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STRAWBERRY-CULTIVAR IDENTIFICATION USING RANDOMLY AMPLIFIED POLYMORPHIC DNA (RAPD) MARKERS

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Short Communication

Strawberry-cultivar Identification using Randomly Amplified Polymorphic DNA (RAPD) Markers

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With 1 figure and 1 table

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Abstract

Accurate and rapid cultivar identification is important for breeders'-rights protection, especially for vegetatively propagated plants. The objective of this study was to test the feasibility of developing cultivar-specific RAPD markers in commercial strawberries (*Fragaria ananassa* Dutch). Efforts were focused on distinguishing between two newly developed Volcani cultivars, 'Ofra' and 'Dorit', and six other cultivars, 'Douglas', 'Chandler', 'Oso Grande', 'Dover', 'Nurit' and 'Parker'. Reproducible RAPD fingerprints were generated, each containing at least one polymorphic DNA product. A combination of 10 polymorphic DNA products exhibited cultivar-specific patterns enabling the distinction between closely related varieties, such as 'Ofra' (which is the progeny of 'Dorit' and 'Parker') and 'Dorit' (which is the progeny of 'Nurit' and 'Dover'). This study shows that RAPD markers can help in the protection of breeders' rights to strawberry cultivars.

Key words: *Fragaria ananassa* — DNA fingerprinting — breeders'-rights protection

Commercial strawberry cultivars are generated, essentially, by cross hybridization between lines which express defined and promising traits. This is followed by a process of testing and selection of lines combining the desired traits. As strawberry plants are vegetatively propagated, each cultivar is essentially a clone. Confirmation of breeders' rights requires reliable and, preferably, rapid procedures for cultivar identification. Strawberry-cultivar identification is, at present, based mainly on morphoiogical traits. The use of isozymes for cultivar identification has also been considered (BRINGHURST et al. 1981). However, compared with morphological and biochemical characteristics, the DNA genome provides a significantly more powerful source of genetic polymorphism (BECKMANN and SOLLER 1986). The introduction of restriction-fragment-length polymorphism (RFLP) methodology to plant breeding has significantly improved the opportunities for revealing genetical molecular markers, and these opportunities are now being successfully exploited in various crop species (TANKSLEY et al. 1988). A novel procedure for the detection of DNA polymorphism, randomly amplified polymorphic DNA (RAPD), has been developed more recently (WILLIAMS et al. 1990). RAPD markers are distributed throughout the genome and the procedure for their detection is rapid, thus allowing the use of detection kits in the field or in packing houses. This procedure does not require the use of radioactive material.

The objective of this study was to examine the possibility of producing cultivar-specific RAPD markers for commercial strawberries in order to protect breeders' rights concerning the cultivars 'Ofra' and 'Dorit', and test the resolution power of the method with respect to closely related cultivars and lines on an octoploid crop species.

Two leading Israeli early season cultivars, 'Dorit' and 'Ofra', and six other commercial cultivars, 'Douglas', 'Chandler', 'Oso Grande' 'Dover', 'Nurit' and 'Parker' were utilized in this study. 'Douglas', 'Chandler', 'Oso Grande' and 'Parker' belong to the University of California breeding programme and are genetically related. 'Ofra' is the progeny of 'Dorit' and 'Parker'. Thus, 'Ofra' and 'Dorit' are genetically close. 'Dorit' is the progeny of 'Nurit' and 'Dover' ('Dover' is a cultivar from Florida and 'Nurit' is a Volcani cultivar). The plants were grown at the Volcani Center. From each plant, 2–3 young leaves (about 5 g) were collected individually, and kept at −80°C until DNA extraction. A total of 12 plants of each cultivar were sampled and tested on three independent occasions.

DNA was prepared as described by DOYLE and DOYLE (1990) from leaves collected separately from 12 plants of each cultivar or from pools of three plants.

PCRs were performed using an MJ Research Inc., Programmable Thermal Controller (USA). The PCRs shown in Fig. 1a were essentially performed as described by KANGFU and PAULS (1992). The thermal controller was programmed for 30 s, at 94°C followed by 35 cycles of 5 s at 94°C, 25 s at 35°C and 30 s at 70°C. The extension step in the last cycle was set at 35 s. The PCRs shown in Figure 1 (b–d) were performed according to the conditions described by WILLIAM et al. (1990), except that 1.5 units of Taq DNA polymerase (Boehringer-Mannheim GmbH, Germany) were used in each reaction. Reaction products were resolved by electrophoresis in 1.8 % agarose gels and visualized using ethidium bromide staining and UV light illumination. The 'OF' and 'OG' primer series (20 primers in each) were purchased from Operon Technologies Inc., USA. The primer 1037 was as described by CAETANO-ANOLLES et al. (1991).

