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Parentage Identification of 'Daebong' Grape (*Vitis* spp.) Using RAPD Analysis

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Abstract

The RAPD data were used to assess genetic similarity among 7 grape cultivars. Of the 100 random primers tested on genomic DNA, 10 primers could be selected for genetic analysis, and the selected primers generated a total of 115 distinct amplification fragments. A similarity matrix was constructed on the basis of the presence or absence of bands. The 7 grape cultivars analyzed with UPGMA were clustered into two groups of A and B. The similarity coefficient value of cultivars was high. The mean similarity index for all pairwise comparisons was 0.851, and ranged from 0.714 ('Rosaki' and 'Black Olympia') to 0.988 ('Kyoho' and 'Daebong'). After due consideration of differences in cultural and morphological characteristics of these two theoretically identical cultivars, it could be deduced that 'Daebong' is a bud sport of 'Kyoho' cultivar.

Introduction

Traditional methods of identifying grape cultivars have relied on morphological characters whose expression is affected by developmental and environmental factors. Since the first published description of randomly amplified polymorphic DNA (RAPD), this polymerase chain reaction (PCR)-based technique has been adopted as a convenient and powerful means of detecting genetic differences among closely related organisms (Welsh and McClelland, 1990; Williams et al., 1990). The need for practical and objective means for identifying grape cultivars has encouraged researchers to investigate DNA-based techniques (Bott et al., 1995; Bourquin et al., 1993; 1995; Striem et al., 1990, 1996; Thomas et al., 1994; Meredith et al., 1999; Xu and Bakalinsky, 1996; Xu et al., 1995; Bowers and Meredith, 1996; Ronning and Schnell, 1995).

'Kyoho', a tetraploid cultivar derived from 'Ishihara Wase' (4x)X'Centennial' (4x), is a very important grape cultivar, occupying 14.4% of total Korean vineyard areas (MAF, 1997). Although 'Daebong' was assessed slightly lower in fruit quality and sugar content compared to its presumed original cultivar 'Kyoho' (RDA, 1999a), the cultivar is popular in some growing areas such as Okcheon occupying 7% in planted area (RDA, 1999b) due to its much improved berry set.

Under this study, a phenetic analysis of RAPD data obtained from 7 *Vitis* cultivars was presented. The objective of our study was to assess genotype identification, and the estimation of genetic relationship by RAPD assay in 'Daebong' cultivar along with the original 'Kyoho' family cultivars.

Materials and Methods

Plant materials

Seven grapes cultivars ('Daebong', 'Kyoho', 'Campbell Early', 'Rosaki', 'Ishihara Wase', 'Centennial', and 'Black

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Olympia') were collected from Okcheon Grape Experiment Station located in Okcheon, Chungbuk province. Genetic composition and the origin of cultivars are shown in Table 1.

DNA extraction and RAPD conditions

One hundred mg young leaves were ground in a mortar and pestle to a fine powder by using liquid nitrogen. Genomic DNA was extracted according to the protocol of plant genomic DNA miniprep system (VIOGENE, CA, USA). DNA concentrations were determined spectrophotometrically. In a preliminary experiment, the influence of template DNA and primer concentration was tested with 2 primers (UBC 537 and UBC 546), 5 DNA concentrations (10, 20, 30, 50, and 100 ng) and 5 primer concentrations (10, 20, 30, 50 and 100 pmol), respectively.

Amplification

Genomic DNA was amplified using 100 different RAPD primers (The University of British Columbia) and the primers which have yielded optimal results are shown in Table 2. The reaction included 40 mM KCl, 10 mM Tris-HCl (pH 9.0), 0.1% (v/v) Triton X-100, 1.5 mM MgC_{12}, 250 μM each dATP, dCTP, dTTP, dGTP, 1 unit Taq DNA polymerase (Bioneer, Cheongweon, Korea), 20 pmol primer, and 50 ng genomic DNA, in a final volume of 20 L. Reactions were amplified immediately. Amplifications were performed in a DNA thermal cycler (Model 480; Perkin Elmer, Norwalk, Conn., USA) preheated to 94℃, 5 min, cycling parameters were 45 cycles of 94℃, 1 min; 36 ℃, 1 min; and 72℃, 2 min. After the last cycle, the samples were kept at 72℃ for 10 min and then cooled to 4℃. PCR products were separated by electrophoresis on a 1% agarose gel in 0.5x TAE buffer (0.045 M Tris, 0.001 M EDTA, acetic acid), and visualized by ethidium bromide staining.

