IDENTIFICATION OF DNA POLYMORPHISM IN CULTIVATED GROUNDNUT (*Arachis hypogaea* L.)

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Abstract

Initial studies in the germplasm of cultivated groundnut using RFLP and RAPD revealed very limited polymorphism (Kochert *et al.,* 1991, Halward *et al*., 1992) and led to the opinion that cultivated groundnut has a very narrow genetic base. However, the studies by He and Prakash (1997) indicated the possibility of exploiting DAF and AFLP techniques for the detection of polymorphism in groundnut. In the present study, Amplified Fragment Length Polymorphism (AFLP) was done using 64 combinations of primers specific to *EcoRI* and *Mse I* with the selective nucleotides in 12 groundnut cultivars. Primer combinations were ranked based on their ability to amplify different fragments, and having more than 10% polymorphism and selected five primer pairs. These primer pairs were further tested in four genotypes viz. TMV 7, Chandra, RS 1 and UF 70-103 for confirmation. All the selected primers could give amplification of the fragments and the banding pattern showed polymorphism. The five primers when tested with cultivars which are selections from Robut 33-1, also shown polymorphism.It could be concluded that the five primers selected were suitable for detecting polymorphism and can be further used in varietal identification and finger printing, and to tag genes of economic importance in breeding programmes.

Introduction

In the genus *Arachis*, considerable variability with regard to morphology and economically important traits like resistance to pests and diseases have been documented (Abdou *et al* 1974, Subrahmaniam *et al*, 1985, Stalker and Moss, 1987, Mehan *et al* 1992). However, the morphological variability exhibited in the cultivated species doesn't reflect at the biochemical and molecular level till recent times. The initial studies in the germplasm of cultivated groundnut using RFLP and RAPD concluded the existence of a very limited polymorphism (Kochert *et al* 1991, Halward *et al* 1991, 1992, Paik-Ro *et al* 1992) and concluded that cultivated groundnut has a very narrow genetic base. Recently, He and Prakash (1997) established DNA polymorphism in the primary gene pool of cultivated groundnut using the AFLP and DAF techniques. Hopkins *et al* (1999) reported 6 SSR primers capable of detecting polymorphism in cultivated groundnut. The present study attempts to identify primers suitable for deducing DNA polymorphism in the cultivated groundnut and validate them for their suitability in assessing the relatedness of groundnut cultivars

Materials and Methods

Seeds of the cultivars were obtained from the genetic resources section of NRCG. For screening of the primers 12 cultivars (Table 1) of four habit types were used. The cultivars Chandra, RS1, TMV7, UF70-103 were used for confirmation of the results. Four cultivars viz. ICGS1, ICGS11, ICGS 44 and Kadiri 3 (all selections from Robut 33-

1) were used to validate the primers for their capability to assess the polymorphism in the related cultivars.
Approximately 2g of leaf material from the plants grown in dark (etiolated) were used for isolation of genomic DNA. Fresh leaves were ground under liquid nitrogen and the urea based method for DNA isolation described by Chen and Dellaporta (1994) was used with slight modification. The DNA quantity and quality were determined using the spectrophotometric method.

Table 1 List of cultivars used for screening primers for DNA polymorphism.

Cultivar	Habit type	Pedigree
MH 2	Valencia	Selection from Gujarat dwarf mutant
M H 4	Valencia	Not known to authors
Gangapuri	Valencia	Not known to authors
TMV 10	Virginia bunch	Natural mutant from Argentina
Kadiri 3	Virginia bunch	Selection from Robut 33-1
Kadiri 2	Virginia bunch	Nigerian culture MK 374
Chandra	Virginia runner	Selection from Ah 114
UF 70-103	Virginia runner	Introduction from USA
RS 1	Virginia runner	Selection from local collection
Spanish Improved	Spanish	Selection from Spanish groundnut
TMV 7	Spanish	Selection from Tenesse white
JL 24	Spanish	Selection from EC 94943

AFLP procedure was followed as described by Vos *et al* (1995) and the product manual supplied by Life Technologies Inc (GIBCO BRL, India) with minor modifications in dilutions. