

RAPD markers for hybrid seed purity testing in tomato (*Solanum lycopersicum* L.)

Tomato (*Solanum lycopersicum* L., formerly *Lycopersicon esculentum* Mill.), a member of the Solanaceae family, is the second most important vegetable in terms of total production and has worldwide commercial distribution. The total production of tomato in India¹ during 2002–03 was 7.62 mt from an area of 0.5 m ha, with average productivity of only 15.9 t/ha. Hybrid tomato varieties under optimum management give economically higher yield (50–100 t/ha) than open-pollinated varieties (30–60 t/ha). They also offer certain other inherent advantages like uniform quality products, gene combinations for disease resistance and proprietary control of breeder/s in the form of trade secret of parental lines². In the recent past, tomato hybrids have become popular among Indian farmers, and presently 50% of the area under tomato cultivation is covered under hybrids¹. In general, tomato hybrid seeds are being produced by hand emasculation and pollination. As a result, the cost of hybrid seed is high owing to huge investments in labour, time and cost involved in the purity testing of hybrid seeds through conventional grow-out-test (GOT) before it reaches the farmers. In addition, the results of GOT based on seedling morphology are often misleading due to the masking of the expression of specific morphological or physiological traits during seedling development. The usefulness and potential of PCR-based rapid

and cost-effective markers like RAPD for hybrid purity testing have successfully been demonstrated in several vegetable crops, including tomato^{3–6}.

Two potential hybrids of tomato, viz. NTH-1 (DVRT × Flora Dade) and NTH-7 (DVRT-2 × 97/754) developed at the Indian Institute of Vegetable Research (IIVR), Varanasi under the National Agricultural Technology Project (NATP) on Development of Vegetable Hybrids were used in this study. Thirty-day-old seedlings of the two hybrids and their parental lines were transplanted on raised beds at a spacing of 60 cm × 45 cm. Young apical leaves were collected from 20 randomly selected plants of both the hybrids and their respective parents. Total DNA was isolated from each of these plants using the DNeasy Plant Mini Kit (Qiagen, GmBH, Germany) following a previously developed protocol^{7,8}. The DNA samples were quantified using a Hoefer DyNA Quant 200 Fluorometer (Amersham Biosciences, Hong Kong). For PCR analysis, the master mix consisted of 1.0 µl dNTPs (containing 10 mM each dNTP), 2.5 mM MgCl₂, 0.5 µl Taq polymerase (2.5 units/µl) with the supplied polymerase buffer (Bangalore Genei Pvt Ltd, Bangalore), 0.5 µl primer (1 µM) and 50 ng genomic DNA. The amplification programme consisted of one initial denaturation cycle (94°C for 60 s) followed by 35 cycles of 5 s at 94°C for denaturation, 25 s at 35°C for

primer annealing, and 30 s at 70°C for primer extension and DNA synthesis. At the beginning of the cycling profile, the reaction was held for 3 min at 94°C. In the final cycle, the extension period was for 5 min at 70°C. The amplification products were electrophoresed in a 1.2% agarose gel, stained with ethidium bromide and analysed using Alpha ImagerTM 3400 Gel Documentation System (Alpha Innotech, USA). Reproducibility of the RAPD markers was examined by carrying out five independent PCR reactions and gel analyses.

For NTH-1 hybrid, a total of 394 decamer oligonucleotide primers from Operon Technologies (Sigma, St Louis, USA) were initially used to identify polymorphs between the pooled DNA samples from ten plants of each parental line (DVRT-1 and Flora Dade). Three hundred and seventy-five (95.1%) primers generated repeatable amplicons and among them, six (1.6%) generated polymorphic fragments between the parents. One of these six primers, namely OPB-16 generated a unique 1193 bp amplicon in the male parent (Flora Dade), and four fragments of 630 bp, 900 bp, 1790 bp and 2011 bp, common to both the parents.

