Randomly Amplified Polymorphic DNAs in the Cultivated Strawberry, *Fragaria ×ananassa*

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Abstract. Eight strawberry cultivars or advanced selections from the Univ. of California, Davis, breeding program were screened for polymorphisms using the polymerase chain reaction (PCR) and 43 random 10-base DNA primers. Over 60% of the primers screened resulted in replicable polymorphic banding patterns (amplification profiles), and a subset of ten primers that exhibited high levels of amplification profile polymorphism was used to identify each of the eight genotypes uniquely. There was also a significant product–moment correlation ($r = 0.64$, $P < 0.01$) between number of shared amplification profile phenotypes and pairwise coefficient of coancestry. This technology shows high promise as a means of verifying the identity of cultivars and developing a genetic map of the octoploid cultivated strawberry.

Molecular markers are playing an increasingly important role in genetic studies of horticultural crops. Isozymes, restriction fragment length polymorphisms (RFLPs), and randomly amplified polymorphic DNAs (RAPDs) have a wide array of uses including genetic mapping, tagging genes of interest, pedigree verification, cultivar identification, and measuring levels of genetic diversity (O'Brien, 1990). Isozymes and RFLPs have been the most widely employed genetic markers, but interest in RAPDs is rapidly growing as they are often highly polymorphic and their identification requires no radioactivity (Grattapaglia et al., 1992).

Randomly amplified polymorphic DNA profiles represent base differences or chromosomal rearrangements that are uncovered after polymerase chain reaction (PCR) amplification using random oligonucleotide primers (Williams et al., 1990). Randomly amplified polymorphic DNA markers are generally expressed as dominant traits, because amplification of a product proceeds with the presence of a pair of sequences homologous to that of the primer on either one or both homologous chromosomes. Randomly amplified polymorphic DNAs have been used to distinguish genotypes of asparagus, beans, peas, peppers, watermelons (Weeden et al., 1992), broccoli, cauliflower (Hu and Quiros, 1991), lettuce (Paran et al., 1992), tomato (Klein-Lankhorst et al., 1991), and wheat (Vierling and Nguyen, 1992).

Isozymes have been used to characterize strawberry cultivars; however, only 60% of the genotypes from the Univ. of California breeding program could be distinguished using three enzyme systems (Arulsekar et al., 1981; Bringhurst et al., 1981). This lack of resolution limits the utility of allozymes in cultivar identification and breeding. In contrast, Davis (1993) has described high levels of RAPD diversity in the diploid *F. vesca* and Rowland (personal comment) has observed high levels of variation among octoploid *F. ×ananassa* cultivars. We undertook this survey to determine if similar levels of RAPD diversity might also be found within a breeding population of *F. ×ananassa*.

Materials and Methods

Eight cultivars or selections were chosen for study from the Univ. of California Strawberry Improvement Program (Table 1). These genotypes represent a highly related subset of the germplasm available in this program; many are siblings or parent–offspring pairs. A related sample of genotypes was chosen to challenge the resolution power of the molecular markers. Coefficients of coancestry estimated using SAS Procedure INBREED (SAS Institute, 1988) ranged from 0.113 to 0.386 for pairwise comparisons of these eight genotypes (Table 1), and several of the genotypes in this sample share a common parent or are related as offspring–parent.

Total cellular DNA was isolated from fully expanded leaves of greenhouse-grown plants using a modification of a CTAB procedure (Haghighi and Hancock, 1992). DNA was amplified in 25 μ l volumes using 10-base primers (from Operon Technologies, Alameda, Calif.). Reaction conditions were 1 ng- μ l⁻¹ strawberry DNA, buffer (50 mM KCL, 10 mM Tris-HCl pH 8.3), 2.0 mM MgCl₂, 200 μ M dATP, dCTP, dGTP, dTTP (Perkin Elmer), 0.2 μ M primer, and 0.026 units/ μ l Taq DNA polymerase (Perkin Elmer). The thermo-cycle profile was preceded with an initial denaturation soak for 5 min at 94C. DNA was amplified for 47 cycles in a Perkin Elmer thermal cycler programmed for 1 min at 94C, 1 min at 40C, and 2 min at 72C, followed by a 5-min soak at 72C. The DNA fragments were run in 1.0% agarose gels and visualized by ethidium bromide staining.

Results and Discussion

The PCR conditions were optimized using a variety of reaction conditions. From an initial group of 43 primers, 28 (65%) were useful in distinguishing among the eight genotypes; 11 of the remainder lacked polymorphism in this sample and four failed to produce amplification profiles. Of the 28 primers that produced polymorphic amplification product profiles, the ten with the greatest level of polymorphism were used to further evaluate the power of this technology for discriminating among genotypes and obtaining quantitative relationships.

Each primer yielded a wide array of strong and weak bands (Fig. 1, Table 2); only reproducible fragments with intense bands were scored in our comparisons. The different amplification profiles

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Table 1. Coefficients of coancestry (above diagonal) and the number of shared RAPD amplification profiles (below the diagonal) for eight strawberry clones and ten marker systems.

