DNA Fingerprints in Plants Using Simple-sequence Repeat and Minisatellite Probes

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Abstract. Plant genomes contain polymorphic repetitive sequences that can be used as DNA markers. Minisatellites (16 to 64 bp per repeat) and simple-sequence repeats (2 to 6 bp per repeat) are the most polymorphic markers found in plant and animal genomes. In this study, the hybridizations between genomic DNA and variable number of tandem repeat probes were examined in *Arabidopsis thaliana* L. (Heyn), onion (*Allium cepa* L.), tomato (*Lycopersicon esculentum* L.), wheat (*Triticum aestivum* L.), avocado (*Persea americana* Mill.), litchi (*Chinensis* Sonn.), mango (*Mangifera indica* L.), and *Carica* species. Some of the probes detected polymorphic sequences in all the species, but others were useful only for one or two species. None of the probes gave clear band patterns in either onion or wheat. The in-gel hybridization method was similar to Southern blot hybridization using the simple-sequence repeat probes.

Eukaryotic genomes contain repetitive sequences, some of which can serve as genetic markers. Most polymorphic repetitive sequences are minisatellites and simple-sequence repeats (also called microsatellites). The polymorphism of this repetitive DNA results from the variable number of tandem repeats (VNTR) of a core sequence (Nakamura et al., 1987). Minisatellite regions contain a core sequence from 16 to 64 bp long and were the first used to detect multiple-band patterns that are highly polymorphic in humans and, thus, are used for DNA fingerprints (Jeffreys et al., 1985). These band patterns were generated by hybridization of probes to genomic DNA digested with a restriction enzyme (the restriction sites flank the repeat motif). The simple-sequence repeat regions consist of 2- to 6-bp sequences (Weising et al., 1991) and are a major source of genomic variation (Tautz et al., 1986).

After digestion and electrophoresis, two techniques can be used to prepare the DNA for fingerprints: Southern blotting (gel is blotted onto a membrane that is then hybridized with the probe) or in-gel hybridization (gel is dried and hybridization with simple-sequence repeats takes place directly in the gel). Both techniques have been used to produce polymorphic DNA fingerprint patterns in many species, including humans (Ali et al., 1986; Jeffreys et al., 1985), various other animals (Epplen, 1988; Hillel et al., 1989, 1992), and plants (Beyermann et al., 1992; Dallas, 1988; Lavi et al., 1991; Nybom and Hall, 1991; Sharon et al., 1992; Tzuri et al., 1991; Vosman et al., 1992; Weising et al., 1991). The in-gel hybridization approach (Schaefer et al., 1988) for simple-sequence repeat DNA fingerprints is more efficient and faster than Southern blot hybridization by saving blotting and prehybridization procedures. In the human genome, many thousands of simple-sequence repeat loci have been detected and used for mapping (National Institute for Health—Center d'Etude du Polymorphisme Humain Collaborative Mapping Group, 1992; Weissenbach et al., 1992).

Despite many species examined for DNA fingerprints, to our knowledge no information is available for *Arabidopsis*, litchi, mango, onion, and wheat, and limited information is available concerning avocado (Lavi et al., 1991), *Carica* (Sharon et al., 1992), and tomato (Vosman et al., 1992).

Our study evaluated DNA fingerprints obtained by either simple-sequence repeat or

minisatellite probes in *Arabidopsis*, onion, tomato, wheat, avocado, litchi, mango, and *Carica*. Using Southern blotting for simple-sequence repeat fingerprints was compared with in-gel hybridization.

Materials and Methods

The following plant material was used in this study: *Arabidopsis*—'Columbia', 'Landsberg', and 'Niderzants'; avocado—'Fuerte', 'Nabal', 'Reed', and 'Sharwill'; *Carica* species (Manshardt and Wenslaff, 1989)—*C. quercifolia*, *C. papaya*, *C. pentagona*, and *C. pubescens*; litchi—'Fay Zee Siu', and 'Vai Chee'; mango—'Yasmin', '1/5', and 'Palmer'; and tomato—'Hazer 1', 'Hazer 2', 'Hazer 3', 'Hazer 4', 'Hazer 5', and 'Hazer 6'.

