Identification of DNA polymorphism in cultivated groundnut using random amplified polymorphic DNA (RAPD) assay

V. Subramanian, S. Gurtu, R.C. Nageswara Rao, and S.N. Nigam

Abstract: Construction of a genetic linkage map is necessary to apply marker-assisted selection tools in a crop improvement program. Except for the recent studies from two laboratories, most of the previous studies have shown little or no DNA polymorphism in cultivated groundnut (*Arachis hypogaea* L.). In the present study, 70 selected genotypes, representing variability for several morphological, physiological, and other characters, were studied for polymorphism employing random amplified polymorphic DNA (RAPD) assay with 48 oligonucleotide primers. Of the 48 oligonucleotide primers only 7 (14.6%) yielded polymorphic amplification products. The total number of bands from the 7 primers was 408, of which 27 were polymorphic. Detection of polymorphism in cultivated groundnut opens up the possibility of development of its molecular map by judicious selection of genotypes that show DNA polymorphism. This approach will be useful for developing marker-assisted selection tools for genetic enhancement of groundnut for desirable traits.

Key words: *Arachis hypogaea* L., RAPD, DNA polymorphism, oligonucleotide, random primers.

Résumé : La production de cartes génétiques est une étape incontournable en vue de l'utilisation de la sélection assistée de marqueurs dans le cadre de programmes d'amélioration génétique. À l'exception d'études récentes en provenance de deux laboratoires, la plupart des études antérieures avaient montré peu ou pas de polymorphisme chez l'arachide (*Arachis hypogaea* L.). Dans la présente étude, 70 génotypes choisis, montrant de la variabilité au niveau de plusieurs caractères morphologiques, physiologiques et autres, ont été examinés pour le polymorphisme au moyen de la technique RAPD (ADN polymorphe amplifié au hasard) en utilisant 48 amorces. Des 48 amorces, seules sept (14,6 %) ont produit des amplicons polymorphes. Le nombre total de bandes produites par ces amorces était de 408, dont 27 étaient polymorphes. La détection de polymorphismes chez l'arachide cultivée ouvre la voie au développement d'une carte génétique moléculaire en choisissant judicieusement des génotypes parentaux montrant du polymorphisme. Cette approche sera utile pour développer des outils de sélection assistée en vue de la sélection de caractères d'intérêt chez l'arachide.

Mots clés : *Arachis hypogaea* L., RAPD, polymorphisme d'ADN, amorces oligonucléotidiques aléatoires.

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Introduction

Groundnut (*Arachis hypogaea* L.) is an important oilseed crop grown mainly under rainfed regions of the semi-arid tropics (SAT). The yield of the crop in the SAT is very low (<900 kg·ha^{-1}) due to prevalence of stresses such as drought, diseases, and pests. ICRISAT Center in India maintains a global groundnut germplasm collection of about 15 000 accessions of cultivated species and 457 accessions from about 40 wild species, for selection of genotypes with genes associated with desirable traits, including tolerance to various biotic and abiotic stresses. Although molecular tools such as DNA markers are becoming increasingly important as effective tools in crop breeding programs, their application in groundnut enhancement is lagging behind because of limited knowledge of the genome. Extensive variation for morphological and physiological characteristics exists in both wild and cultivated groundnut (Halward et al. 1992), but abundant DNA polymorphism has been observed only in wild diploid *Arachis* species (Kochert et al. 1991; Halward et al.

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V. Subramanian. Maharastra Hybrid Seeds Company (MAHYCO) Ltd., R&D, Kallakal, Andhra Pradesh 502 334, India.
S. Gurtu and S.N. Nigam.[1] Genetic Resources and Enhancement Division, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT[2]), Patancheru, Andhra Pradesh 502 324, India.
R.C. Nageswara Rao. Queensland Department of Primary Industries, P.O. Box 23, Kingaroy Q 4610, Australia.

[1]Author to whom all correspondence should be addressed (e-mail: s.nigam@cgiar.org).
[2]Mention of commercial products of companies does not imply endorsement or recommendation by ICRISAT over others of similar nature.