In case of hybrid NTH-7, the 253 primers screened, 51 (20.15%) generated amplicons, out of which only six primers (11.8%) generated polymorphic fragments. The OPB-19 primer generated a total of six fragments of 2186 bp, 1661 bp,

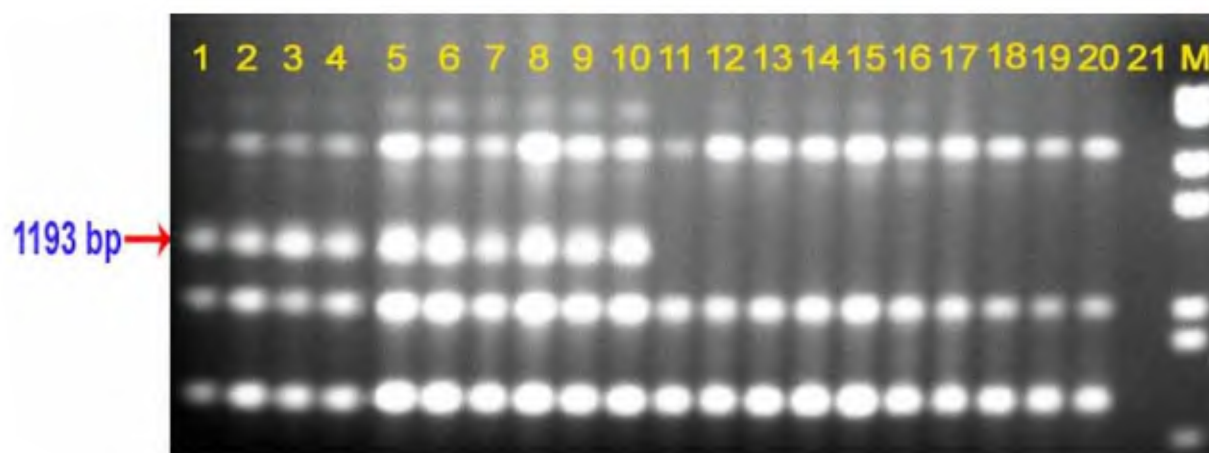


Figure 1. RAPD marker (OPB16₁₁₉₃) present in individual male plant (Flora Dade, lanes 1–4) hybrids (NTH-1, lanes 5–10), and absent in female plant (DVRT-1, lanes 10–20). M,

979 bp, 554 bp, 397 bp and 327 bp. Five of these fragments were common to both parents (DVTR-2 and 97/754). However, the 2186 bp fragment was specific to the male parent (97/754). The low level of polymorphism detected through RAPD markers in the parental lines of NTH-1 (1.6%) and NTH-7 (11.8%) reflects the status of relatively low genetic variability between the tomato-inbred lines. Narrow genetic base of Indian tomato inbred lines revealed from RAPD analysis has been reported earlier⁷. Low incidence of RAPD polymorphism (4–8%) in tomato has also been reported in exotic parental lines^{3–5}.

Reproducibility of the OPB16₁₁₉₃ marker (Figure 1) was confirmed in the five independent PCR reactions. The OPB16₁₁₉₃ marker was absent in all the 20 female plants (DVTR-1) and present in all the 20 male plants (Flora Dade) and hybrids (NTH-1; Figure 1). Hence, the marker could be commercially used to detect selfed-seeds in the hybrid seed lot of NTH-1. Like OPB16₁₁₉₃, reproducibility of the OPB19₂₁₈₆ marker was also confirmed. It was found to be present in 20 individual plants each of the male (97/754) and hybrid (NTH-7), and absent in female plants (DVTR-2). Therefore, this male-specific marker (OPB19₂₁₈₆) was found to be useful to detect the percentage of selfed seeds in the hybrid seed lots of NTH-7, which would be time- and cost-effective than the GOT.

These results revealed that the RAPD markers are effective for the determination of genetic purity of tomato hybrid seeds. From the seed lots of both the hy-

brids (NTH-1 and NTH-7), hybrid crops comprising 100 plants were raised as a part of the routine evaluation process. There was no contamination in hybrid seed lots because based on morphological features, all the 100 plants of each hybrid were judged to be true hybrids. Although non-reproducibility is considered to be one of the major drawbacks in using RAPD markers, in this study, case-specific utilization of RAPD markers in practical plant breeding has been demonstrated for genetic purity testing of two potential tomato hybrids. The usefulness and potential of RAPD markers for purity testing of hybrid seeds have also been demonstrated in tomato^{3–6} and in several other vegetable crops like pepper^{9,10}, watermelon³, cauliflower¹¹, cabbage¹², Chinese cabbage¹³ and broccoli¹⁴.

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NAMRATA SINGH
MAJOR SINGH*
SANJEET KUMAR
RAJESH KUMAR
VINEETA SINGH
H. C. PRASANNA
MATHURA RAI

*Indian Institute of Vegetable Research,
P. B. No. 5002,
P.O. BHU,
Varanasi 221 005, India*
*For correspondence.
e-mail: majorsingh@sify.com