BIOLOGIA PLANTARUM 46 (2): 301-304, 2003

BRIEF COMMUNICATION

Primer screening and optimization for RAPD analysis of cashew

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Abstract

Primer screening and optimization for random amplified polymorphic DNA (RAPD) analysis of cashew (*Anacardium occidentale* L.) was investigated. Among four series (A, B, D and N) of 10-mer primers, A-series performed better amplification of fragments than other series. The maximum amplification fragments was obtained using OPA-02, OPA-03, OPA-09, OPB-06, OPB-10, OPD-03, OPD-05 and OPN-03 primers. The primers OPA-02 and OPN-03 produced maximum number of DNA fragments in *Anacardium occidentale* cv. H-320. Primers (OPB-08 and OPN-05 performed a least number of amplification fragments. RAPD profile also indicate that some primer did not produce good amplification. The primer OPA-02 amplified 12 number of polymorphic bands in 20 cultivars of cashew. Only one DNA fragment was produced in *A. occidentale* cv. Vridhachalam - 2 (M-44/3) by using the primer OPA-02.

Additional key words: *Anacardium occidentale*, DNA amplification, polymerase chain reaction (PCR).

Emergence of agriculture and horticulture as a profitable industry and globalization of world trade in recent years has necessitated formulation of legal instruments for plant cultivar protection, registration and certification. The characterization of germplasm is essential not only for the identification of various species, varieties and cultivars but also to determine their genetic relationships. Random amplified polymorphic DNA technique has been widely used in many plant species for cultivar analysis, population studies and genetic linkage mapping (Williams *et al.* 1990). Optimization of the RAPD method depends on selection of primers. Although, the RAPD method uses arbitrary primer sequences, many of these primers must be screened in order to select primers that provide useful amplification product. The amplification and the type of reaction buffer on RAPD profiling of various species have been reported (Levi *et al.* 1993, Hilton *et al.* 1997). Few reports are available on the use of high G + C percentage in primers used in RAPD analysis in *Cassava* and *Nepenthes* (Wong *et al.* 1997, Lim *et al.* 2000).

Cashew (*Anacardium occidentale* L.) is commercially grown for its kernels although cashew nut shell liquid and cashew apples are also valuable byproducts. It is considered to be the most important edible nut in the world trade (Ascenso 1986a,b). Cashew crop has been considered useful for soil conservation and afforestation of waste lands. Cashew is a cross-pollinated crop and therefore highly heterozygous exhibiting a wide variation for a number of characters such as growth, yield and quality of nut (Ascenso 1986b). There is an urgent need to characterise the genetic resources through molecular markers for successful breeding program. Here, we report results of primer screening and optimization of RAPD profiling of cashew for better screening of accession, parents and progeny identification.

Fresh leaves of *Anacardium occidentale* cvs. BPP-2, BPP-3, BPP-4, BPP-8 (H-2/16), Vridhachalam-2 (M-44/3), Vridhachalam-3 (M-26/2), ULLAL-2, ULLAL-3, Vengurla-2, Vengurla-3, Vengurla-4, Vengurla-7, H-320, Madakkathara-1 (BLA-39-4), Dhana (H-1608), Kanaka (H-1598), Priyanka (H-1591), Jhargram-1, NRCC-1 and NRCC-2 were collected in an ice box from the Cashew orchard maintained by the Orissa University of Agriculture and Technology, Bhubaneswar, Orissa, India. The leaves (2.5 g) were