Analysis

Each individual was amplified at least twice; reproducible, polymorphic bands were scored as 1 (band present) or 0 (band absent). Percent band sharing between pairs of cultivars was calculated as the number of shared bands divided by the total number of bands. A similarity matrix was constructed with the Dice similarity coefficient (Dice, 1945), which represents the proportion of shared bands and is identical to the similarity coefficient of Nei and Li (1979). Cluster analysis was performed with Molecular Analyst Fingerprinting program (ver 1.1).

Results and Discussion

While 100 UBC primers were used initially, 10 primers gave optimal amplified DNA, generating a total of 115 bands in 'Kyoho', 'Daebong', 'Campbell Early', 'Rosaki', 'Ishihara Wase', 'Centennial', and 'Black Olympia'. Polymorphisms shown among grape cultivars were 89 bands (77.4%). All 10 primers produced at least one vari-

Table 1. Genetic composition and origin of seven grape cultivars used for the RAPDs.

Cultivar	Genetic composition	Origin
Kyoho	*Vitis labrusca, V. vinifera*	Ishihara Wase × Centennial
Daebong	*V. labrusca, V. vinifera*	Bud sport of Kyoho × ?
Campbell Early	*V. labrusca, V. vinifera*	Moore Early × (Belvidere × Muscat Hamburg)
Rosaki	*V. vinifera*	
Ishihara Wase	*V. labrusca, V. vinifera*	Tetraploid bud sport of Campbell Early
Centennial	*V. vinifera*	Tetraploid bud sport of Rosaki
Black Olympia	*V. labrusca, V. vinifera*	Kyoho × Kyogei

Table 2. RAPD primers used to analyze genetic variation among 'Kyoho', 'Daebong', 'Campbell Early', 'Rosaki', 'Ishihara Wase', 'Centennial', and 'Black Olympia'. Percent polymorphism reflects the number of total bands from each primer that distinguishes at least one cultivar.

Primer	No. of bands	No. of polymorphic bands	Percent polymorphism
526	10	9	90.0
537	15	14	93.3
540	18	13	72.2
546	12	8	66.7
560	9	7	77.8
584	9	6	66.7
588	7	6	85.7
594	7	7	100.0
599	15	10	66.7
600	13	9	69.2

able locus in all cultivars (Table 2). Optimal template DNA concentration and primer concentration settled was 50 ng and 20 pmole, respectively. However, DNA concentration was not affected in these results (Figure 1 and Figure 2). The RAPD reaction is competitive because of the low annealing temperature and short primer and template (Heun and Helentjaris, 1993). The competitive aspect of RAPD analysis may explain in part why minor changes in almost any aspect of the amplification reaction have been reported to affect the outcome: DNA quality and quantity (Williams et al., 1993), choice of DNA polymerase (Schierwater and Ender, 1993), Mg concentration (Park and Kohel, 1994; Williams et al., 1993), choice of thermal cycler (Penner et al., 1993), primer concentration (Williams et al., 1993), use of ethidium bromide vs. silver for detection of products (Caetano-Anolles et al., 1992), and presence of RNA (Yoon and Glawe, 1993). Primers were evaluated for their ability to identify any one cultivar as illustrated by the example of primers 537, 540 and 546 shown in Figure 3. Both 'Kyoho' (Lane 1) and 'Daebong' (Lane 2) were distinguishable by amplification fragments of primer 537. The 7 cultivars were identified by means of the six primers that were selected for their ability to generate

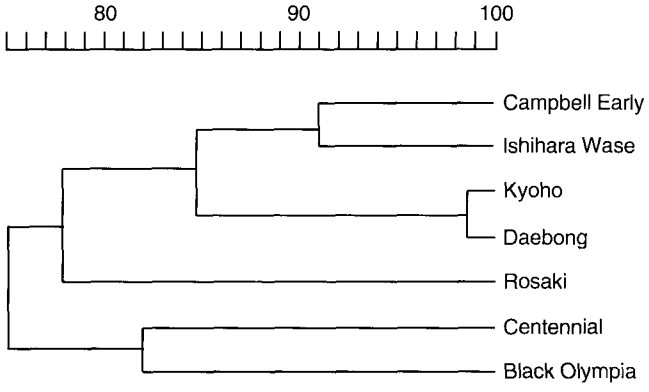

Figure 1. Electrophoretic separation of RAPD amplification products from 'Kyoho'. A: template DNA concentration (10, 20, 30, 50, 100 ng), B: primer concentration (10, 20, 30, 50, 100 pmole), M: 1kb DNA ladder.

