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Identification of apple cultivars using RAPD markers

B. Koller, A. Lehmann, J. M. McDermott, and C. Gessler

[1] Institute of Plant Sciences, Section Phytomedicine, Swiss Federal Institute of Technology. Universitätstr. 2, CH-8092 ETH-Zürich, Switzerland

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Summary. Eleven apple cultivars were differentiated using randomly amplified polymorphic DNA (RAPD) markers obtained by the polymerase chain reaction (PCR). The variability of the technique and of the origin of the DNA extract was analyzed. A set of bands consistent in their presence or absence was chosen to create a differentiating band pattern. A key is proposed by which one can differentiate apple cultivars using commercially available prime.

Key words: RAPD/PCR fingerprint – DNA – Apple – Breeding – Identification

Introduction

The capability to distinguish apple cultivars as they are being multiplied and grown is extremely important, as the correct identification is usually not possible until fruit are produced (Weeden and Lamb 1985). The characterization of cultivars requires a large set of phenotypic data that is often difficult to assess and sometimes variable due to environmental influences. At the present time the term cultivar means that all trees carrying a particular cultivar name are phenotypically equal and originate from the same ancestor by vegetative reproduction. This implies that all trees of a certain cultivar have basically the same genome.

Although isoenzyme systems have been useful in cultivar identification (Weeden and Lamb 1985), they are limited by the number of informative markers and give no direct assessment of the potential variation present in the genome. In addition, certain systems are prone to environmental or developmental variation. Direct assessment of genetic variation at the DNA level avoids such difficulties. Restriction fragment length polymorphisms (RFLPs) have been used to identify apple clones and seedlings (Nybom and Schaal 1990; Watillon et al. 1991), but the technique is laborious and not suited for studies of a large number of samples (Williams et al. 1990). Randomly amplified polymorphic DNA (RAPD) markers generated by the polymerase chain reaction (PCR) can be used to differentiate between morphologically indistinguishable strains and varieties (Welsh and McClelland 1990; Welsh et al. 1991; Goodwin and Annis 1991). DNA profiles based on arbitrarily primed PCR are both time and cost effective (Hedrick 1992). Furthermore, the availability of markers will aid in mapping genes coding for agronomically important characters. Such a molecular aid will increase the efficiency and reduce the time-scale of plant breeding (King et al. 1991; Wolfe and Gessler 1992).

In the investigation presented here we tested the reliability of the RAPD-PCR system as a tool for the identification of apple cultivars.

Materials and methods

The following apple cultivars were used in this study: 'Arlet', 'Cox Orange', 'Florina', 'Gala', 'Glockenapfel', 'Golden Delicious', 'Idared', 'Ingold', 'Ontario', 'Red Delicious' and 'Spartan'.

DNA isolation

Apple leaves were frozen immediately in liquid nitrogen and stored at −80 °C. DNA was extracted as described by Dellaporta et al. (1983) with the following modifications: after

Correspondence to: C. Gessler

RNAse treatment 1 vol chloroform-isoamylalcohol (24:1) was added, mixed, and centrifuged at 6500 *g* for 10 min. The upper aqueous phase was transferred to a new tube, and 5 *M* NaCl was added to a final concentration of 0.2 *M*. Two Volumes of cold ethanol (−20 °C) was added, mixed, and allowed to stand for 30 min at 4 °C. After centrifugation at 6500 *g*, the supernatant was discarded and the DNA pellet was rinsed in 500 µl 70% ethanol. After centrifugation the ethanol was discarded and the pellet air dried. The DNA was dissolved in 100 µl TE buffer pH 7.4.