Received 18 June 2001, *accepted* 4 January 2002.
Acknowledgement: The authors wish to thank the Department of Forest and Environment, Government of Orissa for necessary facilities.
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ground to a fine powder in liquid nitrogen in a mortar using a pestle. The fine powder was resuspended in 10 cm^3 of preheated DNA extraction buffer [1 M boric acid (pH 8.0), 2 mM EDTA, 1.4 M NaCl, 1.5 % hexadecyltrimethylammonium bromide (CTAB), 0.2 % β-mercaptoethanol (v/v)]. The mixtures were subsequently heated at 60 °C for 3 h. This was followed by addition of 10 cm^3 of chloroform and spin at 8 000 *g* for 20 min. After centrifugation, DNA was precipited with 5 M NaCl and ethanol. The crude DNA pellet was resuspended in 1 cm^3 of Tris-EDTA (TE) buffer. Subsequently, it was treated with 0.003 cm^3 RNase (10 mg cm^3) and incubated for one hour at 37 °C. DNA purification was made through clean *Genei Kit* (*M/S Bangalore Genei*, Bangalore, India). The DNA was washed in 70 % ethanol and resuspended in 0.05 cm^3 of sterile distilled water. DNA quantifications were performed by UV-spectrophotometer (*UVIDEC-650, Jasco*, Tokyo, Japan) and also visualising under UV radiation, after electrophoresis on 1.0 % agarose gel compared with λ-DNA digested by Hind-III as marker. The resuspended DNA was then diluted in sterile distilled water to concentration 5 mg cm^{-3} for use in RAPD analysis.

A set of 24 random decamer oligonucleotides (series A, B, D and N) purchased from *Operon Technologies Inc.* (Alameda, USA) was used as primers for the amplification of RAPD fragments. Polymerase Chain Reactions (PCR) were carried out in a final volume of 0.025 cm^3 containing 20 ng template DNA, 100 μM each deoxynucleotide triphosphate, 20 ng of decanucleotide primers (*M/S Operon Technology, Inc.*, Alameda, USA), 1.5 mM $MgCl_2$, 1× Taq buffer (10 mM Tris-HCl, pH 9.0, 50 mM KCl, 0.01 % gelatin) and 0.5 U Taq DNA polymerase (*M/S Bangalore Genei*, Bangalore, India). Amplification was achieved in a PTC 100 thermal cycler (M.J. Research, USA) programmed for a preliminary 4 min denaturation step at 94 °C, followed by 45 cycles of denaturation at 94 °C for 1 min, annealing at 37 °C for 1 min and extension at 72 °C for 2 min, finally extension at 72 °C for 10 min. Amplification products were separated alongside a molecular mass marker (1 kb ladder, *MBI Fermentas Inc.*, Amherst, USA) by electrophoresis on 1.2 % agarose gels run in 0.5 × TAE (Tris acetate EDTA) buffer, stained with ethidium bromide and visualized under UV light. Gel photographs were scanned through *Gel Doc System 2000* (*BioRad*, Hercules, USA). Data were recorded as presence (1) or absence (0) of bands from the examination of photographic negatives. Each amplification fragment was named by the source of the primer (*Operon, Advanced Biotechnologies*), the kit letter or number and the primer number.

The present investigation offers a primer screening and optimization for RAPD profile of *Anacardium occidentale*. Different series of Operon 10-mer primers were tested for amplification by using polymerase chain reactions. The results showed that A-series primers produced relatively more amplification fragments as compared to B, D and N-series (Fig. 1). The amplification generated by primers (OPB-08 and OPN-05) produced least number of fragments by using the cultivar H-320. The primers OPA-02 and OPN-03 produced maximum number of DNA fragments; the size of the DNA fragments ranged from 400 - 2900 base pairs. Primer OPA-02 amplified nine number of DNA fragments whereas OPN-03 produced eight number of bands in *Anacardium occidentale* cv. H-320. The result also noted that some decamer primers (OPB-11, OPD-05) did not show any amplification by using the cultivar H-320 (Fig. 1). The number of fragments were varied from one series of primer to other series of primer. The primers OPA-02, OPA-03, OPA-04, OPA-07, OPA-08, OPA-09, OPB-06, OPB-07, OPB-08, OPB-09, OPB-10, OPB-11, OPD-01, OPD-02, OPD-03, OPD-04, OPD-05, OPD-06, OPN-01, OPN-02, OPN-03, OPN-04, OPN-05 and OPN-06 produced good amplification of RAPD fragments ranged from 350 to 2900 base pairs (Table 1). RAPD analysis involved 20 cultivars with eleven selected primers produced 80 consistent RAPD markers, ranging in size from 200 to 3 000 base pairs; out of which 16 bands were monomorphic and rest were polymorphic (data not shown). Twenty cultivars of *Anacardium occidentale* with two selected primers (OPA-02 and

Table 1. Primers used for PCR amplification of *Anacardium occidentale* cv. H-320.

