Amplified fragment length polymorphism analysis of grapevine rootstock genotypes in Turkey

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ABSTRACT. Amplified fragment length polymorphism (AFLP) technique was used to assess genetic relationships among 20 grapevine rootstocks in Turkey. Discrimination of the rootstocks with 10 primer combinations yielded 1366 bands on AFLP gels; 65% of them were polymorphic. The rootstocks revealed two main clusters; one of them comprised two (Malégue and Harmony) and the other 18 genotypes. The *Ber x Rip* hybrid*s* Cosmo 2 and Cosmo 10 formed a group with a high internal similarity ratio (0.909); they also formed a group with other *Ber x Rip* hybrids, 5C, 8B, SO4, and 420A Mgt, with a similarity ratio higher than 0.690 (subcluster II). Rootstock 5BB was placed in another subcluster (subcluster III). Among five *Ber x Rup* rootstocks, 110R-99R (0.853) and 1103P-140Ru (0.837), which were located in different subclusters, formed a dual group, as expected. Rootstock 779P, which had almost 0.800 similarity with the dual group of 110R-99R, formed another group. The 44-53 Malégue and Harmony rootstocks formed a group with the lowest similarity ratio (0.668) (subcluster I) and 41B-Fercal formed another dual group with a high similarity ratio (0.813). The

distinction capacity of single- and double-*Eco*RI-*Mse*I primers was evaluated; primers AC/CTA, TC/CAC, AG/CTC, and AG/CAG discriminated the 20 rootstocks, with a similarity value below 0.910. The best primers for discrimination of rootstock varieties were AG/CAG and AG/CTC, while the primers TC/CAC and AC/CTA could also be useful for clonal discrimination of genotypes.

Key words: AFLP; Molecular analysis; Grapevine rootstock

INTRODUCTION

The use of different rootstocks and breeding studies is indispensable when characteristics such as tolerance to abiotic stress conditions are considered, in addition to their use against phylloxera and nematodes. DNA identification and discrimination studies of rootstocks comprising American species and their hybridization within themselves and also with *Vitis vinifera* are important especially in stages of vegetative propagation and in other biotechnological studies.

In addition to ampelographic studies and isozyme identifications (Walker and Boursiquot, 1992; Walker and Liu, 1995), sequence characterized amplified region (Xu and Bakalinsky, 1996), specific polymerase chain reaction (Bauer and Zyprian, 1997), restriction fragment length polymorphism (Guerra and Meredith, 1995; Bourquin et al., 1995), and especially random amplified polymorphic DNA (RAPD) (This et al., 1997) and simple sequence repeat (SSR) (Sefc et al., 1998; Lin and Walker, 1998) markers have been used in rootstock identification. In these investigations, in addition to determining genetic relationships, the distinctive capacity of markers in rootstocks were defined, and suitable RAPD primers and SSR loci were suggested.

Amplified fragment length polymorphism (AFLP), which scans large areas within the genome, has been used in the discrimination of *V. vinifera* genotypes (clones, synonyms, varietal groups, etc.), yielding a high similarity ratio like many other plant species, but to our knowledge its application for the purpose of rootstock genetic identifications has not been reported.

This research constitutes the first application of the AFLP technique to grapevine rootstocks to reveal the genetic relations of 20 rootstocks using 10 primer combinations. In addition, primers and primer combinations that could be used in rootstock identification, mainly in clonal discrimination, were recorded.

MATERIAL AND METHODS

Plant material

The leaves and shoots of 20 rootstock genotypes were obtained from Ankara University Faculty of Agriculture, Kalecik Viticulture Research Station, and Manisa Viticulture Research Institute of the Ministry of Agricultural and Rural Affairs. Rootstocks used for AFLP analysis, their hybridization combinations and sample collections are displayed in Table 1.

Table 1. Rootstocks used for AFLP analysis, their hybridization combinations and sample collections.

