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Partial molecular characterization of some kiwi fruit cultivars by RAPD markers

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Molecular variability among seven cultivars of *A. deliciosa* var. *deliciosa* was investigated through RAPD markers. Thirty four decamer primers were screened generating polymorphic patterns of amplified DNA for these cultivars. Twenty one selected primers gave clear and reporducible patterns. A total of 430 bands were produced and 29.37% of them were polymorphic. The patterns distinguished between the cultivars and their analysis established an approach to classification within *A.deliciosa* var. *deliciosa* based on RAPD markers. The dendrogram clearly differentiated male from female cultivars. While abbot and allison female cultivars were closely related, bruno and abbot female cultivars showed maximum dissimilarity.

Kiwi fruit (*Actinidia deliciosa* var. *deliciosa*) is a commercial fruit crop, originating in the hills and mountains of Southern and Central China[1]. It was first developed as commercial crop in New Zealand. It is also known as China's miracle fruit and Horticultural wonder of New Zealand. It has also gained popularity in India. Presently, seven cultivars available in India belong to *A. deliciosa* var. *deliciosa* and are maintained by vegetative propagation. These commercially grown cultivars are dioecious in nature and are considered to be hexaploids ($x = 29$)[2,3].

There is need for a reliable and precise verification of the cultivars identity for growers. This also represents an important aspect in the fruit industry, particularly as the sale of fruit trees and planting orchards represent major investments in terms of time and money. Recently, the importance of many molecular markers like RAPD (randomly amplified polymorphic DNA), RFLP (restriction fragment length polymorphism) and microsatellites in taxonomic classification and cultivar typing in fruit trees has been reported in several plant species. RAPD-PCR technique has an edge as it requires extremely small quantity of genomic DNA, is easy to employ and eliminates need of blotting and radioactive labelling[4]. RAPD markers have been used successfully for identification of genetic relationships of papaya[5] peach[6,7], apple[8], grapes[9,10] lemon[11] etc.

Crowhurst *et al.*[11] employed RFLP technique to study the origin of kiwi fruit and reported *A. chinensis* to be the progenitor of *A. deliciosa* and these two species were grouped together, whereas *A. eriantha* and *A. latifolia* paired on a separate branch. Crowhurst and Gardener[3] isolated and cloned repetitive DNA sequences from various species in *Actinidia* and found a repeat element of 468 base pairs which occurred in a tandem array of 50 kb length. This sequence was present in *A. deliciosa* and *A. chrysentha* but not in *A. chinensis*.

Cipriani *et al.*[12] tested 80 random primers of OPC, OPE, OPQ and OPS series for finger printing of 13 cultivars of *A. deliciosa*. The polymorphism thus obtained was able to separate blake cultivar from the rest. Shim *et al.*[13] reported seven primers which produced polymorphic banding patterns for 12 genotypes and six primers could distinguish *A. deliciosa* from *A. chinensis*.

In the present communication, seven clonal cultivars of *A. deliciosa* var. *deliciosa* are characterized using RAPD-PCR and grouped according to their genetic relationship with each other. Accordingly, a dendrogram has been developed.

Plant material—The seven cultivars of *Actinidia deliciosa* var. *deliciosa* maintained at the experimental orchard of the department of Pomology, Dr.Y.S. Parmar University of Horticulture and Forestry, Nauni-Solan, formed the experimental material. Out of these two are staminate i.e. male, viz. tomuri and allison and five are pistillate i.e. female, viz. hayward, bruno, monty, allison and abbot. Young green leaves from ten different vines of each cultivar were collected and kept in liquid nitrogen.

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Genomic DNA isolation—Genomic DNA from seven cultivars of kiwi fruit were isolated by CTAB method[14] with certain modifications. Five gram of young leaves frozen in liquid nitrogen were ground to a fine powder and transferred to 15 ml pre-warmed DNA extraction buffer {10 m*M*, tris-HCl (*p*H 8.0), 20 m*M*, EDTA, 1.4 *M*, NaCl, 2%, CTAB (cetyl trimethyl ammonium bromide) and 0.2%, mercapoethanol}. The samples were incubated at 60°C for 1 hr with occasional mixing by gentle shaking. To this, 15 ml of chloroform: isoamyl alcohol. (24:1 v/v) was added, mixed gently by inversion and kept for 20 min, followed by centrifugation at 15,000 rpm for 10 min at room temperature. The aqueous phase was recovered and mixed with 2/3 volume of isopropanol to precipitate DNA, which was recovered with a glass hook, washed with 70% ethanol and dried overnight. DNA was finally suspended in 2 ml of TE buffer (10 m*M*, tris-HCl (*p*H 8.0), 0.1 m*M*, EDTA). DNA was extracted from all the samples and ten samples of each cultivar from different vines were pooled together for further steps.