Forty-one primers were used for the RAPD screening of 'Ofra', 'Dorit', 'Douglas', 'Chandler', 'Oso Grande', 'Dover', 'Nurit' and 'Parker'. Each primer generated a specific DNA fingerprint containing 4–12 amplified DNA products. Four of these primers produced fingerprints which contained a total of 10 polymorphic DNA products. Primer OG-2 (5′-GGCACTGAGG-3′; Fig. 1a) produced five DNA products (1.1-kb, 0.8-kb, 0.75-kb 0.6-kb and 0.4-kb) that discriminate between each of the eight cultivars tested except between 'Ofra' and 'Dorit'. The next three polymorphic primers (Fig. 1b–d) have been focused on discrimination between 'Ofra' and 'Dorit', as well as 'Douglas' and 'Chandler'. OF-7 primer (5′-CCGATATCCC-3′ Fig. 1c) generated three polymorphic DNA segments (1.7-kb, 0.85-kb and 0.8-kb long) that differentiate all four cultivars. The 1.7-kb DNA fragment, produced by the OG-5 primer (5′CTGAGACGGA-3′, Fig. 1b), together with the fingerprints produced by the OF-7 primer, discriminate 'Dorit' from 'Ofra'. Primer 1037 (5′-AATCGGGCTG-3′, Fig. 1d) generated a 1.8-kb DNA that discriminates between 'Dorit' and 'Chandler' on the one hand, and 'Ofra' and 'Douglas' on the other hand.

These RAPDs have been repeated consistently, using either independent DNA preparations from 12 plants of each cultivar or DNA extractions from pools of three plants (data not shown). Only consistent and reproducible RAPD products were considered for this analysis. The intensity of the amplified polymorphic DNA segments produced by OG-5 primer in 'Dorit' and by 1037 in 'Chandler' was consistently and significantly lower than that of the DNA counterparts produced by these primers in the other cultivars. It is suggested that these primers anneal to genomic loci which are mismatched, such that priming and extension efficiencies are reduced. Alternatively, these amplified regions may contain secondary structures which hinder the polymerization process.

The combination of the 10 polymorphic DNA products shown in Fig. 1 and detailed in Table 1, exhibits cultivar-specific patterns that enable each cultivar to be identified individually. Table 1 demonstrates the high-resolution power of the RAPD method in strawberry. The variety 'Ofra' is a progeny of 'Dorit' × 'Parker' and, indeed, the presence or absence in 'Ofra' of each RAPD product is closely related to its presence in the parental lines. For example, RAPD OG-2 1.10-kbp product is present in all three lines ('Dorit', 'Parker', 'Ofra'). RAPD OG-2 0.80-kbp product is present in 'Dorit' and not in 'Parker' and it has apparently been transmitted to 'Ofra' from 'Dorit'. Similarly, considering that 'Dorit' resulted from a