Rootstock	Parentage	Sample collection
1. du lot	*Rupestris*	1
2. Harmony	1613C x Dog Ridge	1
3. Ramsey	*Champinii*	1
4. Dog Ridge	*Champinii*	1
5. Fercal	(*berlandieri* x *vinifera*) x 333EM	1
6. 41B	*vinifera* x *berlandieri*	1
7. 1613C	*solonis* x *Othello*	1
8. 99R	*berlandieri* x *rupestris*	1
9. 110R	*berlandieri* x *rupestris*	2
10. 140Ru	*berlandieri* x *rupestris*	1
11. 1103P	*berlandieri* x *rupestris*	1
12. 779P	*berlandieri* x *rupestris*	1
13. 420A Mgt	*berlandieri* x *riparia*	1
14. SO4	*berlandieri* x *riparia*	1
15. 5C	*berlandieri* x *riparia*	1
16. 5BB	*berlandieri* x *riparia*	2
17. 8B	*berlandieri* x *riparia*	1
18. Cosmo 2	*berlandieri* x *riparia*	1
19. Cosmo 10	*berlandieri* x *riparia*	1
20. 44-53 Malégue	*riparia* x (*cordifolia* x *rupestris*)	1

1 = University of Ankara, Kalecik Viticulture Research Station, Ankara, Turkey; 2 = The Ministry of Agricultural and Rural Affairs, Institute of Viticulture, Manisa, Turkey.

DNA isolation and AFLP reaction

DNA was extracted using the procedure described by Lefort et al. (1998). Concentration and purity of the extracted DNA were analyzed using a NanoDrop® ND-1000 spectrophotometer.

The AFLP reaction was performed using a modified procedure from Vos et al. (1995) as previously described by Ergül et al. (2006), and 10 *Eco*RI/*Mse*I primer combinations were used in the analysis (Table 2).

Table 2. Selected primer combinations and polymorphism rates for AFLP analysis of rootstocks.

Primer combination		Total bands	Polymorphic bands	% Polymorphism
*Eco*RI +2-	*Mse*I +3-			
AT	CTC	142	89	62.6
AC	CAC	138	89	64.4
	CTA	149	89	59.7
TG	CAC	135	101	74.8
	CTC	106	69	65.0
TC	CAC	137	81	59.1
	CTG	137	75	54.7
TT	CTC	143	104	72.7
AG	CTC	138	106	76.8
	CAG	141	98	69.5
Total		1366	901	65.9

Data analysis

The MVSP software package version 3.1 (Kovach, 1999) was used to calculate Jaccard's (1908) similarity coefficients among the genotypes. A dendrogram was constructed using UPGMA (unweighted pair-group method with arithmetic mean).

RESULTS

While 10 *Eco*RI/*Mse*I primers yielded 1366 bands of 50-500 bp, 65% of these bands (901 bands) were detected as polymorphic and used in the analysis. The ratio of polymorphism did not change significantly according to primer combinations; the range of difference was from 59.1% (*Eco*RI-TC/*Mse*I-CAC) to 76.8% (*Eco*RI-AG/*Mse*I-CTC) (Table 2). AFLP profiles generated using primer combinations *Eco*RI-AG/*Mse*I-CTC and *Eco*RI-AG/*Mse*I-CAG are shown in Figure 1.
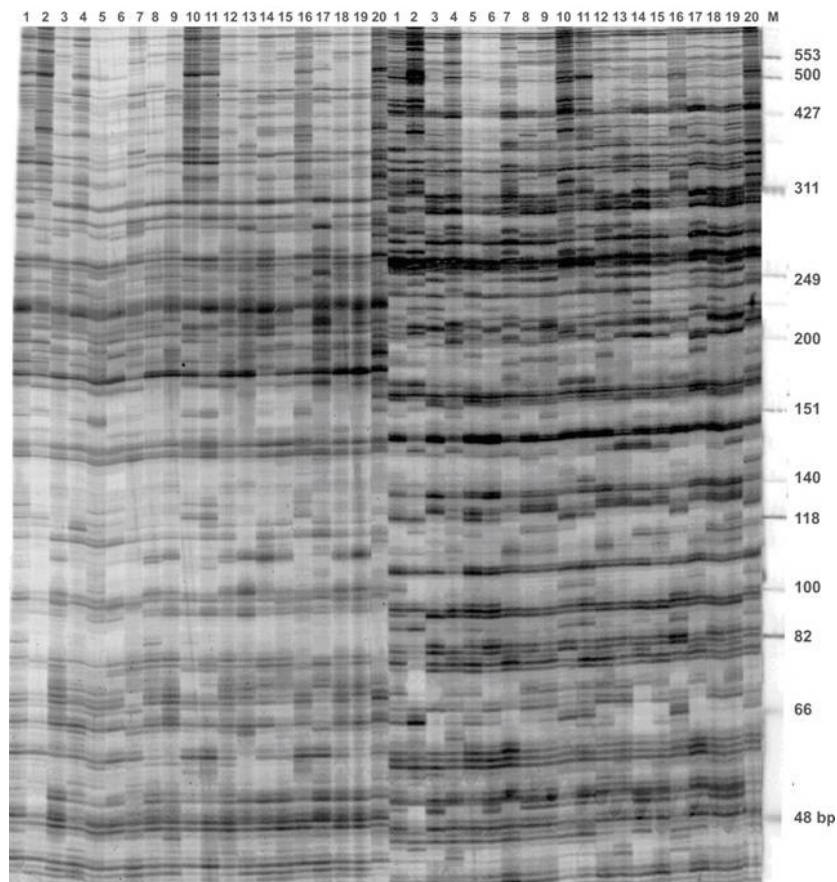