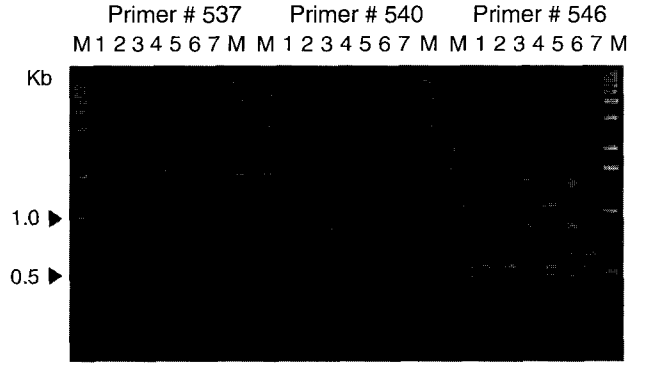

Figure 2. Electrophoretic separation of RAPD amplification products, using primer 537, 540 and 546. Lanes 1: Kyoho, 2: Daebong, 3: Campbell Early, 4: Rosaki, 5: Ishihara Wase, 6: Centennial, 7: Black Olympia, M: 1Kb DNA ladder.a

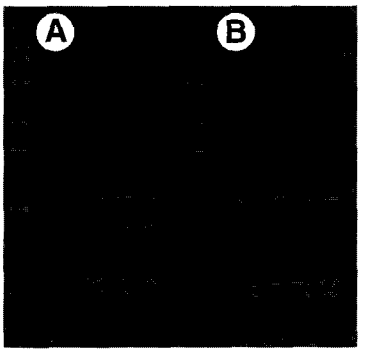

Figure 3. Dendrogram illustrating UPGMA cluster analysis of 7 grape cultivars.

Table 3. Percent bands shared between pairwise combinations of 'Kyoho', 'Daebong', 'Campbell Early', 'Rosaki', 'Ishihara Wase', 'Centennial', and 'Black Olympia'.

Cultivar	Kyoho	Daebong	Campbell Early	Rosaki	Ishihara Wase	Centennial	Black Olympia
Kyoho	100.0						
Daebong	98.8	100.0					
Cambell Early	85.3	86.7	100.0				
Rosaki	80.6	79.2	74.2	100.0			
Ishihara Wase	84.0	85.3	91.3	77.3	100.0		
Centennial	74.8	74.8	75.6	76.0	74.0	100.0	
Black Olympia	73.6	73.6	80.3	71.4	77.3	82.6	100.0

unique polymorphic amplification fragments. The base sequences of these primers are listed in Table 2. The mean similarity index for all pairwise comparisons was 0.851 and ranged from 0.714 ('Rosaki' and 'Black Olympia') to 0.988 ('Kyoho' and 'Daebong') (Table 3).

Similar results were obtained with unweighted pair group method with arithmetic mean (UPGMA). When 7 *Vitis* hybrids were analyzed with UPGMA (Molecular Analyst Fingerprinting program (ver. 1.1) Bio-Rad), they were clustered into two groups of A and B. The clusters produced by UPGMA are illustrated as a dendrogram in Figure 1. The branch point at which any two cultivars diverge indicates their degree of similarity.

It must be emphasized that the dendrogram illustrates the relative similarity within and between clusters of cultivars, but does not imply an evolutionary path by which the cultivars might have originated.

Very close relationship between 'Kyoho' and 'Daebong' is evident in Figure 1. All were consistently revealed by all the clustering methods used. Some of these relationships confirm previously known or suspected genealogy, though others were not anticipated. The similarity coefficient value of *Vitis* was shown high (0.714 ~0.988). According to the results, it could be deduced that 'Daebong' is a bud sport of 'Kyoho' cultivar.

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