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Determination of grafting compatibility of grapevine with electrophoretic methods

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Abstract

This study was carried out to determine the efficiency of electrophoretic methods in predicting graft incompatibility of grape cultivars with American rootstocks. Three isoenzyme systems (peroxidase, PER; esterase, EST; acid phosphatase, AcPH) and total protein profiles were obtained in 15 grape cultivars (*Vitis vinifera* L.) and 12 American rootstocks. Compatibility levels were determined by the band similarities. Field compatibilities were also calculated. Results showed that incompatibility exists between different cultivar–rootstock combinations. AcPH and total protein profiles of the cultivar–rootstock combinations could be suggested to use for forecasting graft incompatibility.

Keywords: Grapevine; Graft incompatibility; Isoenzymes; Protein; Electrophoresis

1. Introduction

A graft union is considered to be successful when several functional phloem and xylem connections cross the graft surface (Moore, 1984; Gersani, 1985; Wang and Kollmann, 1996; Schoning and Kollmann, 1997). However, incompatible grafts can grow several years without any external symptom of incompatibility (Errea and Felipe, 1993; Hartmann et al., 1997), indicating the presence of functional vascular connections (Mosse, 1962).

Different levels of compatibility between grapevine rootstock and *Vitis vinifera* cultivars were found to be existing (Lider et al., 1978; Fallot et al., 1979; Hidalgo and Candela, 1980; May, 1994; Kazaliev et al., 1997; Çelik et al., 2003; Zink and Schropp, 2004; Todić et al., 2005) and they were mostly noted during the adaptation experiments of scion–rootstock combinations conducted in different ecologies. For instance, Kocsis and Bakonyi (1994) found in their study of interaction between the woods of rootstock and cultivar in hot room callusing that Fercal was compatible with the three cultivars used while 5 BB gave the poorest results with them. Ungurenau (1995) obtained the best results of affinity from the vines grafted onto 140 Ru. Kaserer and Schoeffl (1993) showed the effects of six rootstocks on a local variety and found that 5 C, SO4 and 5 BB were best rootstocks for highest yield.

An early and accurate prediction of graft incompatibility has great importance because incompatible combinations could be avoided while compatible ones could be selected (Petkou et al., 2004). The involvement of certain enzymes in the cellular behavior during the first steps of graft formation has been studied in different species; although the specific role and effects on incompatibility is still not clear (Deloire and Hebant, 1982; Quesada and Macheix, 1984; Pina and Errea, 2005). The complexity of incompatibility and the mechanism behind the reactions have been investigated in several ways: in vitro pear and quince combinations (Moore, 1984), or between callus cultures of many different Prunus species (Gebhardt et al., 1982), peroxidase activity and the production of phenolic compounds in Prunus (Schmid et al., 1982; Treutter, 1987; Bauer et al., 1989; Rodrigues et al., 2001) and in pear-quince graftings (Musacchi et al., 2000) and the analysis of cyanogenic glycosides in some incompatible Prunus combinations (Gur et al., 1968; Gur and Blum, 1973; Moing et al., 1987).

Isoforms of enzymes separated by electrophoresis were one of the earliest in vitro methods used for the prediction of graft incompatibility. Santamour et al. (1986) reported that isoenzyme analysis of scions and rootstocks could be used to predict incompatibility before grafting in different cultivars

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of Acer, Quercus and Castanea. They stated that when stock and scions' phenotype of peroxidase isoenzyme, the enzyme responsible for the polymerization of *p*-coumaryl alcohols to lignin (Whetten et al., 1998; Quiroga et al., 2000), matched, grafting resulted in a compatible union. In contrast, if isoenzyme phenotypes of graft partners were different, callus formation was impaired at the graft union (Santamour, 1988a, 1988b). Past research with other plant species showed that analysis of isoenzymes, especially peroxidases, and protein spectra between rootstock and scion before grafting could be used to predict intraspecific compatibility or incompatibility (Copes, 1973; Tubbs, 1973; Schmid and Feucht, 1985; Moreno et al., 1994; Gülen et al., 2002; Fernandez-Garcia et al., 2004; Pedersen, 2006).

