggested the findings rootstock can adapt more easily to this stress factor than the other parts of the graft graft. Some researchers reported that in tomato grafts, peroxidase activity increased day by day after the graft (Fernandez-Garcia, 2004). Similarly in another study compared peroxidase activity in melon at 14 and 24 days after grafting (Aloni *et al*., 2008). Parallel to their findings, peroxidase activity was increased at the 12th month in the study. Some researchers suggested that homogenetic and heterogenetic grafts give different reaction to grafting (Feucht, 1983; Olmstead, 2004). Similarly in the study after the 8 months later grafting highest peroxidase activity at the graft union was obtained from '0900 Ziraat'/'Gisela 5' combination which was the heterogenetic graft. Additionally, the homogenetic combination '0900 Ziraat'/'Kuş kirazi' had the lowest peroxidase activity. It was suggested that lignifications finished in homogenetic combination earlier than in heterogenetic combination. This result parallels those reported by another researchers (Schmidt and Feucht, 1981).

Conclusions

The present results suggest that peroxidase activity can be used as a method to predict graft incompatibility. Especially before grafting it can be useful to detect combinations that might show delayed incompatibility. For cherry varieties, using genetically different *Prunus* as rootstocks could be accepted as a risk factor for graft incompatibility compared to grafting with rootstocks of *Prunus avium*. This is the first report on graft compatibility for these cherry combinations. Further combinations and graft incompatibility need to be thoroughly investigated in cherry varieties.

Acknowledgements

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

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