Table 1. Characteristics of 25 selected *Arachis hypogaea* genotypes used in the study.

No.	Genotype	Major characteristics[a]	Origin[b]	Subsp.[c]	Variety[d]	Status[e]
9	ICG 11325	Resistant to BW	India	FST	HYP	INSP
11	ICGV 86606	Resistant to BW	IC, India	FST	VUL	BL
19	ICG 1705	Resistant to LLS	Peru	FST	FST	LR
20	ICG 1712	Resistant to LLS	Brazil	FST	FST	BL
21	ICG 2716	Resistant to LLS	Uganda	FST	FST	UN
22	TMV 2	Susceptible to BW, LLS	India	FST	VUL	RC
23	JL 24	Susceptible to PBNV	India	FST	VUL	RC
24	J 11	Susceptible to BW, LLS	India	FST	VUL	RC
25	ICGS 11	Susceptible to BW, LLS	IC, India	FST	VUL	RC
27	ICGV 86031	Tolerant to drought	IC, India	FST	VUL	BL
28	ICGV 86707	High WUE, low P	IC, India	HYP	HYB	BL
29	Chico	Low WUE, high P, short-duration, non-dormant	U.S.A.	FST	VUL	BL
30	TAG 24	High WUE, high P	India	FST	VUL	RC
32	TMV 2-NLM	High WUE, low P, medium-duration	India	HYP	HYB	BL
33	ICGV 91123	Short-duration	IC, India	FST	VUL	BL
34	ICGV 92206	Short-duration	IC, India	FST	VUL	BL
37	M 13	Long-duration, dormant	India	HYP	HYB	RC
38	ICGV 86158	Short-duration, dormant	IC, India	HYP	VUL	BL
39	ICGV 87378	Short-duration, dormant	IC, India	FST	VUL	BL
41	ICG 6327	Resistant to GRV	Burkina Faso	FST	VUL	BL
42	ICGV 92209	Susceptible to GRV	IC, India	FST	VUL	BL
66	ICGV 88448	High oil content (53%)	IC, India	HYP	HYB	BL
68	ICG 1171	Average oil (45%)	India	FST	VUL	BL
70	ICGV 93094	High 100-seed mass (130 g)	IC, India	HYP	HYB	BL
71	ICG 4906	Low 100-seed mass (16 g)	Sri Lanka	HYP	HYP	LR

[a]BW, bacterial wilt; LLS, late leaf spot; PBNV, peanut bud necrosis virus; WUE, water-use efficiency; P, partitioning of photosynthates to pods; GRV, groundnut rosette virus.
[b]IC, ICRISAT (International Crops Research Institute for the Semi-Arid Tropics, Patancheru, Andhra Pradesh, India).
[c]FST, *Arachis hypogaea fastigiata*; HYP, *Arachis hypogaea hypogaea*.
[d]HYP, *hypogaea*; VUL, *vulgaris*; FST, *fastigiata*; HYB, *hypogaea* bunch.
[e]INSP, interspecific derivative; BL, advanced breeding line; LR, landrace; UN, unknown; RC, released cultivar.