Lachaund (1975) suggested that incompatibility could be avoided, to a certain extent, where similarity of protein composition between the partners would increase the probability of graft success. The comparison of protein profiles of graft combinations to predict graft incompatibility using SDS-PAGE was studied in Prunus species (Huang et al., 1984; Schmid and Feucht, 1985) and in *V. vinifera* (Masa, 1985, 1986, 1989). Poëssel et al. (2006) showed by using proteome analysis of 2D-PAGE analysis that some constituent proteins of leaves could be good candidates as compatibility markers.

Electrophoretic mobility of enzymes and total proteins were determined in grapevine by many scientists (Masa, 1985, 1986, 1989; Altındişli et al., 1995; Kara et al., 1995a, 1995b). Electrophoretic determination of graft incompatibility using total proteins was tested by Masa (1985) between the scions Airen, Bobal, Garnacha, Tempranillo and Viura and the rootstocks 420 A, 41 B, 99 R, 110 R, 161-49 and 196-17 C. The affinity indexes between scion and rootstock (K_{S-R}) and the rootstock and scion (K_{R-S}) using Safanov and Veidenberg (1969) formula was calculated from the relative electrophoretic mobilities of total proteins. He found that the final results agreed well with the field behavior and stated that that the results can be generalized because there is no dependence on the environment, and that the application of this method could allow learning a priori which rootstock might be compatible with a given cultivar. In his study in 1986, Masa carried out trials for determining the affinity between scion and rootstock by comparison of enzymes (acidic and alkaline phosphatase, peroxidase, esterase). He concluded that the two phosphatases were best suited. Masa (1989) also investigated the degree of compatibility between cv. Albarino and six rootstocks (420 A, 42 B, 99 R, 110 R, 161-49 and 196-17) using total proteins, acid and alkaline phosphatases and peroxidases. He calculated the index of affinity between the cultivar and the rootstocks (and vice versa) based on the relative electrophoretic mobility of total proteins. It was concluded that the cultivar is compatible with 110 R, 41 B and 161-49.

In the light of aforementioned studies, the possibility of using isoenzymes (peroxidase, acid phosphatase and esterase) and total protein profiles in early prediction of graft incompatibility between American grapevine rootstocks and *V. vinifera* cultivars were investigated.

2. Materials and method

2.1. Plant material

Fifteen *V. vinifera* L. cultivars (tablegrape cvs; Alphonse Lavallèe, Amasya, Ata Sarısı, Cardinal, Çavuş, Gülüzümü, Hafizali, Italia, Razakı and winegrape cvs; Emir, Muscat of Hamburg, Kalecik Karası, Narince, Pinot noir, and Riesling) and 12 American grapevine rootstocks (99 R, 110 R, 8 B, 5 BB, 420 A Mgt, SO4, 1103 P, 44-53 M, 1613 C, 140 Ru, 5 C and 41 B) were used to determine grafting compatibility, using the techniques of PAGE and SDS-PAGE in three different enzyme systems (peroxidase EC 1.11.1.7 (PER); esterase EC 3.1.1.1 (EST) and acid phosphatase EC 3.1.3.2 (AcPH)) and total protein, respectively.

One-year-old cuttings of the cultivars and rootstocks, 10–12 mm thick and 40–45 cm long, were maintained from Kalecik Viticultural Research Center, University of Ankara. They were kept in plastic bags at 2 °C until used.

2.2. Method

2.2.1. Field determination of compatibility constant (FCC)

Field compatibility constants (FCC) were obtained from the 12 year old vineyard containing different cultivars (*V. vinifera*) grafted onto different American rootstocks. A formula developed by Perraudine (1962)was used to calculated FCC.

$$\mathrm{FCC} = \frac{C}{A} + \left(C + \frac{A}{2B}\right) + 10\,(12 = \text{indication of ideal compatibility}),$$

where *A*: width of scion 10 cm above graft union, *B*: width of graft union and *C*: width of rootstock 10 cm below graft union.

2.2.2. Enzyme extraction

Cuttings were taken to the laboratory where tissue samples were obtained for electrophoresis. Woody samples were scrapped off with a knife after the woody bark had been removed. The 2 g samples were processed for isoenzyme extraction following the procedure of Arulsekar and Parfitt (1986). The extraction buffer contained 0.05 M Tris (pH 8.0) with 0.007 M citric acid (monohydrate), 0.1% cysteine hydrochloride, 0.1% ascorbic acid, 1.0% polyethylene glycol (M_r 3500), and 1 mM 2-mercaptoethanol. The final pH was about 8.0.