DNA was isolated from leaves by modifying the cetyltrimethyl ammonium bromide (CTAB) method (Murray and Thompson, 1980). The extraction buffer contained 2% CTAB, 0.1 M Tris-HCl (pH 8), 0.02 M ethylenediamine tetraacetic acid (EDTA), 1.4 M NaCl, and 1% 2-mercaptoethanol. Chloroform extraction was performed three times. Two volumes of precipitation buffer were added to the final aqueous phase. The precipitation buffer contained 1% CTAB, 0.05 M Tris-HCl (pH 8), 0.01 M EDTA, and 1% 2-mercaptoethanol. Nucleic acids were dissolved in 1 M CsCl, precipitated with ethanol, and redissolved in 10 mM Tris-HCl (pH 8) and 1 mM EDTA.

Ten-microgram DNA samples were digested with one of the following restriction enzymes: *Dra* I, *Eco* R I, or *Hinf* I (New England Biolabs, Beverly, Mass.) according to the supplier's recommendations. Samples were loaded onto 30- or 20-cm-long (in-gel hybridization or Southern blot hybridization, respectively), 0.8% agarose gels in 0.045 M Tris-borate and 0.001 M EDTA (pH 8) (TBE buffer) and electrophoresed at 1.5 V·cm⁻¹.

Gels for in-gel hybridization were dried on a vacuum gel dryer, denatured in 0.5 M NaOH and 0.15 M NaCl for 30 min, neutralized in 0.5 M Tris-HCl (pH 8) and 0.15 M NaCl for 30 min, and equilibrated in 6× SSC (20× SSC : 3 M NaCl, 0.3 M sodium citrate) before hybridization. Oligonucleotides were 5-prime end-labeled by a T4-polynucleotide kinase reaction (Fritsch et al., 1989) using γ^{32} P-labeled dATP (Amersham International, Amersham, U.K.). Labeled probes were purified through a DE52 column (Whatman, Maidstone, England) according to Schaefer et al. (1988).

Hybridization of dried gels with seven simple-sequence repeat probes was performed for 1 to 2 h in hybridization buffer [5× SSPE (20× SSPE = 3 M NaCl, 0.2 M NaH₂PO₄·H₂O, 0.02 M Na₂EDTA·H₂O), 5× Denhart's solution (100× Denhart's = 2% polyvinyl-pyrrolidone 40, 2% bovine serum albumin, and 2% ficoll 400), 0.1% SDS, 10 mg sonicated, denatured *E. coli* DNA/ml] at the respective hybridization temperature (°C): (GATA)₄ and (AAC)₅ = 35; (GGAT)₄, (CA)₈, (GAA)₆, and (GACA)₄ = 43; and (GTG)₅ = 45. After hybridization, gels were washed in 6× SSC for 20 min at room

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temperature, followed by a wash at the hybridization temperature for 1 to 2 min, followed by another 20-min wash in 6× SSC at room temperature.

Gels for Southern blot hybridization were blotted onto Hybond-N+ membranes according to the supplier's recommendations. Membrane-bound DNA was hybridized to minisatellite probes 33.6 and 33.15 (Jeffreys et al., 1985), which were labeled with ³²P-dCTP by random priming (Feinberg and Vogelstein, 1983), or to simple-sequence repeat probes [(GGAT)₄, (GACA)₄, and (GATA)₄]. Prehybridization, hybridization, and washes were performed according to Hillel et al. (1989).

The efficiency of each probe was tested by two criteria: 1) the level of polymorphism based on the number of bands specific to the tested genetic groups and 2) the quality of DNA fingerprint patterns, based on the ratio between the specific signals and the background.