DNA purification—The isolated DNA was further purified to remove RNA, proteins, polysaccharides and phenols using RNase, proteinase K, phenol: chloroform: isoamyl alcohol (25:24:1), phenol: chloroform (24:1) chloroform: isoamyl alcohol and sodium acetate treatments.

DNA quantification—The purified DNA was quantified by following the protocol given by Pharmacia Biotech DyNA Quant TM 200 fluorometer instruction manual. Finally the isolated DNA was diluted to 25 ng/μl.

Polymerase chain reaction (PCR) conditions—PCR amplification reactions were carried out as described[15] with minor modifications. Reaction mixture (25 μl) contained genomic DNA (50ng), tris-HCL (10 m*M*), $MgCl_2$ (1.9 m*M*) and 100 μ*M* of each dATP, dGTP, dCTP and dTTP (Perkin Elmer-USA), primer (0.4m*M*) (Operon Technologies-USA) and 0.5 units of Ampli Taq DNA polymerase (Perkin Elmer-USA) The tubes were centrifuged for few seconds and placed in a thermocycler (Perkin Elmer-9800) for cyclic amplification using the following parameters: 1 cycle of 5 min at 94°C followed by 45 cycles of 1 min each at 94°C for denaturation, 1 min at 37°C primer annealing and a 2 min extension at 72°C followed by a 5 min cycle at 72°C and finally the machine was held at 4°C till analysis. Amplification products were analyzed by gel electrophoresis on 1.5% agarose gel incorporated with 1 μl/ml of ethidium bromide in 0.5 X TBE buffer. Gels were visualised on a UV transilluminator and photographs were taken with the help of a Polaroid camera.

Data analysis—For RAPD analysis, the bands with same molecular weight and mobility were treated

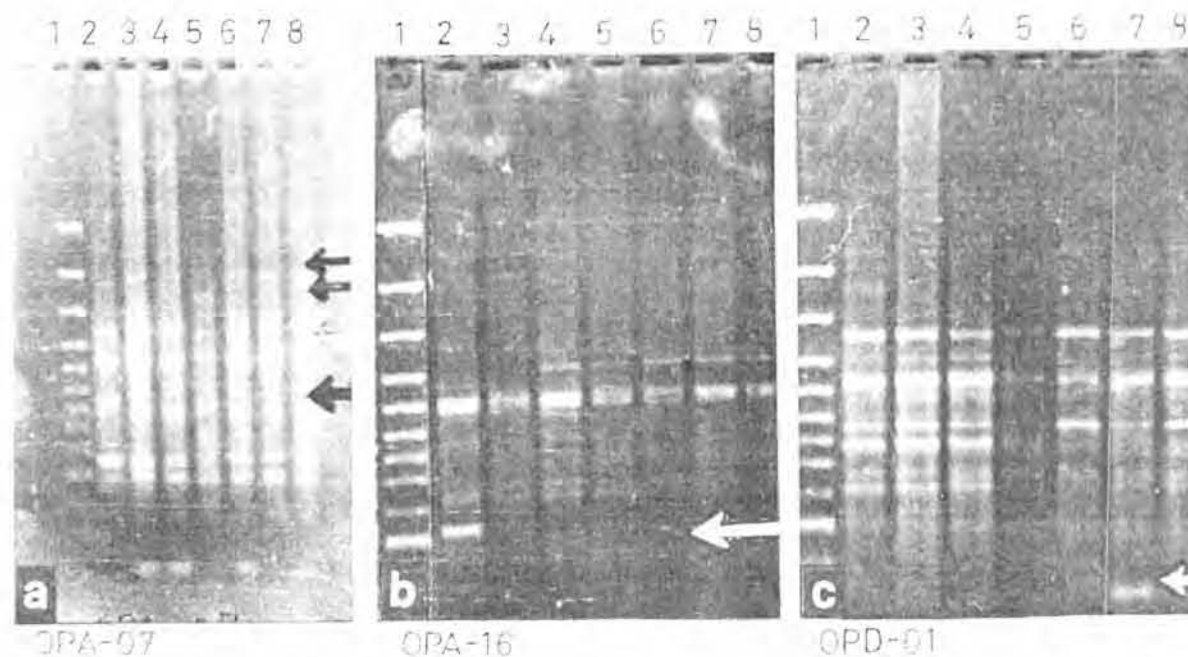

Fig. 1—RAPD banding profile generated by OPA-07, OPA-16 and OPD-01, showing selected RAPD markers $OPA\text{-}07_{850}$, $OPA\text{-}07_{1500}$, $OPA\text{-}07_{2000}$, $OPA\text{-}16_{200}$ and $OPD\text{-}05_{150}$ [Lane 1–100 bp DNA ladder plus molecular weight marker; lane 2–Allison male; lane 3-Tomuri male; lane 4-Hayward female; lane 5-Bruno female; lane 6-Monty female; lane 7-Allison female and lane 8-Abbot female cultivars].