Samples were crushed with liquid nitrogen using a pestle and a mortar. 0.6 mg PVPP (Sigma P 6755) and 30 ml extraction buffer were added and later homogenized at 15 000 *xg* for 20 s in ice. After filtering through four layer cheesecloth, they were centrifuged for 15 min at 14 000 rpm. Supernatant was used as enzyme source and kept under −35 °C until used.

PAGE was performed with a mini protean II cell (Biorad, Hercules, Calif.) according to Laemmli (1970) for the three enzyme systems. PER was detected on 12% separation gel and EST and AcPH were on 9.45% separation gels. Stacking gel concentration was 4%.

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Electrophoresis was run at 4 °C. Samples dyed with bromophenol blue were loaded and run at 100 V until the samples entered into separation gel. Electrophoresis continued at 350 V until the buffer front had migrated 8 cm past the origin in the anode direction. Staining solutions were prepared immediately before the end of the gel run. Gels were immersed in the staining solution and incubated at 37 °C in the dark until the isoenzyme bands were stained. Bands were immediately recorded and photographed. Staining solutions for the enzymes were prepared according to Arulsekar and Parfitt (1986).

2.2.3. Protein extraction

Extraction was performed according to the method by Kozma et al. (1990). The extraction buffer consisted of 0.05 M Tris–HCl, 0.05 mM NaCl, 2% (v/v) 2-mercapthoethanol. Two grams woody tissue without the bark was crushed in a mortar with a pestle with the aid of liquid nitrogen. One gram PVPP and 10 ml extraction buffer were added and homogenized. The filtered extract was kept at 100 °C for 4 min and later centrifuged at 14 000 rpm for 10 min. Supernatant was used as protein source and kept at −35 °C until used.

SDS-PAGE was performed in the total protein analysis. SDS was added to the gels which were prepared for the enzyme systems. Gel run was performed at 25 mA until the dyed samples reached the separation gel and at 15 mA until the front had reached 10 cm past the origin. Silver staining method (Biorad) was used to make the bands visible. Bands were recorded and photographed immediately.

2.2.4. Data collection for enzymatic compatibility constant (ECC)

For each enzyme system and total protein, the distance traveled by the isoenzymes was measured and recorded as R_f values (distance traveled by the band divided by the distance traveled by the bromophenol blue dye front). Enzymatic compatibility constant (ECC) between the grape cultivars and the stocks was calculated for each cultivar/rootstock combinations according to Safanov and Veidenberg (1969) formula.

$$\mathrm{ECC} = \left(\frac{\text{Number of common isoenzyme-protein bands}}{\text{Total number of isoenzyme-protein bands}}\right) \times 100 (> 65 \text{ indicates good compatibility})$$

3. Results

FCC of the vines were determined according to Perraudine's (1962) formula and shown in Table 1. Riesling/41 B combination had the highest FCC (12.03), while the lowest was obtained from the combination of Riesling/SO4 (10.97).

Table 1
Field compatibility constants of the vines according to Perraudine (1962)[a]

	SO4	16-13 C	41 B	1103 P	5 BB	110 R	99 R	140 Ru	8 B	5 C	44-53
Alphonse Lavallee	–	–	11.93	11.81	11.74	11.72	11.94	11.92	11.67	11.66	11.90
Amasya	–	–	11.72	11.51	11.34	11.60	11.75	11.56	11.23	11.25	11.43
Çavuş	–	–	11.64	11.71	11.59	11.84	11.66	11.64	11.52	11.43	11.64
Gülüzümü	–	–	11.83	11.71	11.47	11.57	11.85	11.75	11.43	11.57	11.85
Hafizali	–	–	11.90	11.84	11.59	11.67	11.89	11.50	11.48	11.45	11.69
Emir	11.40	11.91	11.61	11.85	11.63	11.71	–	–	–	–	–
Narince	11.38	11.79	11.47	11.66	11.68	11.58	–	–	–	–	–
Pinot noir	11.56	11.99	11.57	11.75	11.55	11.73	–	–	–	–	–
Riesling	10.97	12.00	12.03	11.98	11.84	11.76	–	–	–	–	–

[a] Value of 12 indicates good compatibility.

