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Short communication

Modified AFLP technique for fingerprinting of *Anemone coronaria*

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Abstract

We successfully generated clear amplified fragment length polymorphism (AFLP) profiles of *Anemone coronaria* using only a single restriction enzyme (*Eco* R I). Four primer combinations generated 571 bands in 37 anemone accessions with an average 142.75 bands per primer combination. Polymorphism among the 37 samples was very high (98.15%). This high polymorphism showed that the one-enzyme AFLP method is effective for the assessment of genetic diversity as well as cultivar differentiation of anemone. We observed 26 bands which differentiated between fingerprints derived from leaf and corm DNA of eight plant individuals. However, the low percentage of such bands showed that anemone corms can also be used to evaluate anemone germplasm using AFLP markers.

Keywords: AFLP; *Anemone coronaria*; fingerprinting; modification; polymorphic bands

Experimental and discussion

Methods

DNA was isolated from *Anemone coronaria* young leaves or corms by the CTAB method (Murray and Thompson, 1980). Leaves were collected from individuals of wild populations in Israel (Elyakim, Dorot, Eilon, Arbel and Adomim; Horovitz, 1973), and the commercial cultivar 'Mona-Lisa'. To compare corm and leaf DNA amplified fragment length polymorphism (AFLP) profiles, eight large corms from the Elyakim, Eilon and Adomim populations were selected randomly, and were cut into two pieces. One piece was soaked overnight and used for DNA isolation, while the other was planted to produce leaf for DNA isolation. For AFLP the enzyme combinations *Eco* R I/*Taq* I (David *et al*., 2001) and *Eco* R I/*Mse* I, and also *Eco* R I on its own (Vos *et al*., 1995; Kashkush *et al*., 2001) were used. For the latter, only *Eco* R I adapters, *Eco* R I pre-selective primer and *Eco* R I selective primers are necessary. AFLP primers were named by the last three selective nucleotides.

AFLP profiles

AFLP profiles from standard two-enzyme, digests consisted of 105–129 faint and poorly scorable bands in the size range 50–300 bp. These profiles were not

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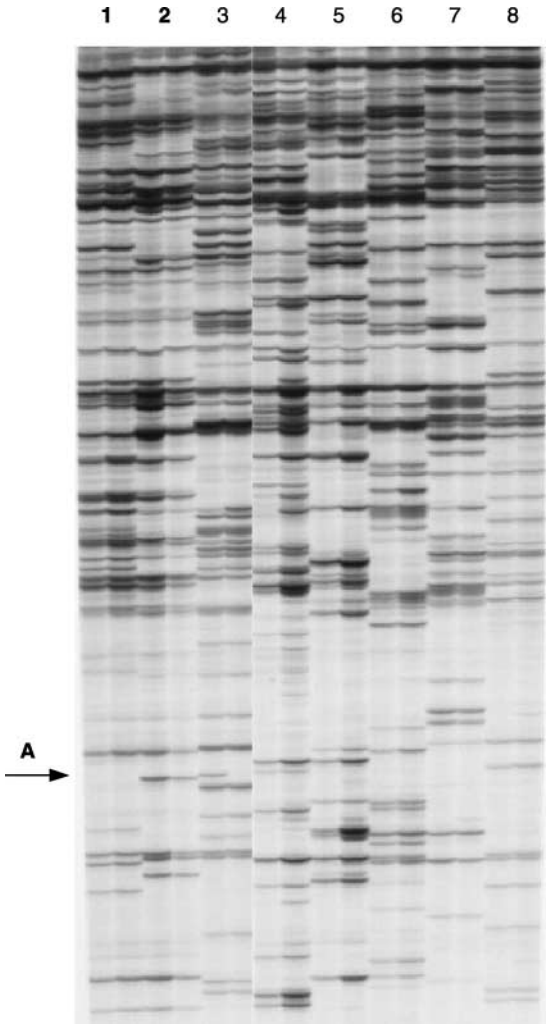


Fig. 1. AFLP patterns (E-AGC/E-ACA) of eight *Anemone coronaria* individuals. Pairs of contiguous profiles are derived from the same individual: the first from corm DNA and the second from leaf DNA. Arrow A indicates a band specific to the corm DNA profile of sample 3.

improved by using more selective primers (e.g. *Eco*R I + 3/*Mse* I + 4, *Eco*R I + 4/*Mse* I + 4, *Eco*R I + 4/*Taq* I + 3). Lockhart and McLenacham (1997) used agarose electrophoresis to display single-enzyme AFLP profiles, but we separated *Eco*R I anemone products on 4% denaturing PAGE gels. This generated clear and reproducible profiles, with longer fragments than produced by the conventional two-enzyme digests. We only scored bands above 200 bp in length.

AFLP fingerprints were generated from corm DNA using three primer combinations (E-AGC/E-ACT, E-AGC/E-ACA, E-ACC/E-AGG). Tissue-specific AFLPs were observed in all eight samples (Fig. 1). In all, we identified 26 such differential bands (DBs), of which 22 were leaf-specific. The origin of DBs is unknown, but since these are relatively rare (2.55% of all AFLP bands scored), we suggest that comparisons using either leaf- or corm-derived DNA are possible for anemone germplasm evaluation and cultivar identification.