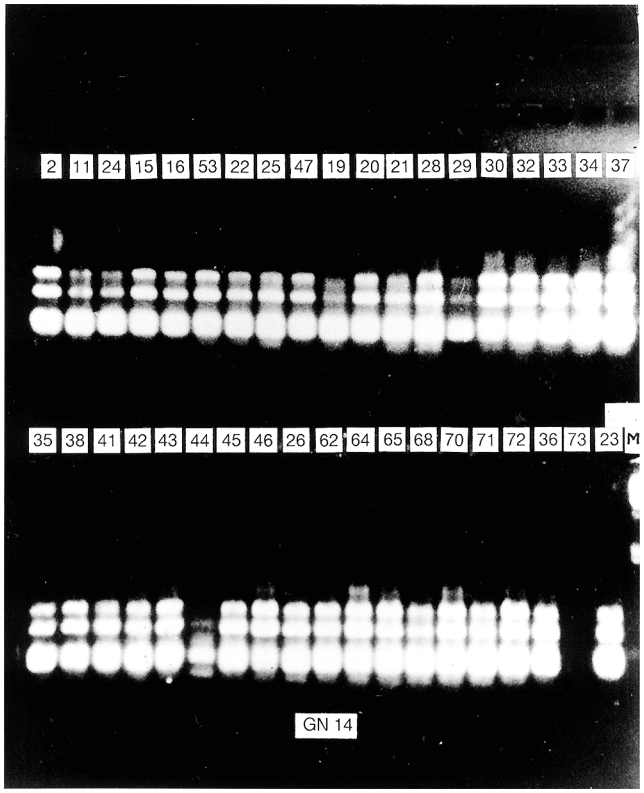
1993). It is surprising that such a diverse variation for phenotypic characteristics in cultivated species did not show significant variability at the DNA level. However, Halward et al. (1991) observed very low levels of DNA polymorphism in cultivated groundnut germplasm, using different techniques such as restriction fragment length polymorphism (RFLP) and polymerase chain reaction (PCR) analysis.

No variation in banding pattern was observed among the cultivars and germplasm lines of *A. hypogaea* using RAPD techniques (Halward et al. 1992), whereas the wild *Arachis* species were uniquely identified with most primers tested. Paik-Ro et al. (1992) also reported that DNA polymorphism could not be detected within or between *A. hypogaea*, *A. monticola*, and the lines of interspecific origin involving *A. hypogaea* and *A. cardenasii* with the 32-endonuclease–probe combination of RFLP. Also, very limited isozyme variation was observed in *A. hypogaea* (Lacks and Stalker 1993; Stalker et al. 1994). Low levels of DNA polymorphism have also been reported in other self-pollinated crops including tomato (Helentjaris et al. 1985), melon (Shattuck-Eidens et al. 1990), and wheat (Joshi and Nguyen 1993). Because of low levels of polymorphism, construction of a genetic map by the different techniques cited above has been difficult for cultivated groundnut. Although significant polymorphism has been observed in diploid *Arachis* species (Garcia et al. 1995), these markers could not be utilized in the improvement of *A. hypogaea* because of the incompatibility problems between species. He and Prakash (1997) reported the presence of DNA polymorphism in cultivated groundnut using the amplified fragment length polymorphism (AFLP) technique. Recently, Hopkins et al. (1999) observed low levels of DNA polymorphism in wild and cultivated groundnut using simple sequence repeats (SSRs). In this paper, we report the results of DNA polymorphism in 70 cultivated *A. hypogaea* genotypes using the RAPD assay involving 48 oligonucleotide primers. The observed polymorphism may be useful for developing molecular markers helpful for screening various traits in groundnut improvement programs.

Materials and methods

The study involved a selection of 70 groundnut genotypes, comprising 40 germplasm accessions originating from 16 countries, 11 advanced breeding lines, and 19 landraces, and representing two subspecies, *hypogaea* and *fastigiata.* These genotypes had variation for several desirable traits including drought tolerance, water-use efficiency, maturity period (crop duration), partitioning, seed dormancy, 100-seed mass, oil content, oleic/linoleic fatty acid ratio, and resistance to late leaf spot disease (*Phaeoisariopsis personata*), bacterial wilt (*Ralstonia solanacearum*), and groundnut rosette virus. Plants were grown in a greenhouse, in pots containing sterilized potting mixture of sand and soil. Fully expanded

Fig. 1. Genomic DNAs from a collection of 38 *Arachis hypogaea* genotypes amplified with RAPD primer GN 14 showed lack of DNA polymorphism.



leaves on the main axis were sampled from 10-day-old plants and frozen in liquid nitrogen until use for DNA extraction. DNA was extracted using the CTAB (cetyltrimethylammonium bromide) method (Saghai-Maroof et al. 1984).