Table 2
Number of isozyme bands obtained through electrophoresis analyses

Cultivars	PER	EST	AcPH	TP	Rootstocks	PER	EST	AcPH	TP
Alphonse L.	5	9	5	8	41 B	5	6	5	7
Amasya	6	6	5	8	99 R	6	7	5	6
Ata Sarısı	5	7	5	8	110 R	6	8	5	6
Cardinal	5	8	5	8	8 B	5	7	5	6
Çavuş	5	6	5	6	140 Ru	6	10	5	6
Emir	4	8	6	6	1103 P	7	10	5	6
Gülüzümü	5	5	5	6	5 BB	6	9	5	6
Hafizali	5	4	4	6	5 C	5	7	5	7
Italia	4	6	4	6	16-13 C	6	6	5	6
Kalecik K.	6	7	5	5	44-53	6	5	5	7
Narince	4	8	5	6	SO4	6	6	5	6
Muscat of Hamburg	4	10	5	6	420 A	6	7	5	6
Pinot noir	5	8	5	5					
Razakı	5	7	4	7					
Riesling	5	6	5	7					

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Table 3
Compatibility constant of the scion/rootstock combination estimated through iszoyme analysis using the Safanov-Veidenberg (1969) index[a]

Enzyme system	Cultivar															
	Stock	Alphonse Lavallee	Amasya	Ata Sarısı	Cardinal	Çavuş	Emir	Gül üzümü	Hafizali	Muscat of Hamburg	Italia	Kalecik Karası	Narince	Pinot noir	Razakı	Riesling
PER	41 B	68	55	80	60	60	45	60	60	45	45	74	45	60	60	60
	99 R	84	83	74	74	74	63	74	74	63	63	83	63	55	74	74
	110 R	84	83	74	74	74	63	74	74	63	63	83	63	55	74	74
	8 B	68	55	80	60	60	45	60	60	45	45	74	45	60	60	60
	140 Ru	84	83	74	74	74	63	74	74	63	63	83	63	55	74	74
	1103 P	79	77	69	69	69	59	69	69	59	59	77	59	52	69	69
	5 BB	84	83	74	74	74	63	74	74	63	63	83	63	55	74	74
	5 C	68	55	80	60	60	45	60	60	45	45	74	45	60	60	60
	16-13 C	84	83	74	74	74	63	74	74	63	63	83	63	55	74	74
	44-53	84	83	74	74	74	63	74	74	63	63	83	63	55	74	74
	SO4	84	83	74	74	74	63	74	74	63	63	83	63	55	74	74
	420 A	84	83	74	74	74	63	74	74	63	63	83	63	55	74	74
EST	41 B	42	50	47	29	33	44	19	42	14	33	31	29	44	31	33
	99 R	38	31	29	41	31	27	17	20	37	16	57	41	27	57	16
	110 R	35	44	54	25	29	38	49	38	57	29	41	63	25	27	29
	8 B	64	47	57	57	31	41	35	40	61	31	71	67	41	57	31
	140 Ru	21	0	12	45	27	23	0	18	40	14	37	23	34	25	27
	1103 P	32	14	25	34	40	34	0	35	30	27	25	23	45	12	40
	5 BB	22	28	26	36	28	24	31	18	42	14	38	47	24	26	14
	5 C	38	31	29	41	31	27	17	20	37	16	57	41	27	43	16
	16-13 C	14	17	16	29	17	15	0	0	14	0	31	15	29	16	25
	44-53	16	0	0	17	0	0	0	0	10	0	25	0	0	17	0
	SO4	42	17	31	44	50	29	19	21	54	33	62	44	29	47	17
	420 A	38	31	43	41	47	41	17	40	37	31	57	54	41	43	31
AcPH	41 B	100	100	100	100	80	92	80	90	80	90	80	80	80	90	80
	99 R	100	100	100	100	80	92	80	90	80	90	80	80	80	90	80
	110 R	100	100	100	100	80	92	80	90	80	90	80	80	80	90	80
	8 B	100	100	100	100	80	92	80	90	80	90	80	80	80	90	80
	140 Ru	100	100	100	100	80	92	80	90	80	90	80	80	80	90	80
	1103 P	100	100	100	100	80	92	80	90	80	90	80	80	80	90	80
	5 BB	100	100	100	100	80	92	80	90	80	90	80	80	80	90	80
	5 C	100	100	100	100	80	92	80	90	80	90	80	80	80	90	80
	16-13 C	100	100	100	100	80	92	80	90	80	90	80	80	80	90	80
	44-53	100	100	100	100	80	92	80	90	80	90	80	80	80	90	80
	SO4	100	100	100	100	80	92	80	90	80	90	80	80	80	90	80
	420 A	100	100	100	100	80	92	80	90	80	90	80	80	80	90	80
Total protein	41 B	81	81	81	81	77	77	77	77	77	77	86	62	69	71	71
	99 R	73	73	73	73	67	67	67	67	83	83	92	67	74	77	77
	110 R	73	73	73	73	67	67	67	67	83	83	92	67	74	77	77
	8 B	73	73	73	73	67	67	67	67	83	83	92	67	74	77	77
	140 Ru	73	73	73	73	67	67	67	67	83	83	92	67	74	77	77
	1103 P	73	73	73	73	67	67	67	67	83	83	92	67	74	77	77
	5 BB	73	73	73	73	67	67	67	67	83	83	92	67	74	77	77

