RAPD Analysis of ‘Koshu’ Grape and Its Self- Pollinated Progenies

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Summary

Random amplified polymorphic DNA (RAPD) analysis was applied to a Japanese grape cultivar ‘Koshu’ and nine selected self- pollinated progenies. The RAPD analysis, using 18 primers, resulted in identical banding patterns with one primer but different banding patterns with the other primers. A total of 108 bands, including three novel bands that were not found in the patterns of the parent, were detected. Percentage similarity between each pair of vines ranged from 68.4% to 90.8%. The similarity values among progenies were comparatively low, whereas those between progenies and parent were comparatively high. Therefore, the data indicate that diversity among the nine vines of the self- pollinated seedlings was as broad as between pre- existing cultivars.

Key Words: ‘Koshu’, novel bands, RAPD, self- pollinated seedling.

Introduction

The Japanese grape ‘Koshu’ was first introduced from China into Japan 800 to 1200 years ago. ‘Koshu’ is considered to be a member of the cluster of oriental cultivars of Vitis vinifera, although the RFLP pattern of ‘Koshu’ showed two weak bands that are not found in other cultivars of Vitis vinifera (Goto- Yamamoto et al., 1998). ‘Koshu’ was originally grown as a table grape in Japan, but over 70% of the crop is now used for wine so that it is the primary wine- grape cultivar of Japan. However, Koshu- wines lack character and aroma and, are, thus, classified world- wide as being mediocre.

In this study, we attempted to breed and select ‘Koshu’ strains for wine making, which have the following characteristics: small cluster and berry, rich character and aroma, and higher acidity. Previously, nine vines were selected from self- pollinated seedlings during sprouting, based on shape and color of young leaves, color of hypocotyl, and differences in DNA confirmed by RAPD analysis. In our study, we used the same method to compare DNA polymorphisms among ‘Koshu’ and its self- pollinated progenies by RAPD.

Materials and Methods

The DNAs of ‘Koshu’ and nine selected self- pollinated progenies were isolated and analyzed by the RAPD method. A total DNA was extracted from young and fully expanded leaves according to the methods of Yamamoto et al. (1991), with some modifications. Eighteen decamer oligonucleotides, OPA- 01, 02, 04, and 06–20 (Operon Technologies, Inc.) were used as primers. Amplification was performed in a Perkin- Elmer 9600 thermal cycler programmed as follows: initial denaturation 2 min at 94 °C, followed by 45 cycles of 1 min denaturation at 92 °C, 1 min annealing at 35 °C, and 2 min extension at 72 °C. The amplified mixtures were electrophoresed through 2.5% agarose gels. The gels were stained with ethidium bromide and the presence or absence of each band was recorded. The amplification was repeated at least two or three times, and only reproducible data were retained.

The percentage similarity (Ye et al., 1998) was calculated as the number of shared bands (either present or absent) between two genotypes divided by the total number of bands in the entire group of genotypes analysed.

Results and Discussion

The DNA analyses of ‘Koshu’ and its nine self- pollinated progenies yielded a total of 108 bands, including three novel ones. The RAPD banding patterns of OPA- 01 and OPA- 11 (Fig.1) revealed that with the former all progenies had banding patterns identical to that of the parent, whereas the latter resulted in unique banding patterns for all progenies and the parent. Moreover, with OPA- 09, and OPA- 11, the progenies 0058, 0012, and 0053 yielded three novel bands which are not present in the parent. Büscher et al.(1994) reported that RAPD analysis of two grape progenies, ‘Kerner’ and ‘Diana’, using 11 primers, identified 2 of 68 bands (2.8%) and one of 66 bands (1.8%) novel bands, respectively, which were not found in the parental cultivars. The novel bands, identified in our study, may have been caused by artifacts or competition (Hansen et al., 1998) during RAPD analysis or transfer during meiosis, which induced a mutation in the DNA sequence. Therefore, the origin of these novel bands is yet to be confirmed, based on their sequences.

The calculated percentage similarities for our samples
Table 1. Percentage similarity among ‘Koshu’ and its nine selected self-pollinated progenies as determined by PCR using 18 primers.

<table>
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<th>0012</th>
<th>0033</th>
<th>0037</th>
<th>0041</th>
<th>0047</th>
<th>0053</th>
<th>0058</th>
<th>0077</th>
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Fig. 1. Amplification patterns of polymorphic DNA from ‘Koshu’ and its self-pollinated progenies generated by primers OPA-01 and OPA-11. M: φ X174/Hae digest. Arrows indicate novel bands that were not found in the parent.

thus similar to that among pre-existing cultivars. Stavrakakis et al. (1997) investigated genetic similarity among eight Greek grape cultivars by RAPD analysis, using 15 different primers. The highest percentage similarity of 86.5% was observed between progenies and parent, while conversely, those between progenies averaged 80.7%. In the present study, because the nine self-pollinated seedlings were selected based on shape and color of young leaves, and color of hypocotyl, we expected a lower similarity between the progenies. Thomas et al. (1993) reported that individual grape cultivars are highly heterozygous. Likewise, we observed high heterozygosity in ‘Koshu’. Since we detected genetic differences among ‘Koshu’ and its self-pollinated progenies, we expect success in identifying a “Neo-Koshu”, adaptable for wine making.

Literature Cited

自家受粉‘甲州’実生株の RAPD について

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摘 要

日本の伝統的ブドウ品種‘甲州’を用いて、親株と選抜した自家受粉実生株9株の RAPD 解析を行った。18 種のプライマーを用いて RAPD を行ったところ、一つのプライマーでは同じバンドパターンを示したが、残りのプライマーではそれぞれ違ったバンドパターンを示し、親のパターンにない3本の新規バンドを含めて、総数108種類のバンドが得られた。
パーセント相合性は、それぞれの株同士68.4％から90.8％に分布していた。子株間の相合性は比較的低く、親株と子株間は比較的高かった。自家受粉実生株9株の間では、既存の栽培品種間と同じ程度の相合性を示す株もあった。