Results and Discussion

Figure 1 presents the DNA fingerprints of *Arabidopsis*, avocado, and litchi using Southern blot hybridization with probe 33.6 and in-gel hybridization with simple-sequence repeat probes. The two minisatellite probes (33.6 and 33.15) hybridized to *Arabidopsis* genome, but only 33.6 detected high-quality patterns of polymorphic bands. The informative bands from probe 33.6 ranged from 3 to 22 kb (Fig. 1A). The simple-sequence repeat probe (GAA)₆ detected informative patterns between 2 to 3.5 kb and a few bands between 10 to 22 kb in length (Fig. 1A). Each of the probes detected relatively few bands, but the polymorphic patterns allow identification of each cultivar. The other simple-sequence repeat probes [(GGAT)₄, (AAC)₅, (GTG)₅, (GATA)₄, and (CA)₈] did not provide informative patterns (Table 1). The fact that probes 33.6 and (GAA)₆ detected polymorphic DNA fingerprint patterns indicates that the small genome of *Arabidopsis* does contain polymorphic repeated sequences. However, it is noteworthy that most of the simple-sequence repeat probes did not hybridize at all to the *Arabidopsis* genome. The recommended enzyme for *Arabidopsis* is *Hind* III, and the probes are 33.6 and (GAA)₆.

The avocado genome contains most of the simple-sequence repeat sequences that we used. Probe (GATA)₄ detected highly polymorphic patterns (Fig. 1B), but (GTG)₅ and (GACA)₄ (Table 1) detected medium-quality polymorphic patterns. Probes (GGAT)₄, (AAC)₅, (CA)₈, and (GAA)₆ provided poor and nonresolvable patterns (Table 1). Regarding the minisatellite probes, 33.6 was useful for avocado fingerprinting, and probe 33.15 generated fingerprints of low quality and low levels of polymorphism. Previously, avocado DNA fingerprints were obtained by minisatellite probes using Southern blot hybridizations (Lavi et al., 1991). In this study, avocado DNA fingerprints were obtained with additional simple-sequence repeats by in-gel hybridization as well. The best DNA fingerprints for avocado

were obtained using probe (GATA)₄, hybridized to genomic DNA digested with *Hinf* I.

In litchi, the simple-sequence repeats (GGAT)₄ and (GATA)₄ (Fig. 1C) and the minisatellite probes (33.15 and 33.6) detected medium levels of polymorphism and bands of reasonable quality (Table 1). The patterns

obtained using probes (AAC)₅ and (GTG)₅ were less resolvable (Table 1). The best probes for litchi DNA fingerprinting were (GGAT)₄ and (GATA)₄ and the minisatellites 33.15 and 33.6. The genomic DNA was digested with either *Hinf* I or *Dra* I.

Figure 2 presents the DNA fingerprints of

Fig. 1. DNA fingerprints of (A) *Arabidopsis*, (B) avocado, and (C) litchi using Southern blot hybridization with probe 33.6 and in-gel hybridization with simple-sequence repeat probes. Lanes: (A) (enzyme-*Hind* III) 1) 'Niderzants', 2) 'Landsberg', and 3) 'Columbia'; (B) (enzyme-*Hinf* I) 1) 'Nabal', 2) 'Reed', 3) 'Sharwill', and 4) 'Fuerte'; (C) (enzyme-*Hind* III) 1) 'Fay Zee Siu' and 2) 'Vai Chee'. Size markers are given in kilobases.

Table 1. Oligonucleotide fingerprinting in eight plant species.