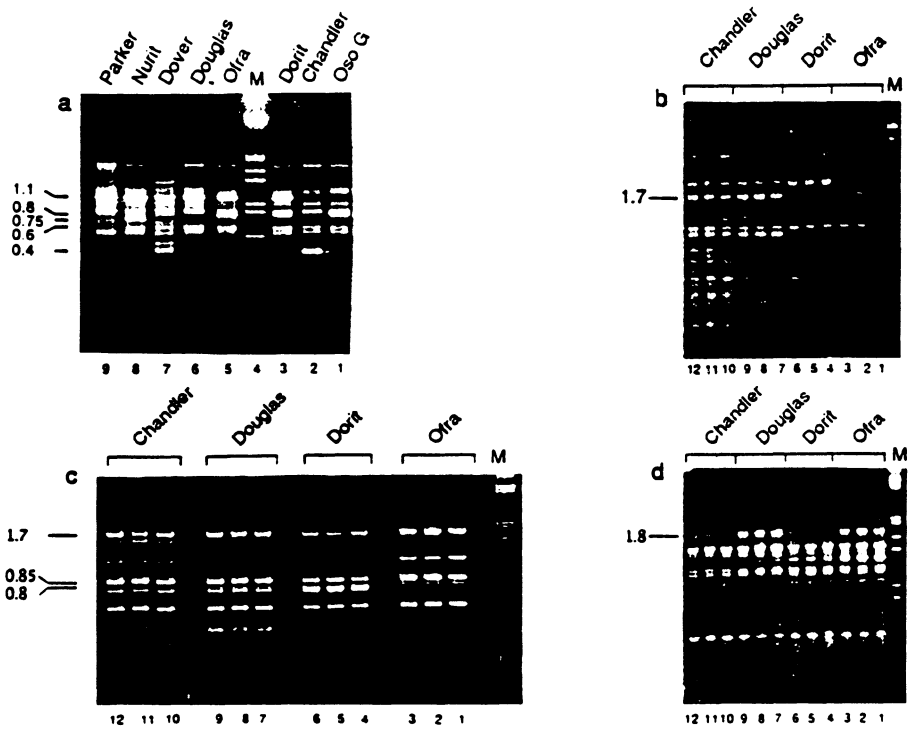

Fig. 1. Agarose-gel electrophoresis of randomly amplified genomic DNA products, generated from representative plants of the eight cultivars indicated (a) and from three individual plants of each of the strawberry cultivars 'Ofra' (1–3), 'Dorit' (4–6), 'Douglas' (7–9), and 'Chandler' (10–12); (b–d). The oligonucleotide primers used were OG-2 (a), OG-5 (b), OF-7 (c), 1037 (d). The sizes (in kbp) of polymorphic DNA products which discriminate between cultivars are indicated on the left. Lane M represents EcoRI and HindIII digested lambda DNA size markers

Table 1. Cultivar-specific RAPD patterns (derived from Fig. 1) which distinguish each of the eight strawberry cultivars tested, individually. + = presence of PCR product (in kbp); − = absence of PCR product; ± = consistent low intensity of PCR product; NT = not tested

Primer	RAPD (kbp)	Cultivar 'Chandler'	'Douglas'	'Dorit'	'Ofra'	'Oso Grande'	'Dover'	'Nurit'	'Parker'
OG-2	1.10	−	+	+	+	−	+	+	+
OG-2	0.80	−	−	+	+	+	+	−	−
OG-2	0.75	−	−	−	−	−	−	+	−
OG-2	0.60	+	+	+	+	+	+	+	−
OG-2	0.40	+	−	−	−	−	+	−	−
OG-5	1.70	+	+	±	+	NT	NT	NT	NT
OF-7	1.70	+	−	−	−	NT	NT	NT	NT
OF-7	0.85	−	+	−	+	NT	NT	NT	NT
OF-7	0.80	+	+	+	−	NT	NT	NT	NT
1037	1.80	±	+	−	+	NT	NT	NT	NT

cross between 'Dover' and 'Nurit', cases of simple inheritance of RAPD products from parents to progeny can be seen. This study indicates the high-resolution power of the RAPD markers for strawberry such that genetically close cultivars can be distinguished. The RAPD approach can, therefore, be used as a useful tool in indentification and protection of strawberry cultivars.

The appearance of RAPD products is often not consistent. Using the RAPD approach for cultivar identification, therefore, requires substantial screening of primers and selection of those primers that, under certain PCR conditions, generate reproducible polymorphic products. Only consistent and reproducible products should be considered. The reproducibility of RAPD products can be even more strongly established by cloning, sequencing the two ends, and then designing fully matched primers for PCR amplification under higher stringency conditions (PARAN and MICHELMORE 1993). The approach described here, combining rapidity, simplicity of operation and high-resolution power, suggests that RAPD provides an advantageous procedure for the identification, certification and protection of commercial strawberry cultivars. It is interesting to note that the present study helped to identify the cultivars 'Ofra' and 'Dorit', and thus prevented unauthorized shipment of both plants and fruits of these varieties. In addition this procedure is currently being used for routine identification of strawberry cultivars in cases of plant mixtures and other cases where quality control of strawberry plants is needed.

Zusammenfassung

Die Identifikation von Erbeersorten mit Hilfe von RAPD-Markern

Eine genaue und schnelle Sortenidentifikation ist insbesondere bei vegetativ vermehrten Pflanzen für den Sortenschutz von großer Bedeutung. Das Ziel dieser Arbeit war es zu prüfen, ob eine Entwicklung von sortenspezifischen RAPD-Markern für Handelssorten von Erdbeeren (*Fragaria ananassa* Dutch) möglich ist. Der Versuch war darauf ausgerichtet, Unterschiede zwischen den beiden am Volcani Center neu entwickelten Sorten 'Ofra' und 'Dorit' und sechs anderen Sorten, 'Douglas', 'Chandler', 'Oso Grande', 'Dover', 'Nurit' und 'Parker', aufzuzeigen. Es wurden reproduzierbare RAPD-Fingerprints entwickelt, von denen jeder mindestens ein polymorphes DNA-Fragment enthielt. Eine Kombination von 10 polymorphen DNA-Fragmenten ergab sortenspezifische Bandenmuster, aufgrund derer die Unterscheidung zwischen eng verwandten Sorten wie 'Ofra' (aus 'Dorit' und 'Parker' hervorgegangen) und 'Dorit' (aus 'Nurit' und 'Dover') möglich war. Die Untersuchung zeigt, daß RAPD-Marker für den Sortenschutz von Erdbeeren geeignet sind.

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