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5 C	81		81	81		77	77	77	77		77		86	62		69	71		71		
16-13 C	73		73	73		67	67	67	83		83		92	67		74	77		77		
44-53	81		81	81		77	77	77	77		77		86	62		69	71		71		
SO4	73		73	73		67	67	67	83		83		92	67		74	77		77		
420 A	73		73	73		67	67	67	83		83		92	67		74	77		77		

[a] Values above 65 indicates good compatibility.

The ideal compatibility was obtained from Riesling vines grafted onto 1613 C and 41 B rootstocks. Although almost all combinations exhibited desired levels of compatibility, cultivars were observed to have wider trunk than the rootstocks, an accepted sign of incompatibility.

Enzyme analyses showed that grape cultivars and American rootstocks had different number of bands. There were 4–6 in cultivars and 5–7 in rootstocks in PER; 4–5 in cultivars and 5 in rootstocks in AcPH; 5–10 bands in the cultivars and the rootstocks. Total protein analysis resulted in 5–8 bands in the cultivars and 6–7 in the rootstocks (Table 2).

ECC of the cultivars and the rootstocks were presented in Table 3. The number of incompatible combinations was high in PER enzyme system. Emir, Muscat of Hamburg, Italia, Narince and Pinot noir failed to show compatibility with any of the rootstocks. In addition, the cultivars, Amasya, Cardina, Çavuş, Gül üzümü, Hafizali, and Riesling, had lower ECC than 65 when they were in combinations with the rootstocks, 41 B, 8 B and 5 C.

ECC in EST indicated all the cultivar/rootstock combinations incompatible, except for Kalecik Karası and Narince grafted on 8B.

Total protein and AcPH analyses generally resulted in that the combinations had satisfactory level of compatibility levels; excluding Pinot noir grafted 41 B, 5C, and 44–53 in total protein analysis. The results agreed well with the field behavior of the cultivars tested.

Success and failure situations of the scion/stock combinations by the ECC values are summarized in Table 4.

4. Discussion

We analyzed by PAGE and SDS-PAGE the profiles of three enzymes' and total protein obtained from the woody extracts of 15 *V. vinifera* cultivars and 12 American rootstocks.

Suggestion of Santamour et al. (1986) that isoenzyme analysis of peroxidase phenotypes of scion–rootstock combinations could be used for prediction of graft incompatibility in early stages was supported by Tubbs (1973) and Bower and Nel (1981).

The results of the peroxidase analysis showed a variation in detection of incompatibility between grape cultivars and American rootstocks. There were compatible combinations as well as the incompatible ones. The ECC results indicating the most common incompatible rootstocks (5 C and 8 B) also had the lowest FCC values. This outcome was also found in the analyses of AcPH and total protein. However, the incompatibility of some cultivars such as Emir, Muscat of Hamburg, Italia, Narince and Pinot noir, with any of the rootstocks indicates the weakness of the electrophoretic method, because these cultivars have known to grow successfully with high yield in vineyards.