Variable	Simple-sequence repeats ^z	<i>Arabidopsis</i>	Avocado	<i>Carica</i>	Litchi	Mango	Onion	Tomato	Wheat
Probe									
(GGAT) ₄	P	—	++	+++	++	++	—	+++	+
	Q	—	+	++	++	++	+	+++	+
(AAC) ₅	P	—	+	—	+	+	+	++	+
	Q	—	+	—	+	++	+	+++	+
(GTG) ₅	P	—	++	+	++	+	+	++	—
	Q	—	++	++	+	++	+	++	—
(CA) ₈	P	+	++	+	—	—	—	++	—
	Q	+	+	+	—	—	+	+	—
(GATA) ₄	P	—	+++	+	++	+	—	+++	—
	Q	—	++	++	++	+	—	+++	—
(GAA) ₆	P	++	—	+	—	—	—	++	+
	Q	+++	—	+	—	—	+	++	+
(GACA) ₄	P	—	++	+++	—	++	—	++	—
	Q	—	++	++	—	++	—	++	—
Minisatellites									
33.6	P	++	++	+	++	+++	+	++	—
	Q	+++	++	+++	++	++	+	++	—
33.15	P	+	—	++	++	—	+	++	—
	Q	++	+	+++	++	—	+	++	—

^zP = polymorphism information based on band sharing (BS) level: —, monomorphic (BS = 100%); +, BS between 85% and 99%; ++, BS between 60% and 84%; +++, BS between 40% and 59%. Q = quality assessed on the ratio of signal to background. Quality ranging from low (—) to high (+++).

Carica species and mango cultivars using Southern blot hybridization. In several *Carica* species and especially in *Carica papaya*, DNA fingerprints were obtained with probes (GGAT)₄ and (GACA)₄ using restriction enzyme *Dra* I (Fig. 2A, Table 1). Both probes gave a medium number of highly polymorphic bands. Probe (AAC)₅ did not hybridize to the *Carica* genome, and probes (GTG)₅, (CA)₈, (GATA)₄, and (GAA)₆ provided noninformative DNA fingerprint patterns (Table 1). The two minisatellite probes tested (33.6 and 33.15) were useful for *C. papaya* DNA fingerprinting. In a previous study, DNA fingerprints of *C. papaya* and other *Carica* species were obtained with minisatellites and simple-sequence repeat probes (CA)₁₂ and (GTG)₅ (Sharon et al., 1992). The polymorphism and band quality detected by these probes were low, and thus, in our study, additional simple-sequence repeat probes were used. As a result, the two probes (GGAT)₄ and (GACA)₄ were useful.

The simple-sequence repeat probes (GACA)₄ (Fig. 2B) and (GGAT)₄ detected a medium level of polymorphism in mango, with band sizes ranging from 2 to 22 kb (Table 1). The simple-sequence repeats (AAC)₅, (GTG)₅, and (GATA)₄ detected low levels of polymorphism, but probes (CA)₈ and (GAA)₆ did not hybridize with the mango genome. The minisatellite probe 33.6 detected highly polymorphic bands of medium quality (Fig. 2B), and probe 33.15 detected only one monomorphic band. The most informative probe for mango fingerprinting was minisatellite 33.6 following digestion with *Eco*R I; the simple-sequence repeat probes (GGAT)₄ and (GACA)₄ were less informative.

All VNTR probes used in this study were hybridized to the tomato genome. Tomato cultivars were digested with *Dra* I or *Hinf* I and hybridized to simple-sequence repeat probes by in-gel hybridization or by Southern blotting. The minisatellite probes were hybridized after Southern blotting. The minisatellite probes 33.6 (Fig. 3A) and 33.15 detected DNA bands characterized by a medium level of polymorphism and medium quality. The simple-sequence repeat probes (GATA)₄ (Fig. 3B) and (GGAT)₄ (Fig. 3C) detected high-quality and highly polymorphic DNA fingerprint patterns, with bands ranging from 1.5 to 22 kb in length. Some of the bands detected by both probes and with the same molecular weight showed the same type of variation. This finding suggests that these fragments have (GGAT)_n and (GATA)_n sequences.

