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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%.