Esterase profiles of the combinations were not informative at all. They indicated that none of the cultivar–rootstock combinations were compatible. This is rather suspicious because grapes have known to not show an immediate incompatibility, but to express different levels of incompat-

Table 4
Success (S) or failure (F) of the combinations of the scions and rootstocks

Enzyme systems												
Scions	Peroxidase				Esterase		Acid phoshatase		Total protein	
	CC				CC		CC		CC	
	S		F		S	F	S	F	S	F
Alphonse Lavallee	41 B	5 BB			41 B	5 BB	41 B	5 BB	41 B	5 BB
	99 R	5 C			99 R	5 C	99 R	5 C	99 R	5 C
	110 R	8 B			110 R	8 B	110 R	8 B	110 R	8 B
	16-13 C	SO4			16-13 C	SO4	16-13 C	SO4	16-13 C	SO 4
	140 Ru	44-53			140 Ru	44-53	140 Ru	44-53	140 Ru	44-53
	1103 P	420 A			1103 P	420 A	1103 P	420 A	1103 P	420 A
Amasya	5 BB	140 RU	41 B		41 B	5 BB	41 B	5 BB	41 B	5 BB
	99 R	420 A	8 B		99 R	5 C	99 R	5 C	99 R	5 C
	110 R	1103 P	5 C		110 R	8 B	110 R	8 B	110 R	8 B
	16-13 C	44-53			16-13 C	SO4	16-13 C	SO4	16-13 C	SO4
	SO4				140 Ru	44-53	140 Ru	44-53	140 Ru	44-53
					1103 P	420 A	1103 P	420 A	1103 P	420 A
Ata Sarısı	41 B	5 BB			41 B	5 BB	41 B	5 BB	41 B	5 BB
	99 R	5 C			99 R	5 C	99 R	5 C	99 R	5 C
	110 R	8 B			110 R	8 B	110 R	8 B	110 R	8 B
	16-13 C	SO4			16-13 C	SO4	16-13 C	SO4	16-13 C	SO4
	140 Ru	44-53			140 Ru	44-53	140 Ru	44-53	140 Ru	44-53
	1103 P	420 A			1103 P	420 A	1103 P	420 A	1103 P	420 A
Cardinal	5 BB	140 Ru	41 B		41 B	5 BB	41 B	5 BB	41 B	5 BB
	99 R	420 A	8 B		99 R	5 C	99 R	5 C	99 R	5 C
	110 R	1103 P	5 C		110 R	8 B	110 R	8 B	110 R	8 B
	16-13 C	44-53			16-13 C	SO4	16-13 C	SO4	16-13 C	SO4
	SO4				140 Ru	44-53	140 Ru	44-53	140 Ru	44-53
					1103 P	420 A	1103 P	420 A	1103 P	420 A
Çavuş	5 BB	140 Ru	41 B		41 B	5 BB	41 B	5 BB	41 B	5 BB
	99 R	420 A	8 B		99 R	5 C	99 R	5 C	99 R	5 C
	110 R	1103 P	5 C		110 R	8 B	110 R	8 B	110 R	8 B
	16-13 C	44-53			16-13 C	SO4	16-13 C	SO4	16-13 C	SO4
	SO4				140 Ru	44-53	140 Ru	44-53	140 Ru	44-53
					1103 P	420 A	1103 P	420 A	1103 P	420 A
Emir			41 B	5 BB	41 B	5 BB	41 B	5 BB	41 B	5 BB
			99 R	5 C	99 R	5 C	99 R	5 C	99 R	5 C
			110 R	8 B	110 R	8 B	110 R	8 B	110 R	8 B
			16-13 C	SO4	16-13 C	SO4	16-13 C	SO4	16-13 C	SO4
			140 Ru	44-53	140 Ru	44-53	140 Ru	44-53	140 Ru	44-53
			1103 P	420 A	1103 P	420 A	1103 P	420 A	1103 P	420 A
Gülüzümü	5 BB	SO4	41 B		41 B	5 BB	41 B	5 BB	41 B	5 BB
	110 R	99 R	8 B		99 R	5 C	99 R	5 C	99 R	5 C
	16-13 C	44-53	5 C		110 R	8 B	110 R	8 B	110 R	8 B
	140 Ru	420 A			16-13 C	SO4	16-13 C	SO4	16-13 C	SO4
	1103 P				140 Ru	44-53	140 Ru	44-53	140 Ru	44-53
					1103 P	420 A	1103 P	420 A	1103 P	420 A
Hafızali	5 BB	140 Ru	41 B		41 B	5 BB	41 B	5 BB	41 B	5 BB
	99 R	420 A	8 B		99 R	5 C	99 R	5 C	99 R	5 C
	110 R	1103 P	5 C		110 R	8 B	110 R	8 B	110 R	8 B
	16-13 C	44-53			16-13 C	SO4	16-13 C	SO4	16-13 C	SO4
	SO4				140 Ru	44-53	140 Ru	44-53	140 Ru	44-53
					1103 P	420 A	1103 P	420 A	1103 P	420 A
Muscat of Hamburg			41 B	5 BB	41 B	5 BB	41 B	5 BB	41 B	5 BB
			99 R	5 C	99 R	5 C	99 R	5 C	99 R	5 C
			110 R	8 B	110 R	8 B	110 R	8 B	110 R	8 B
			16-13 C	SO4	16-13 C	SO4	16-13 C	SO4	16-13 C	SO4
			140 Ru	44-53	140 Ru	44-53	140 Ru	44-53	140 Ru	44-53
			1103 P	420 A	1103 P	420 A	1103 P	420 A	1103 P	420 A