Two cultivars were used to compare the Southern blot and in-gel hybridization methods (Fig. 3B). There was no significant difference between the quality of the DNA fingerprint patterns between the two methods. Probes (AAC)₅ (Fig. 3C) and (GAA)₆ (Table 1) detected medium-quality and medium polymorphic patterns, with bands ranging from 1.5 to 22 kb in length. Probe (GTG)₅ detected high-quality patterns of a medium level of polymorphism ranging from 2.5 to 22 kb (Fig. 3C). Probe (CA)₈ detected medium level of polymorphism but with rather low quality (Table

1). An average total of 155 bands per individual were detected in tomato by the nine VNTR probes, of which an average of 93 were polymorphic. Based on these results, the rec-

ommendation for tomato fingerprinting is the combination of the restriction enzyme *Dra* I and the probes (GGAT)₄ or (GATA)₄. Vosman et al. (1992) suggested that (GATA)₄ is the

Fig. 2. Simple-sequence repeat and minisatellite DNA fingerprints of (A) *Carica* species and (B) mango as produced by Southern blot hybridization. Lanes: (A) (enzyme-*Dra*I) 1) *C. quercifolia*, 2) *C. papaya*, 3) *C. pubescens*, and 4) *C. pentagona*; (B) (enzyme-*Eco*R I) 1) '1/5', 2) 'Yasmin', and 3) 'Palmer'. Size markers are given in kilobases.

Fig. 3. Tomato DNA fingerprints: (A) minisatellite probes (by Southern blot hybridization), (B) simple-sequence repeat probes—a comparison between (left) Southern blot hybridization and (right) in-gel hybridization, and (C) simple-sequence repeat probes (by in-gel hybridization). Lanes: 1) 'Hazera' 1; 2) 'Hazera' 2; 3) 'Hazera' 3; 4) 'Hazera' 4; 5) 'Hazera' 5; and 6) 'Hazera' 6. DNA was digested with *Dra* I. Size markers are given in kilobases.

best probe to produce DNA fingerprints in tomato by Southern blot hybridization. Our study suggests that (GATA)₄ and (GGAT)₄ can be applied using in-gel hybridization as well.

None of the probes used in our study gave satisfactory fingerprints for onion or wheat. For both species, some probes generated low levels of polymorphism [33.15, 33.6, (AAC)₅, and (GTG)₅ in onion; (GGAT)₄, (AAC)₅, and (GAA)₆ in wheat]. The other probes did not hybridize to either the onion or the wheat genomes (Table 1). The hybridization of some simple-sequence repeat probes resulted in smears, indicating many repetitive loci in these genomes. Therefore, searching for microsatellite (Akkaya et al., 1992) markers in these species using these sequences would be reasonable. We assume that the cause of the smear patterns in wheat resulted from its polyploidy and high content of repetitive sequences. We are not aware of DNA studies of onion.

In our study, we used Southern blot hybridization (for tomato, *Carica*, and mango) and in-gel hybridization (for tomato, *Arabidopsis*, avocado, and litchi) for minisatellite hybridization and simple-sequence repeat probes. Both methods produced clear DNA fingerprint patterns. When applying the simple-sequence repeat probes, we recommend using in-gel hybridization, which is a simple and quick procedure. Among the seven simple-sequence repeat probes that we examined in this study, (GATA)₄ and (GGAT)₄ are the most common and useful in plants. In previous studies, mainly (GATA)₄ and (CAC)₅ were useful for identification (Beyermann et al., 1992; Weising et al., 1991). The abundance of the simple-sequence repeats in the plant genome and the ease with which they were applied makes this sequence the best choice for DNA fingerprints. In previous studies, the minisatellite probe 33.6 gave better results in plants than 33.15 (Dallas, 1988; Lavi et al.,

1991); our data confirm this finding. Probe 33.15 was useful only for fingerprinting *Carica* species. The DNA fingerprints described in our study can be used for identification of cultivars and estimation of genetic distances between them.

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