RAPD assay

A set of 48 oligonucleotide random primers, each 10 nucleotides long, was obtained from Operon Technologies, Alameda, Calif. PCR was carried out according to Williams et al. (1990), with a modified reaction mix (Garcia et al. 1995). The reaction mix contained 10 mM Tris–HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 100 mM each of dATP, dCTP, dGTP, and dTTP, 20 ng primer, 15 ng genomic DNA, and one U of Taq polymerase.

Amplification was performed in 0.2-mL tubes placed in a Perkin–Elmer 9600 thermal cycler (Norwalk, Conn.) under the following temperature program: 92°C for 1 min, 35°C for 1 min, and 72°C for 2 min, for 45 cycles. Amplification products were analyzed by electrophoresis in 1.4% agarose gels run at 70 V for 5 min and 40 V until completion (about 3 h) using 1× TBE buffer. The gel was stained with ethidium bromide and visualized by illumination with UV light and photographed.

Results and discussion

The 70 groundnut genotypes chosen for the present study represent a broad spectrum of variation for several phenotypic traits, and in their origin. The characteristics of 25 selected genotypes that show greater diversity for disease reaction, drought, quality traits, and adaptation are given in Table 1. DNA from the 70 genotypes was studied with 48 oligonucleotide primers for RAPD assay. The amplification products observed in 38 genotypes using primer GN14 (5′-GCGAGGCGT-3′) shown in Fig. 1, exhibited the usual pattern of lack of DNA polymorphism in cultivated groundnut. Earlier workers reported similar results in cultivated groundnut (Halward et al. 1991; Halward et al. 1992; Garcia et al. 1995). The lack of polymorphism has also been reported in other self-pollinated crops such as tomato, soybean, and wheat (Helentjaris et al. 1985; Keim et al. 1989; Sharp et al. 1989). Such insufficient variability in DNA polymorphism makes it difficult to construct a genetic map, using a population derived from a cross between tetraploid cultivars (Kochert et al. 1991).

Table 2. Number of amplification products generated and the nucleotide sequence of RAPD primers that showed DNA polymorphism with the 7 random oligonucleotide primers in 25 selected *Arachis hypogaea* genotypes.

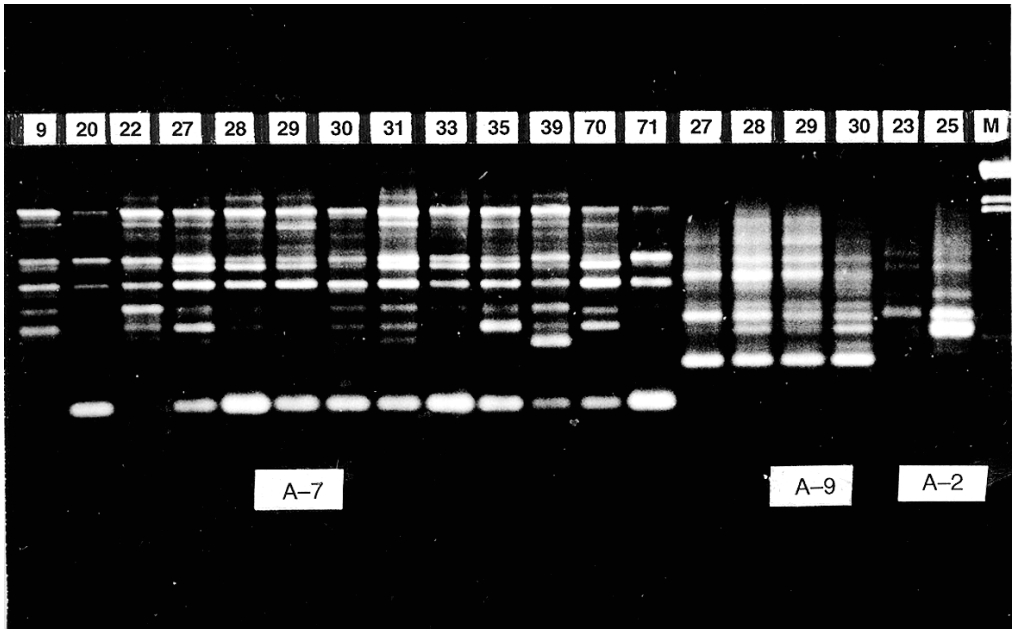
		Number of bands	
Primer code	Primer sequence (5′–3′)	Total	Polymorphic
A-02	TGCCGAGCTG	9 (2)	3
A-07	GAAACGGGTG	125 (13)	9
A-09	GGGTAACGCC	41 (4)	3
A-18	AGGTGACCGT	60 (9)	2
A-20	GTTGCGATCC	25 (4)	5
G-08	TCACGTCCAC	128 (12)	4
G-10	AGGGCCGTCT	20 (4)	1