Figure 1. AFLP profiles of rootstocks generated with the selective primer *Eco*RI-AG/*Mse*I CTC and *Eco*RI-AG/*Mse*I-CAG. The names of the accessions have been listed in Table 1 from left to right [1-20], M: ØX174 DNA/*Hin*fI marker (Promega).

Jaccard genetic similarities between pairs of genotypes in each group can be visualized in the dendrogram obtained from UPGMA analysis (Figure 2). Pairwise similarity ranged from 0.587 to 0.909, and the lowest genetic similarity was found between 420A Mgt-Harmony, while the highest ratio was obtained between rootstocks Cosmo 2-Cosmo 10.
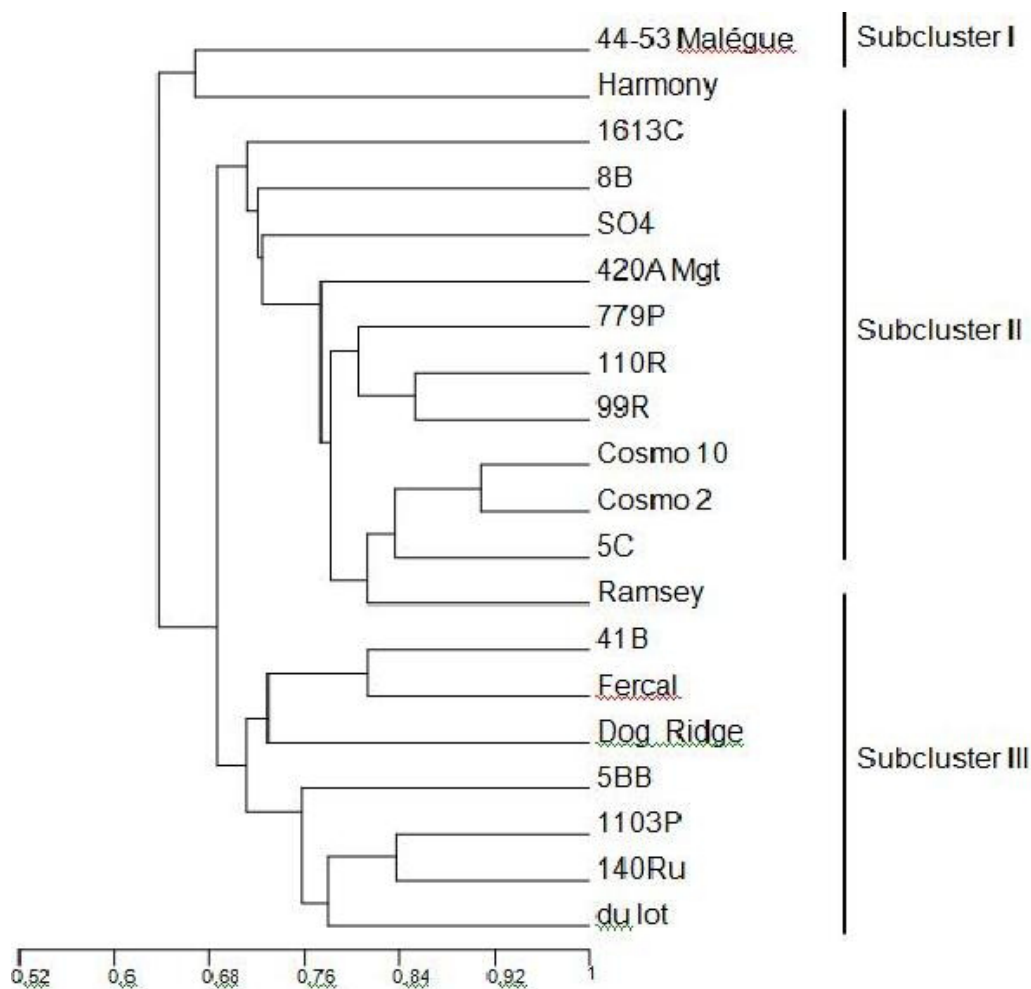
Figure 2. Dendogram showing genetic relationships among 20 rootstock cvs. based on Jaccard's similarity index.

