RFLPs Obtained from an rDNA Probe and Detected with Enhanced Chemiluminescence in Apples

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Abstract. Individual-specific DNA fragment patterns were obtained by hybridization of endonuclease-digested apple (*Malus ×domestica* Borkh.) DNA with a probe (pAR72) derived from the rDNA spacer region of the 'White Angel' crab apple. Fragment detection was carried out with a nonradioactive method, using a horseradish peroxidase-catalyzed luminol oxidation. Paternity could be inferred by comparison of the fragment pattern generated by a seedling with those derived from putative parents.

Several recent investigations have reported on DNA fragment patterns obtained from hybridization of apple DNA with various probes. These fragment patterns are generally stable within cultivars (Nybom, 1990a; Nybom et al., 1990) but highly variable among different genotypes (cultivars or seedlings). They have, therefore, proven very useful for cultivar identification (Nybom et al., 1990; Watillon et al., 1991) and pater-

nity analysis (Nybom, 1990b; Nybom and Schaal, 1990).

However, a serious drawback to the methodology is that fragment detection usually involves the use of radioactive isotopes, e.g. ³²P. Although several nonradioactive fragment-detection methods have been reported, these have been used almost exclusively in analysis of human DNA (Macedo et al., 1989; Pollard-Knight et al., 1990; Zischler et al., 1989). For plant DNA, fragment detection has been accomplished by using biotin (Hueros et al., 1990), digoxigenin (Weising et al., 1991), and alkaline phosphatase with chemiluminescence (Kreike et al., 1990). However, these studies have not involved a detailed analysis of complex DNA fragment patterns.

In a previous study, DNA extracted from

apple seedlings derived by open pollination between cultivars Golden Delicious (GD), Red Delicious (RD), and Jonathan (JO) was digested with the restriction enzyme *TaqI*, and in some cases also with *RsaI*, and electrophoresed in an agarose gel (Nybom and Schaal, 1990). The resulting gels were Southern-blotted onto Nytran nylon filters (Schleicher & Schuell, Dassel, Germany) and hybridized initially to a ³²P-labelled M13 minisatellite probe. The highly variable DNA fingerprints obtained enabled deductions about paternity in 56 out of 64 cases (Nybom and Schaal, 1990). In the present study, filters with the *RsaI*-digested samples were hybridized to the pAR72 probe derived from an rDNA spacer region of the 'White Angel' crab apple (Simon and Weeden, 1991). DNA fragments were detected with the enhanced chemiluminescence kit from Amersham International (Amersham, Great Britain), which uses a horseradish peroxidase-catalyzed luminol oxidation (Durrant et al., 1990). Probe labelling, hybridization (in the presence of 0.5 M NaCl and 5% blocking agent), and washing were carried out according to the manufacturer's instructions. The detection reaction was performed by placing the nylon filter, dried of surface moisture, on a Whatman no. 3 filter paper soaked with the detection reagents. Both were quickly enclosed in a Photogene development folder (Life Technologies, Gaithersburg, Md.), the edges of which were then sealed with waterproof tape, and the filter was exposed to Fuji X-ray film in a photographic cassette. Exposure was from 15 min to 2 h to detect fragments of differing hybridization intensity.

Altogether, 32 seedlings, divided on to two filters, were analyzed. Each seedling had >10 strongly hybridizing fragments and 10 to 15 weaker ones (Fig. 1). No seedlings were completely identical. Nine fragments, existing in one or two of the parental cultivars but not in all three, were scored on one filter, and eight fragments were scored on the other filter due to somewhat lower probe activity. JO had fragments 2, 3, 6, 8, and 9; GD fragments 4 and 7; RD fragments 1 (could not be scored on one of the filters), 3, 5, and 7. By comparison of the fragment profile of a seedling with those of its putative par-

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Fig. 1. DNA of apple cultivars (GD = 'Golden Delicious', JO = 'Jonathan', RD = 'Red Delicious') and 16 apple seedlings (A-P) digested with *RsaI*, hybridized with the pAR72 rDNA probe, and detected by enhanced chemiluminescence. Size markers (lane 1), 4.4 kb, 2.3 kb, and 2.0 kb, obtained by digesting λ DNA with *HindIII*. Markers left of photo denote the nine fragments used for the paternity test.

ents, paternity could be deduced for the majority of the seedlings.

Two out of five seedlings previously assigned to a cross of JO $\times$ GD (Nyborg and Schaal, 1990) were reconfirmed. However, the remaining three lacked diagnostic fragments since all their fragments were also found in JO.

Of nine seedlings previously assigned to JO $\times$ RD, six were reconfirmed, whereas the remaining three had only JO fragments.

One seedling was assigned to GD $\times$ JO in concordance with the previous study.

Six of nine seedlings, previously assigned to GD $\times$ RD, were reconfirmed. Two of the remaining seedlings in this group had one fragment found in both JO and RD and, thus, paternity could not be deduced. The third had a fragment not found in any of the three cultivars and was therefore probably derived by pollination from an outside source.

In two seedlings derived from JO, only JO fragments were found in the previous study, suggesting that these seedlings originated through selfing. One of these seedlings again had only JO fragments. However, the other seedling had a fragment found in GD and RD but not in JO, showing that it was actually derived through cross-pollination.

Two JO seedlings previously exhibited fragments not found in any of the three cul-

tivars. Now one of them had only JO fragments, whereas the other also had a fragment found in GD and RD. However, no "alien" fragments were found this time.

Of the two JO seedlings not resolved after M13 hybridization, one now had only JO fragments, whereas the other also had an alien fragment. Finally, two seedlings not analyzed previously due to poor hybridization could now be assigned to GD $\times$ RD.

We conclude that this rDNA probe may become a valuable complement to the non-specific minisatellite probes previously used to obtain individual-specific fragment patterns (Nyborg 1990a, 1990b; Nyborg et al., 1990; Nyborg and Schaal, 1990) and that enhanced chemiluminescence is a feasible alternative to radioactive labelling for plant DNA characterization. DNA fingerprinting, which is already a valuable tool in plant breeding (Nyborg 1991), will thus become more easily accessible for many breeding and research facilities.

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