Table 1 — Identification of kiwifruit cultivars by the presence(1) and absence(0) of selected RAPD markers

Kiwifruit cultivars	OPA-07 (850)	OPA-07 (1500)	OPA-07 (2000)	OPA-16 (200)	OPD-05 (150)	OPU-01 (2500)
Allison (male)	1	0	0	1	0	0
Tomuri (male)	0	0	1	0	0	0
Bruno (female)	0	1	0	0	0	0
Allison (female)	0	0	0	0	1	0
Abbot (female)	0	0	0	0	0	1

*Marker notation refers to the kit (last letter and the primer number) purchased from Operon Technologies. The number in parenthesis refers to the size (base pair) of amplified DNA fragment.

as identical fragments. The data matrices were analysed by the SIMQUAL program of NTSYS-Pc (Version 1.8) and similarities between cultivars were estimated using Jaccard's coefficient to develop a dendrogram by UPGMA method.

A total of 34 primers were screened to amplify genomic DNA of seven cultivars of kiwi fruit and only 21 (OPA-01, 02, 06, 07, 08, 09,11,12,16,18,19, OPB-01, 02, 20, OPC-05, OPD-03, 05 OPN-01, 02, 05, and OPU-01) were found to be polymorphic. Total number of bands amplified from polymorphic primers was 430 with the band size ranging from 150 bp to 5 kbp.

In the present study, no single primer was able to distinguish among all the cultivars. However, amplification by different primers was found to be informative and produced cultivar specific patterns. With OPA-07 primer, one band of 2000 bp was present only in tomuri male and another band of 1500 bp was present in bruno female cultivar (Fig. 1a). Allison male could also be characterized by the presence of $OPA\text{-}16_{200}$ marker (Fig. 1b). Abbot female could be characterized by the presence of $OPU\text{-}01_{2500}$ marker (not shown in the figure) and $OPD\text{-}01_{150}$ differentiated allison female cultivar from rest of the cultivars (Fig.1c). Many markers were identified which could differentiate between tomuri male and allison male cultivars. Thus the above mentioned primers can be used to identify individual cultivars. However, use of additional primers would probably allow the identification to be more clear and precise.

The results are in accordance with Cipriani *et al.*[12], who tested 80 primers to distinguish blake cultivar from the rest. They could distinguish all the cultivars with OPQ-20 except for abbot, hayward and topstar.

In order to quantify the level of polymorphism, a similarity index was generated. All of the cultivars fell in the range of 0.67 to 0.9 and depicted that allison female and abbot female cultivars have maximum

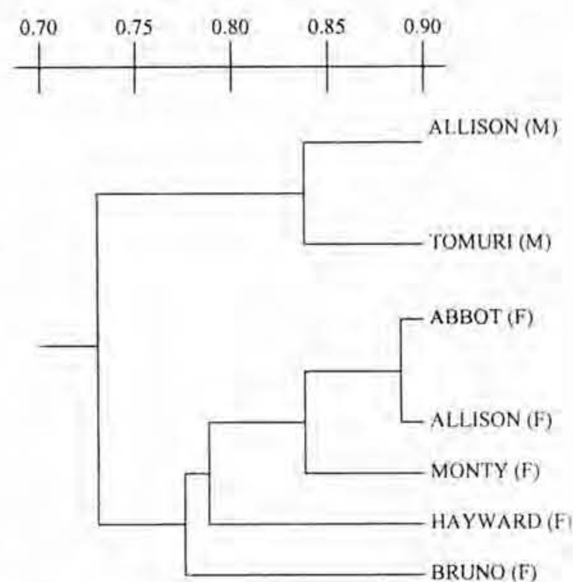

Fig. 2—Genetic relatedness among seven cultivars of *Actinidia deliciosa* var. *deliciosa* based on all 34 primers

similarity of 90%, whereas minimum similarity (maximum dissimilarity) of 67% was found between allison male and bruno female cultivars. Finally a dendrogram was constructed and the first major bifurcation divided the seven cultivars into two clusters. One cluster consisted of two male cultivars whereas the second cluster belonged to five female cultivars. This indicates a high level of polymorphism between males and females which is expected in the genus *Actinidia*, as it is dioecious (Fig. 2).

This study has shown that primers OPA-07, OPA-16, OPD-05 and OPU-01 (Table 1) could be used to distinguish bruno, allison female, abbot, allison male and tomuri male cultivars. Eight primers can differentiate between allison and tomuri males, whereas allison male and allison female can be differentiated by OPA-08. Though no individual marker could be identified for hayward and monty cultivars yet they have been clearly differentiated from others in the dendrogram. It is expected that testing of more primers may

result in one to one band specificity with the cultivar under reference so that the unknown bulk of different genotypes may be rationally grouped into known or new genotypes.

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