Amplification conditions

Amplification reaction volumes were 25 µl, each containing 10 m*M* TRIS-HCl pH 8.3, 50 m*M* KCl, 2.5 m*M* $MgCl_2$, 100 µ*M* each of dATP, dCTP, dGTP, and TTP (Boehringer), 0.28 µ*M* primer, 5 ng genomic DNA and 1 U Taq DNA polymerase (Boehringer). Amplification was performed in a Perkin Elmer Cetus Gene Amp PCR System 9600 programmed as follows: 2 cycles of 30 s at 94 °C, 30 s at 36 °C, 120 s at 72 °C; 20 cycles of 20 s at 94 °C, 15 s at 36 °C, 15 s at 45 °C, 90 s at 72 °C; 19 cycles of 20 s at 94 °C (increased 1 s/cycle), 15 s at 36 °C, 15 s at 45 °C, 120 s at 72 °C (increased 3 s/cycle), followed by 10 min at 72 °C.

The amplification products were electrophoresed in 1.5% agarose (Biorad) gels with 1 × TPE (0.09 *M* TRIS-phosphate, 0.002 *M* EDTA) and stained with ethidium bromide.

The following primers were used: primer P2 5′ACGAGGGACT; E6 5′AAGACCCCTC.

Results

The arbitrarily primed DNA profiles of five separate DNA samples with two primers is illustrated in Fig. 1. This analysis was performed on DNA extracted from five 'Golden Delicious' trees grown on M26 rootstock to show within-cultivar variation of the amplification results. For each primer the DNA profiles are uniform over the five trees.

The results of the PCR amplifications were robust over a wide range of DNA concentrations. With more than a 2000-fold change in DNA concentration the variation in the PCR amplifications were primarily quantitative (Fig. 2). This consistency is especially true for bands that are strongly amplified. To show repeatability, PCR amplifications were performed four separate times with two separate, arbitrary primers on extracts from a set of 2 cultivars. Variation in the DNA profiles could be observed among the sequential PCR runs. Some bands in the DNA profiles (Fig. 3) were consistently amplified in each run, while others varied considerably.

A set of 11 arbitrarily chosen apple cultivars was subjected to several arbitrarily primed PCR runs.

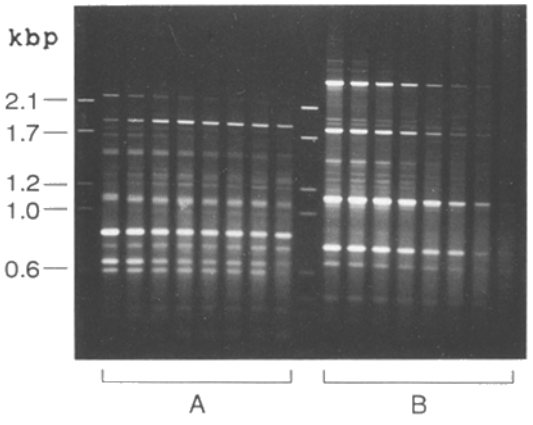

Fig. 2. Effect of DNA concentration in PCR reaction mixture. From *left to right*: each lane is the three-fold dilution of the previous lane. DNA was extracted from cv 'Glockenapfel'. In *A*, primer P2, in *B*, primer E6 was used. The highest DNA concentration was 55 ng DNA in 25 µl reaction mixture (*left lanes* of *A* and *B*)

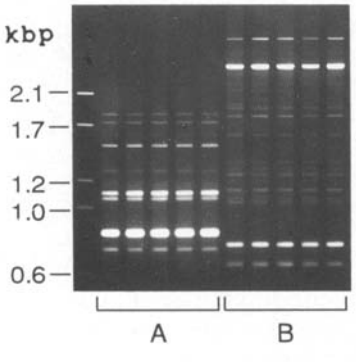

Fig. 1. Amplified DNA polymorphisms of five 'Golden Delicious' scions. *A* and *B* were made with two different primers (P2; E6) in the same PCR run. PCR patterns using a particular primer do not differ between the inoculants. Electrophoresis was performed on agarose gel (1.5%)