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Table 4 (*Continued*)

Scions	Peroxidase				Esterase				Acid phoshatase				Total protein			
	CC				CC				CC				CC			
	S		F		S	F			S			F	S			F
Italia			41 B	5 BB		41 B	5 BB		41 B	5 BB			41 B	5 BB		
			99 R	5 C		99 R	5 C		99 R	5 C			99 R	5 C		
			110 R	8 B		110 R	8 B		110 R	8 B			110 R	8 B		
			16-13 C	SO4		16-13 C	SO4		16-13 C	SO4			16-13 C	SO4		
			140 Ru	44-53		140 Ru	44-53		140 Ru	44-53			140 Ru	44-53		
			1103 P	420 A		1103 P	420 A		1103 P	420 A			1103 P	420 A		
Kalecik Karası	41 B	5 BB				41 B	5 BB		41 B	5 BB			41 B	5 BB		
	99 R	5 C				99 R	5 C		99 R	5 C			99 R	5 C		
	110 R	8 B				110 R	8 B		110 R	8 B			110 R	8 B		
	16-13 C	SO4				16-13 C	SO4		16-13 C	SO4			16-13 C	SO4		
	140 Ru	44-53				140 Ru	44-53		140 Ru	44-53			140 Ru	44-53		
	1103 P	420 A				1103 P	420 A		1103 P	420 A			1103 P	420 A		
Narince			41 B	5 BB		41 B	5 BB		41 B	5 BB			41 B	5 BB		
			99 R	5 C		99 R	5 C		99 R	5 C			99 R	5 C		
			110 R	8 B		110 R	8 B		110 R	8 B			110 R	8 B		
			16-13 C	SO4		16-13 C	SO4		16-13 C	SO4			16-13 C	SO4		
			140 Ru	44-53		140 Ru	44-53		140 Ru	44-53			140 Ru	44-53		
			1103 P	420 A		1103 P	420 A		1103 P	420 A			1103 P	420 A		
Pinot noir			41 B	5 BB		41 B	5 BB		41 B	5 BB			16-13 C	5 BB		41 B
			99 R	5 C		99 R	5 C		99 R	5 C			8 B	140 Ru		5 C
			110 R	8 B		110 R	8 B		110 R	8 B			99 R	110 R		44-53
			16-13 C	SO4		16-13 C	SO4		16-13 C	SO4			420 A	1103 P		
			140 Ru	44-53		140 Ru	44-53		140 Ru	44-53			SO4			
			1103 P	420 A		1103 P	420 A		1103 P	420 A						
Razakı	5 BB	140 RU	41 B			41 B	5 BB		41 B	5 BB			41 B	5 BB		
	99 R	420 A	8 B			99 R	5 C		99 R	5 C			99 R	5 C		
	110 R	1103 P	5 C			110 R	8 B		110 R	8 B			110 R	8 B		
	16-13 C	44-53				16-13 C	SO4		16-13 C	SO4			16-13 C	SO4		
	SO4					140 Ru	44-53		140 Ru	44-53			140 Ru	44-53		
						1103 P	420 A		1103 P	420 A			1103 P	420 A		
Riesling	5 BB	140 RU	41 B			41 B	5 BB		41 B	5 BB			41 B	5 BB		
	99 R	420 A	8 B			99 R	5 C		99 R	5 C			99 R	5 C		
	110 R	1103 P	5 C			110 R	8 B		110 R	8 B			110 R	8 B		
	16-13 C	44-53				16-13 C	SO4		16-13 C	SO4			16-13 C	SO4		
	SO4					140 Ru	44-53		140 Ru	44-53			140 Ru	44-53		
						1103 P	420 A		1103 P	420 A			1103 P	420 A		

ibility (Lider et al., 1978; Hidalgo and Candela, 1980; Zink and Schropp, 2004; Todić et al., 2005), such as different growth rates between rootstock and scion.