	75.121-101	81.16-604	83.25-2	85.22-1	Capitola	Chandler	Douglas	Seascape
75.121-101	---	0.174	0.160	0.372	0.372	0.113	0.180	0.182
81.16-604	0	---	0.279	0.386	0.278	0.254	0.387	0.330
83.25-2	1	1	---	0.219	0.273	0.188	0.288	0.251
85.22-1	3	2	3	---	0.325	0.184	0.283	0.256
Capitola	6	1	2	2	---	0.184	0.283	0.256
Chandler	0	2	2	1	0	---	0.328	0.236
Douglas	0	4	3	2	0	5	---	0.382
Seascape	1	3	4	3	1	5	5	---

Fig. 1. DNA profiles generated by primers C (top), E (middle) and G (bottom). The lanes and their contents are: 1 (Size markers from HindIII digests of lambda DNA), 2-3 (Chandler), 4-5 (Capitola), 6-7 (75.121-101), 8-9 (85.22-1), 10-11 (Douglas), 12-13 (Seascape), 14-15 (81.16-604), 16-17 (83.25-2). The fragments used in the statistical analysis are marked by arrows.

were given an alpha-numeric designation, where a letter was used to represent each primer and a number to denote the different DNA patterns obtained with that primer (Table 3).

The cultivars and selections were readily distinguished by their DNA amplification profiles (Tables 2 and 3). For this subset of 10 primers, 28 of the 54 fragments identified were polymorphic. From two to six unique amplification profiles were found with each primer. All of the primers distinguished at least 50% of the genotypes in pairwise comparisons and some separated all but a few cultivars (Table 3). Amplification profiles from primer A (5'-CCGCATCTAC) differed in 26 of 28 pairwise genotypic comparisons, and profiles from primers F (5'-GGACCCAACC) and H (5'-AGCGCCGTCA) distinguished 25 of 28.

The number of shared amplification profiles ranged from 0 to 6 of 10 for all pairwise comparisons (Table 1). The product-moment correlation between the number of shared profiles and pairwise coefficient of coancestry was $r = 0.64$ ($P < 0.01$). Although the number of genotypes evaluated was rather small, this result suggests that these highly diverse markers may be a powerful tool for detecting patterns of relatedness in strawberry populations. Genetic similarities generated by selection from a common ancestral base, rather than coancestry, might be detected by appropriate comparisons within and among breeding populations adapted to differing production environments.

Many of the accessions here are close relatives, and are expected to share a large fraction of their genomes. However, the power of resolution even for these closely related items is exceptional. For example, Capitola and 85.22-1 share selection 75.121-101 as a common parent, but have different amplification profiles for 8 of the 10 primers listed in Table 3. In addition, the two derivative genotypes differ from their common parent at 4 of 10

Table 2. Primers used and number of different DNA fragments and profiles observed among eight strawberry clones. There were 28 possible pairwise comparisons between each of the clones.

Primer	Sequence	No. fragments observed		No. DNA profiles	No. distinct pairwise comparisons
		Total	Polymorphic		
A	CCG CAT CTA C	7	5	6	26
B	GTC CCG ACG A	3	1	2	15
C	TGG ACC GGT G	7	6	4	22
D	GTT GCC AGC C	4	1	3	19
E	ACC GCG AAG G	5	2	4	21
F	GGA CCC AAC C	9	5	5	25
G	GTC GCC GTC A	6	2	4	22
H	AGC GCC ATT G	4	2	5	25
I	GAG AGC CCA C	4	2	3	20
J	AGA TGC AGC C	5	2	3	17

Table 3. Amplification profiles observed among the eight strawberry genotypes. A letter was used to represent each primer and a number to denote the different amplification profiles obtained with that primer.

Genotype		Amplification profile								
75.121-101	A3	B2	C3	D2	E2	F2	G3	H3	I2	J2
81.16-604	A4	B1	C1	D1	E1	F4	G1	H2	I3	J1
83.25-2	A2	B2	C4	D3	E4	F5	G4	H5	I1	J1
85.22-1	A4	B2	C4	D2	E3	F1	G4	H3	I3	J3
Capitola	A2	B2	C2	D2	E2	F2	G2	H2	I2	J2
Chandler	A1	B1	C1	D1	E1	F1	G1	H1	I1	J1
Douglas	A5	B1	C1	D1	E3	F3	G4	H4	I1	J1
Seascape	A6	B2	C4	D1	E3	F3	G1	H1	I1	J1

and 7 of 10 of the primers, respectively. The coefficient of coancestry between offspring and parent is identical to that between pairs of full-sibs, and on average the likelihood of discriminating among such relatives is the same. Thus, the variation detected in our sample was sufficient to discern among the closest relatives likely to be encountered in most strawberry breeding programs.

Based on these studies, it is clear that amplification profiles from only a few different primers are necessary to distinguish strawberry cultivars and that numerous markers should be available for genetic studies. Randomly amplified polymorphic DNAs show particular promise as a tool in strawberry genetic studies, because high levels of diversity were observed in a relatively narrow breeding population. Further, the power of this technique to differentiate among close relatives suggests that its use should overcome the limitations cited using isoenzyme polymorphisms (Arulsekhar et al, 1981; Bringham et al, 1981).

We have only just begun to evaluate the inheritance patterns in each amplification profile, but preliminary indications are that the majority of the individual bands within an amplification profile segregate as if controlled by a single diploid locus with one or two alleles, even though the commercial strawberry is an octoploid. Resolution of the inheritance of the markers will facilitate statistical models that can infer parent genotypes; with a large number of segregating markers even more distant relationships could be resolved. These models can then be used to select germplasm in breeding programs.

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