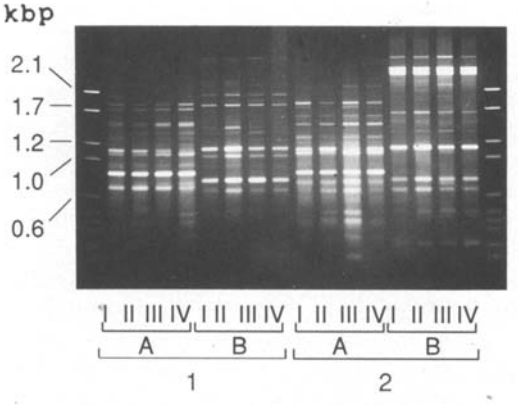

Fig. 3. Amplified DNA polymorphisms of extracts from cvs 'Arlet' (*1*) and 'Florina' (*2*) using two primers (*A* primer P2, *B* primer E6). Four different PCRs (I, II, III, IV) were performed at separate times. The DNA concentration was 5 ng DNA in 25 µl reaction mixture

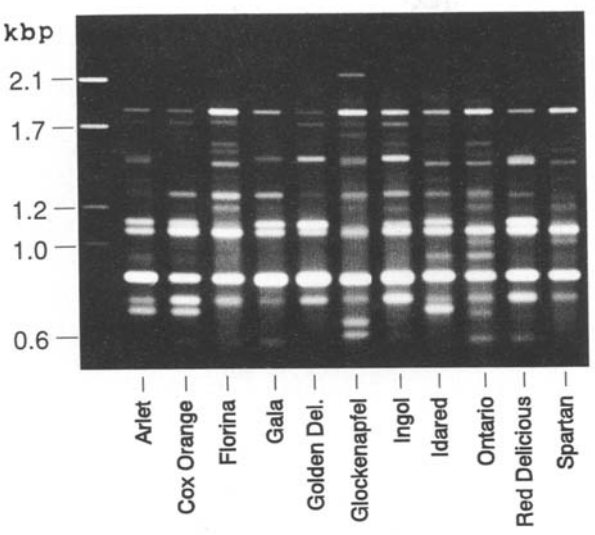

Fig. 4. Banding patterns of 11 different apple cultivars in a simultaneous PCR using primer P2. The DNA concentration was 5 ng DNA in 25 µl reaction mixture

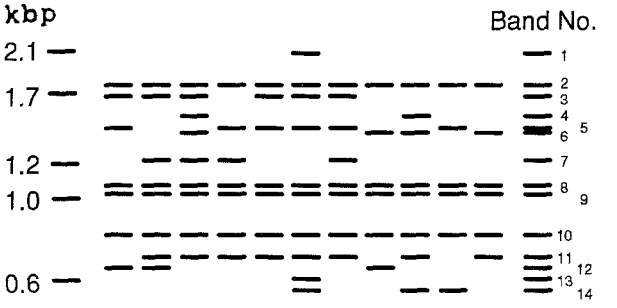

Fig. 6. Banding pattern system created by selecting consistent bands from Fig. 5

Visible in the banding patterns of these 11 cultivars were both bands that appeared consistently throughout all cultivars as well as bands that appeared in only some cultivars (Fig. 4). Some variation in the banding pattern of a single cultivar was observed when the amplifications were repeated at five separate times (Fig. 5). For each apple cultivar we identified a set of bands that appeared consistently in all five repetitions, disregarding those bands that appeared in all of the cultivars. This set provided 14 RAPD markers that can be used to clearly distinguish among the 11 cultivars. This informative variation is schematically represented in Fig. 6. Scoring for the presence or absence of these markers results in a unique binary code for each cultivar (Table 1).