Name of primers	Sequence (5' - 3')	Number of bands	Size range of bands [bp]
OPA-02	5'-TGCCGAGCTG-3'	9	1900-400
OPA-03	5'-AGTCAGCCAC-3'	5	1750-450
OPA-04	5'-AATCGGGCTG-3'	3	1200-700
OPA-07	5'-GAAACCGGTG-3'	3	1350-500
OPA-08	5'-GTGACGTAGG-3'	3	1200-500
OPA-09	5'-GGGTAACGCC-3'	3	1900-900
OPB-06	5'-TGCTCTGCCC-3'	3	1500-600
OPB-07	5'-GGTGACGCAG-3'	3	1100-800
OPB-08	5'-GTCCACACGG-3'	4	1200-500
OPB-09	5'-TGGGGGACTC-3'	1	1200
OPB-10	5'-CTGCTGGGAC-3'	4	1900-400
OPB-11	5'-GTAGACCCGT-3'	1	400
OPD-01	5'-ACCGCGAAGG-3'	0	0
OPD-02	5'-GGACCCAACC-3'	5	1900-500
OPD-03	5'-GTCGCCGTCA-3'	4	2700-500
OPD-04	5'-TCTGGTGAGG-3'	2	1000-500
OPD-05	5'-TGAGCGGACA-3'	3	1200-400
OPD-06	5'-ACCTGAACGG-3'	0	0
OPN-01	5'-CTCACGTTGG-3'	3	1500-700
OPN-02	5'-ACCAGGGGCA-3'	2	1200-500
OPN-03	5'-GGTACTCCCC-3'	8	2900-600
OPN-04	5'-GACCGACCCA-3'	5	2300-600
OPN-05	5'-ACTGAACGCC-3'	1	350
OPN-06	5'-GAGACGCACA-3'	3	700-500

OPA-03) showed different amplification pattern (Fig. 2*A*,*B*). Most of the cultivars produced 8 - 9 amplified DNA fragments by using primer OPA-02 and OPA-03 (Fig. 2*A*,*B*). Only one fragment was produced in *Anacardium occidentale* cv. Vridhachalam-2 (M-44/3) by using OPA-02 primer. The polymorphism generated by PCR reaction by using different primers might be later useful in assisting the identification of cultivar/variety for breeding program. Primer screening and optimization for RAPD analysis has been used for the analysis of diversity and identification of duplicates within the large germplasm collection (Virk *et al.* 1995), identification of varieties/species (Prince *et al.* 1995), phylogenetic relationship (Nair *et al.* 1999), and conservation and management of genetic resources (Bretting and Widrelechner 1995).