Basically, the dendrogram was separated into two branches comprising 44-53 Malégue-Harmony (subcluster I) and the other 18 genotypes. The 18 genotypes were divided further into two branches comprising 1613C, 8B, SO4, 420A Mgt, 779P, 110R, 99R, Cosmo 10, Cosmo 2, 5C, and Ramsey (subcluster II) and 41B, Fercal, Dog Ridge, 5BB, 1103P, 140Ru, and du lot (subcluster III) (Figure 2).

DISCUSSION

Genetic relationship and similarity ratio

When the dendrogram distribution of rootstock genotypes was evaluated on the

basis of species and hybridization combinations, it was noticed that, while the hybrid of *Ber x Rip*; Cosmo 2-Cosmo 10 (0.909) formed a group with 5C, rootstocks 8B, SO4, 420A Mgt were grouped with a similarity ratio higher than 0.690 (subcluster II). 5BB, which is commonly used in Turkey and has yielded no genetic variation across nurseries based on RAPD analysis (Ergül and Ağaoğlu, 2001), took part in subcluster III separated from other *Ber x Rip* rootstocks. When the five *Ber x Rup* rootstocks were examined, it was seen that 110R-99R (0.853) and 1103P-140Ru (0.837) that were located in different subclusters formed a dual group as expected.

779P, by showing almost 0.800 similarity with the dual group of 110R-99R, formed another group (Figure 2). While 44-53 Malégue and Harmony rootstocks constitute a group with the lowest similarity ratio (0.668) (subcluster I) far apart from other genotypes, *vinifera* x American hybrid, 41B-Fercal formed another dual group showing a high similarity ratio (0.813). While Ramsey-Dog Ridge (0.794) that took place in the *Champini* group distributed in different subclusters, *rupestris* selection du lot, comprised a group with *Ber x Rup* rootstocks (1103P-140Ru) (subcluster III). 1613C, which manifested single branching, was placed in subcluster II, with the rootstocks 8B, SO4, 99R, 779P, Cosmo 2, Cosmo 10, and 5C having a similarity ratio higher than 0.700 with these rootstocks.

Distinctive capacity of the AFLP markers for rootstock genotyping

Although rootstocks have been identified previously by Upadhyay et al. (2007), only 7 (Dog Ridge, 41B, 1613C, 99R, 110R, 1103P, SO4) of the rootstocks used in the present study have been analyzed in the earlier study. In addition, *Eco*RI primer with 3 nucleotides applied in the previous research by Upadhyay et al. (2007) yielded a lower level of polymorphism compared to the *Eco*RI primer with 2 nucleotides used in this study.

Therefore, the discrimination of commonly used rootstocks in viticulture with a minimum number of primers, which constitutes the basic purpose of this study, was not investigated in previous studies. The *Eco*RI-*Mse*I primer combinations used in the study, discriminated the 20 rootstocks from each other successfully with a similarity ratio below 0.909. However, in order to display the capacity of AFLP markers and to recommend a minimum primer number for genetic analysis in rootstock discrimination, similarity ratios of single- and double-combination primers were determined. While Cervera et al. (1998) defined the genotypes showing similarity higher than 0.900 as identical, Imazio et al. (2002) identified 0.910 similarity between two different varieties, Pinot noir and Elbing. Also, Fossati et al. (2001) found 0.920 similarity between two different varieties, Rossera and Urban.

When the results of these previous studies and the ratio of 0.909 obtained for Cosmo 2-Cosmo 10 in the present study were taken into consideration, 0.910 was accepted as the upper threshold for the similarity index between genotypes, and the genotypes having values above 0.910 were regarded as "indistinguishable" (Table 3).