Four primer combinations were used to genotype 37 diverse individuals. Each primer combination generated clear fingerprints (Fig. 2). The four primer combinations used generated 571 scorable bands of over 200 bp (Table 1) with a mean band number per lane of 44.25. Over 98% of the scorable bands were polymorphic across the germplasm sample, showing that single-enzyme AFLP is informative for genotyping and identifying anemone germplasm.

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Table 1. Characteristics of *Anemone coronaria* AFLP fingerprinting

	Primer combinations[a]					
	1	2	3	4	Total	Average
Total bands in one gel	153	121	168	129	571	142.75
Polymorphic bands	148	121	165	126	560	140
Polymorphism (%)	96.73	100	98.21	97.67		98.15
Bands in one lane	58.2	33	40.2	45.6		44.25

[a] Primer combinations: 1, E-ACT/E-AAC; 2, E-AGC/E-ACT; 3, E-ACC/E-AGG; 4, E-AGC/E-ACA.

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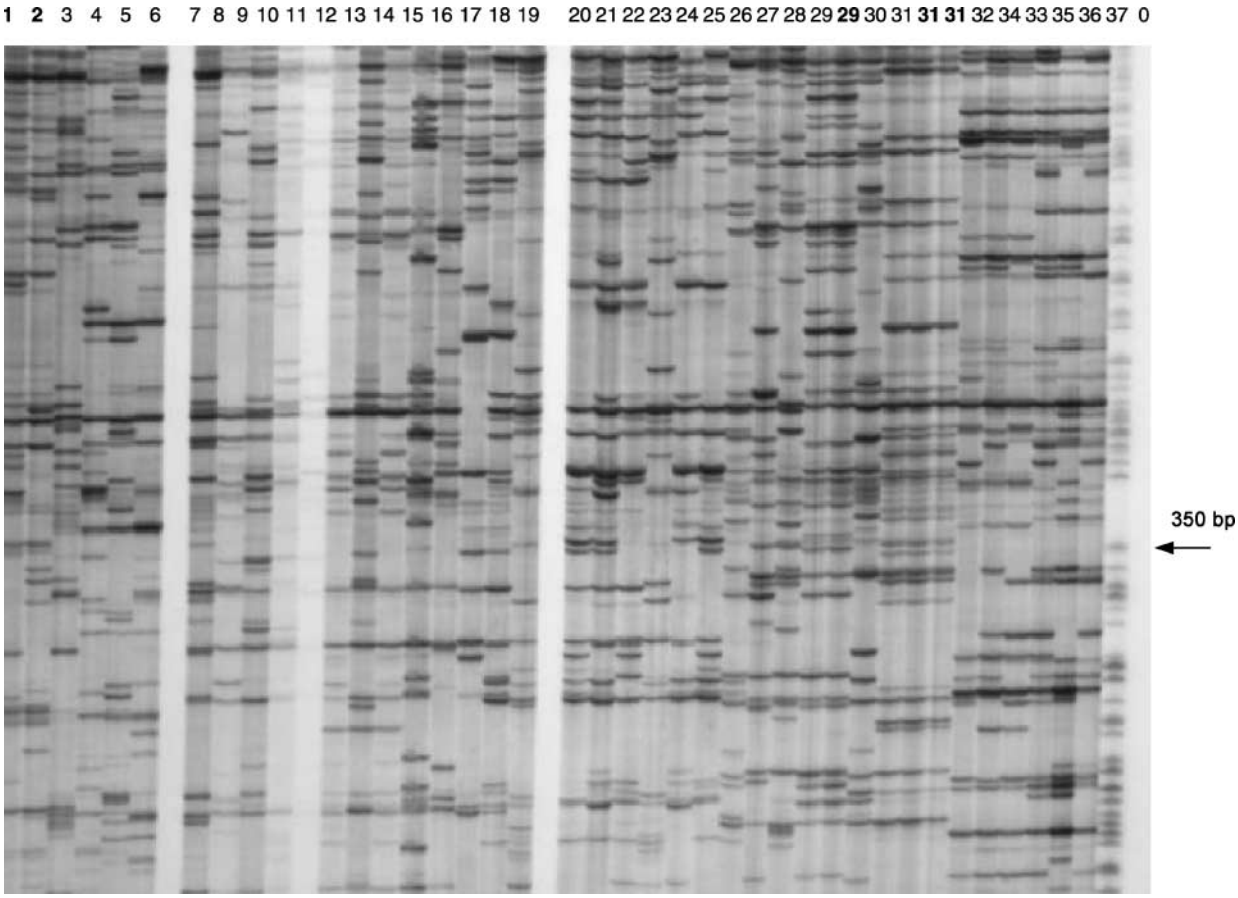


Fig. 2. E-AGC/E-ACT AFLP profiles of 37 *Anemone coronaria* plants. Lanes 1–3, Elyakim; 4–6, Dorot; 7–16, Eilon; 17–25, Arbel; 26–31 (sample 29 and 31 are, respectively, repeated twice and three times from the step of DNA digestion and ligation), Adomim; 32–34, Mona-Lisa red; 35–37, Mona-Lisa blue. Fragment size marker is the G of T7 sequence version 2.0 DNA sequencing kit (Amersham Pharmacia Biotech., Buckinghamshire, UK).

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