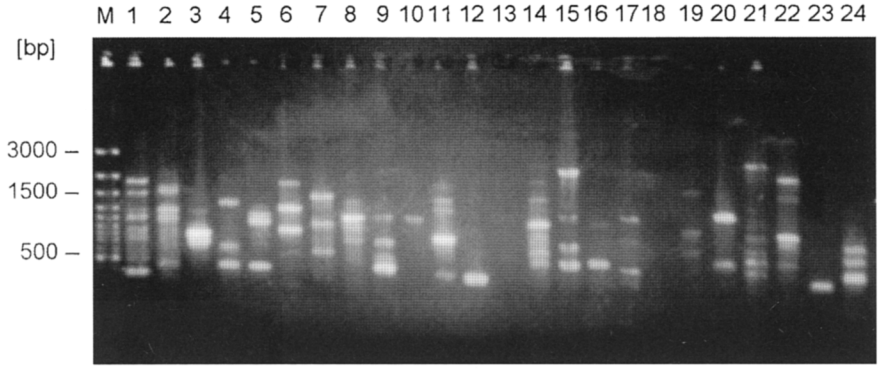

Fig. 1. PCR amplification profiles of *Anacardium occidentale* cv. H-320 with different primers. *Lane 1* - molecular mass marker [bp], *lane 2* - OPA-02, *lane 3* - OPA-03, *lane 4* - OPA-04, *lane 5* - OPA-07, *lane 6* - OPA-08, *lane 7* - OPA-09, *lane 8* - OPB-06, *lane 9* - OPB-07, *lane 10* - OPB-08, *lane 11* - OPB-09, *lane 12* - OPB-10, *lane 13* - OPB-11, *lane 14* - OPD-01, *lane 15* - OPD-02, *lane 16* - OPD-03, *lane 17* - OPD-04, *lane 18* - OPD-05, *lane 19* - OPD-06, *lane 20* - OPN-01, *lane 21* - OPN-02, *lane 21* - OPN-03, *lane 22* - OPN-04, *lane 23* - OPN-05, *lane 24* - OPN-06.

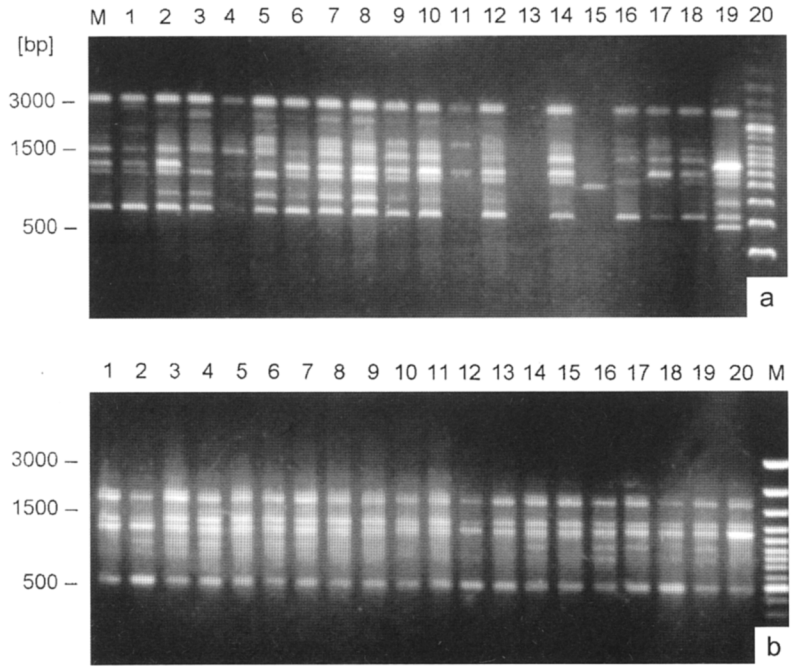

Fig. 2. RAPD patterns of 20 cultivars of *Anacardium occidentale* generated by primer OPA-02 (5'-TGCCGAGCTG-3') (*a*) and OPA-03 (5'-AGTCAGCCAC - 3') (*b*). M - molecular mass ladder [bp]. *Lane 1* - BPP-2, *lane 2* - BPP-3, *lane 3* - BPP-4, *lane 4* - BPP-8 (H-2/16), *lane 5* - Vridhachalam-2 (M-44/3), *lane 6* - Vridhachalam-3 (M-26/2), *lane 7* - ULLAL-2, *lane 8* - ULLAL-3, *lane 9* - Vengurla-2, *lane 10* - Vengurla-3, *lane 11* - Vengurla-4, *lane 12* - Vengurla-7, *lane 13* - H-320, *lane 14* - Madakkathara-1 (BLA-39-4), *lane 15* - Dhana (H-1608), *lane 16* - Kanaka (H-1598), *lane 17* - Priyanka (H-1591), *lane 18* - Jhargram-1, *lane 19* - NRCC-1, and *lane 20* - NRCC-2.

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