Discussion

We have demonstrated that arbitrarily chosen commercial decamer primers can be used to generate amplified segments of genomic DNA that can differentiate apple cultivars. This method is rapid and simple, and produces repeatable results. By prescreening 24 10-mer primers for their informativeness we found one primer that detects enough genetic variation among the 11 apple cultivars to allow for complete

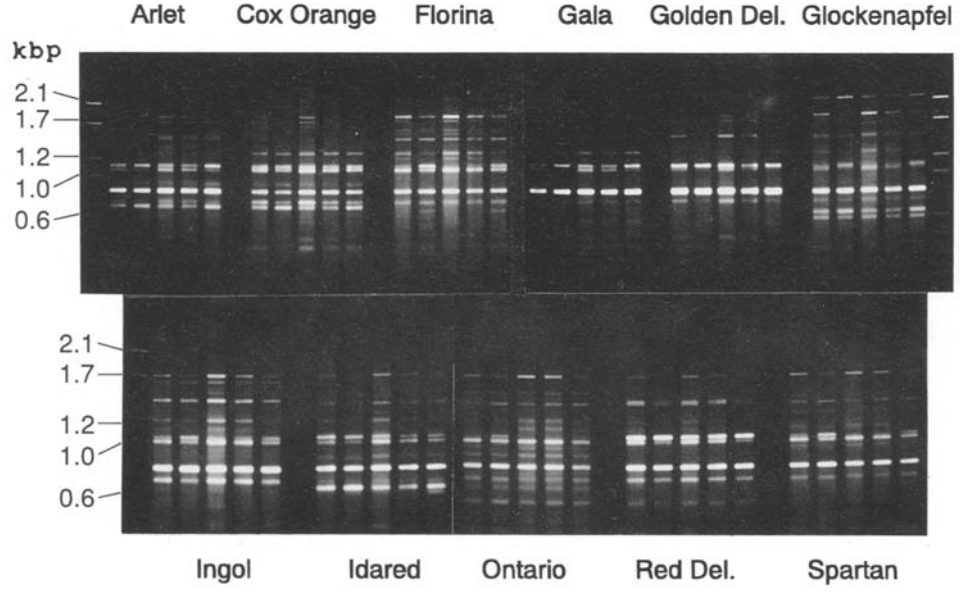

Fig. 5. Amplified DNA polymorphisms of extracts from 11 different apple cultivars. The PCR was repeated 5 separate times using primer P2. The DNA concentration was 5 ng DNA in 25 µl reaction mixture

Table 1. System for differentiation of 11 apple cultivars based on the presence (1) or absence (0) of chosen RAPD bands (Fig. 6)

	Band number													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Arlet	0	1	1	0	1	0	0	1	1	1	0	1	0	0
Cox Orange	0	1	1	0	0	0	1	1	1	1	1	1	0	0
Florina	0	1	1	1	0	1	1	1	1	1	1	0	0	0
Gala	0	1	0	0	1	0	1	1	1	1	1	0	0	0
Golden Delicious	0	1	1	0	1	0	0	1	1	1	1	0	0	0
Glockenapfel	1	1	1	0	1	0	0	1	1	1	1	0	1	1
Ingol	0	1	1	0	1	0	1	1	1	1	1	0	0	0
Idared	0	1	0	0	0	1	0	1	1	1	0	1	0	0
Ontario	0	1	0	1	0	1	0	1	1	1	1	0	0	1
Red Delicious	0	1	0	0	1	0	0	1	1	1	0	0	0	1
Spartan	0	1	0	0	0	1	0	1	1	1	1	0	0	0

differentiation. By selecting only strongly (and therefore consistently) amplified DNA segments as informational bands, variations in minor bands resulting from different amplifications can be excluded.

The 14 bands allow a theoretical differentiation of 16384 band combinations, which is more than sufficient for all known apple varieties. However, the narrow gene pool of apple and the close relationship among many cultivars will require additional markers, generated by more primers, to fully characterize and distinguish a larger set of cultivars.

Given the results so far it should be possible to establish a standard set of primers that can be used to distinguish and characterize most of the common apple cultivars. If this system were to be generally used it would be useful to generate a set of amplified DNA fragments corresponding to the informative markers to serve as size standards for evaluating the presence or absence of particular bands. This would facilitate the comparison of results from different research groups.

The use of RAPD analysis in the identification and characterization of apple cultivars and breeding lines would be of considerable help to breeding institutes and nurseries. These markers would also be of use in the European apple genome mapping project (EAGMAP: King et al. 1991).

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