The results of the electrophoretic analyses of enzymes were supported by the works of Çelik et al. (1998) in grapevine, in tomato, Gülen et al. (2002, 2005) in pear-quince grafts, Masa (1986, 1989) in grapevine, Moore and Walker (1981) in Sedum and Solanum grafts and Schmid and Feucht (1985) in Prunus grafts, which stated the possible use of enzymes in predicting graft incompatibility. However, there are conflicting reports regarding their role in graft compatibility. Copes stated the possibility of using electrophoretic methods in a practical graft testing program, but he did not found a correlation between graft incompatibility and enzyme activity or the presence or absence of isoenzyme bands of catalase, acid phosphatase or leucine aminopeptidase. D'Khili et al. (1995) stated from their study on in vitro micrografts and on green grafts of compatible and incompatible combinations that peroxidase activity should not be used and generalized for rapid characterization of incompatibility from histoenzymological results. Rodrigues et al. (2001) concluded that peroxidase and the total phenol activity influenced the union between plum scion and rootstock (Marianna and Myrobalan, respectively); after grafting, the incompatible degree is related with high peroxidase activity and total phenols. Kawaguchi and Taji (2005) also reported that peroxidase activities increased in Sturt's Desert Pea (*Swainsona formosa*) scions grafted onto incompatible rootstocks.

Compatibility constants obtained from the profiles of AcPH and total protein showed that these two biochemicals could be used for early prediction of graft incompatibility. The results generally agree with the field compatibility constants, making them more reliable to use. Analysis of protein spectra between

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stock and scion has been pointed out by many scientists (Lachaund, 1975; Gülen et al., 2002; Pedersen, 2006) to predict compatibility.

Studies for determination of best scion–rootstock combination in grapevine to a given environment have long been carried out either in nurseries or in the vineyards. It was seen from these studies that best combination of cultivar–rootstock changed and one rootstock which was found superior to another was inferior when grafted with another cultivar (Sarooshi et al., 1982; Legin and Walter, 1986; Hobockova, 1994; Kocsis and Bakonyi, 1994; Kazaliev et al., 1997). The results were conflicting and therefore, could not be generalized for all circumstances. The attempt of using electrophoretic methods in forecasting graft incompatibility faces the same challenges because, researchers should keep in mind, enzymes are prone to environmental conditions (Royo et al., 1997). The technique for preparation and analysis and the plant organ used may have some influence on the results. Zymograms might differ depending on the growth stages of the plants, where it has been obtained (greenhouse vs. open field), seasonal variation, location, or the year of study (Gogorcena et al., 1993; Royo et al., 1993, 1997).

5. Conclusion

The use of enzymes in predicting graft incompatibility is not easy because results are difficult to interpret. The results of the analyses suggest that acid phosphatase and total protein can be used in determination of graft compatibility in grapevine. Peroxidases and esterase enzyme systems are not to be used for prediction of graft compatibility. Researchers should keep in mind that grapes do not show immediate incompatibility, a situation more encountered between any other woody fruit crops such as pear and quince, and that grafting incompatibility between grapevine rootstock and scions may have resulted from virus at the graft union (Sarooshi et al., 1982) or even *Agrobacterium vitis* infected material (May, 1994; Creasap et al., 2004) or from a virus or viral agent in the scion (Legin and Walter, 1986; Uyemoto and Rowhani, 2003).

Laboratory studies have to be supported by long term field observations of growth, yield and quality, and virus indexing is needed. Inclusion of well known incompatible combinations, such as *V. rotundifolia* (cv. Carlos and Male) and *V. vinifera* (cv. Cabernet Sauvignon) (Bouqet, 1980), as the control in these type of studies may help gaining more information. Difference of opinion on which enzyme is most reliable for early detection of graft compatibility warrants further studies that provides more definitive results.

In addition to the researches that rely on enzyme systems, both physiological and genetic foundations of graft incompatibility need to be thoroughly investigated.

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