When Table 3 was evaluated according to this information, it could be seen that AC/CTA, TC/CAC, AG/CTC, AG/CAG primers could discriminate 20 rootstocks alone with a value below 0.910. When the other single primers were examined, the primers AT/CTC, AC/CAC, TG/CAC, TG/CTC, TC/CTG, and TT/CTC yielded similarity above 0.910 in the rootstocks Dog Ridge-Fercal, Cosmo 2-Cosmo 10, Cosmo 2-Cosmo 10, Cosmo 2-Cosmo 10,

Table 3. Rootstocks showing similarity above 91% (=0.910) with the single- and double-primer combinations that were defined as indistinguishable.

	AT/CTC	AC/CAC	AC/CTA	TG/CAC	TG/CTC	TC/CAC	TC/CTG	TT/CTC	AG/CTC	AG/CAG
AT/CTC	DR-Ferc. (0.979)									
AC/CAC	C2-C10 (0.911)	C2-C10 (0.934)								
AC/CTA		C2-C10 (0.917)								
TG/CAC	C2-C10 (0.914)	C2-C10 (0.938)	C2-C10 (0.920)	C2-C10 (0.941)	C2-C10 (0.957)					
TG/CTC	C2-C10 (0.918)	C2-C10 (0.944)	C2-C10 (0.924)	C2-C10 (0.948)	C2-C10 (0.927)					
TC/CAC		C2-C10 (0.919)		C2-C10 (0.922)						
TC/CTG		C2-C10 (0.931)	C2-C10 (0.916)	C2-C10 (0.934)	C2-C10 (0.939)	C2-C10 (0.917)	140-1103 (0.920) C2-C10 (0.928)			
TT/CTC		C2-C10 (0.922)		C2-C10 (0.925)	C2-C10 (0.930)		C2-C10 (0.920)	C2-C10 (0.912)		
AG/CTC				C2-C10 (0.915)	C2-C10 (0.914)					
AG/CAG										

DR-Ferc. = Dog Ridge-Fercal; 140 = 140Ru; 1103 = 1103P.

140Ru-1103P, and Cosmo 2-Cosmo 10, respectively, and these primers were considered as "unable to discriminate" for the rootstock combinations.

When the distinctive capacities of two primers were examined among rootstock genotypes, it could be deduced that AC/CTA, TC/CAC, TC/CTG, TT/CTC, AT/CTC; TC/CAC, TT/CTC, AC/CTA; TT/CTC, TC/CAC; except TG/CAC, TG/CTC, other combinations of AG/CTC and all combinations of AG/CAG primer discriminated 20 rootstocks with a similarity value below 0.910. When the other 23 dual primer combinations were examined they were regarded as "unable to discriminate" for Cosmo 2-Cosmo 10 rootstocks, but on the other hand, they could be able to discriminate 18 rootstocks. Rootstock numbers, single and double primers able to discriminate are displayed in Table 4.

Table 4. The number of rootstocks that could be discriminated by single- and double-primer combinations (based on a value of 91% (=0.910)).

	AT/CTC	AC/CAC	AC/CTA	TG/CAC	TG/CTC	TC/CAC	TC/CTG	TT/CTC	AG/CTC	AG/CAG
AT/CTC	19									
AC/CAC	19	19								
AC/CTA	20	19	20							
TG/CAC	19	19	19	19						
TG/CTC	19	19	19	19	19					
TC/CAC	20	19	20	19	19	20				
TC/CTG	20	19	19	19	19	19	18			
TT/CTC	20	19	20	19	19	20	19	19		
AG/CTC	20	20	20	19	19	20	20	20	20	
AG/CAG	20	20	20	20	20	20	20	20	20	20

This study aimed to determine the distinctive capacity of AFLP markers in rootstock identification, and as a result, 4 single- and 20 double-primer combinations were shown to be successful in the discrimination of 20 rootstocks (Table 4). If the upper threshold of the similarity ratio, which was kept low and restricted to 91% (=0.910) to maintain the easy discrimination of close genotypes (Cosmo 2-Cosmo 10), were increased within certain limits (for example up to 92% (=0.920)), the number of primers and primer combinations that could be used in genotype differentiation would also increase.

The results of the present study suggest that AFLP markers, being highly reproducible and efficient in the determination of polymorphism, provide an excellent tool in the discrimination of rootstock genotypes using single- or double-primer combinations. The study also suggests that the primers that could have priority in the identification of rootstock varieties are AG/CAG or AG/CTC, while the primers TC/CAC and AC/CTA could also be used in clonal discrimination of genotypes.

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