Note: Numbers in parentheses indicate the number of genotypes that yielded amplification products.

Molecular markers using RAPD assay have been used in constructing linkage maps and identification of markers associated with disease resistance (Rafalski et al. 1991; Martin et al. 1991; Michelmore et al. 1991). Lanham et al. (1992) demonstrated that RAPDs are a convenient and effective marker system for *Arachis* species. The RAPD assay was chosen for the present study since the procedures involved were simple, not requiring probes or Southern-blot hybridization, as in the case of RFLP. Of the 48 primers tested with 70 genotypes, amplified fragments with polymorphism were observed for only 7 (14.6%) primers. The 7 primers that showed DNA polymorphism are denoted A-02, A-07, A-09, A-18, A-20, G-08, and G-10. The nucleotide sequences of these primers are given in Table 2. Other primers, although having produced amplification fragments, did not show polymorphism. The amplification is reproducible by strictly adhering to the specific protocols (Joshi and Nguyen 1993). As an example, the presence of DNA polymorphism using three random oligonucleotide primers A-07, A-09, and A-02 that produced polymorphic bands only in 13, 4, and 2 groundnut genotypes, respectively, is shown in Fig. 2. Out of the total of 408 bands that resulted from 48 genotypes using 7 primers, 27 were polymorphic. Primer A-02 yielded polymorphism in only 2 genotypes, whereas, with the primers A-07 and G-08, polymorphism was observed in 13 and 12 genotypes, respectively (Table 2). The number of polymorphic bands produced per primer ranged from 1 in G-10 to 9 in A-07. A low level of DNA polymorphism in cultivated groundnut was reported using SSRs with six primer pairs (Hopkins et al. 1999).

The genotypes shown in Fig. 2 had variable combination of a number of agronomic traits. The demonstration of DNA

Fig. 2. Genomic DNAs from 15 selected *Arachis hypogaea* genotypes (see Table 1) amplified with RAPD primers A-07, A-09, and A-02, showing DNA polymorphism. Lane M corresponds to the molecular weights (ranging from 23.13–2.07 kbp) identified from λDNA–*Hin*dIII digest. Note: Genotypes 27, 28, 29, and 30 showed polymorphic bands with both the primers A-7 and A-9.



polymorphism in cultivated groundnut in this study is significant, and this is the third report (after He and Prakash 1997; Hopkins et al. 1999). The observed polymorphism could be attributed to selection of genotypes with diverse characteristics including geographic origin, as well as to specific primers used in the RAPD assay. These genotypes will be useful for developing mapping populations. Only 3 genotypes (9, 42, and 66 in Table 1) out of 48 showing amplification products have wild *Arachis* species in their parentage. The remaining 45 originate from cultivated groundnut, *A. hypogaea*. The polymorphism of about 6.6% observed in the present study represents inherent variability among genotypes at the DNA level. However, associating polymorphic DNA fragments with different traits requires further study using the selected trait-specific cultivars and their progenies.

In conclusion, the present study indicated the presence of DNA polymorphism in cultivated groundnut using RAPDs. This opens up a possibility for developing a molecular genetic map that will lead to the application of marker-assisted selection tools to genetic enhancement of cultivated groundnut.

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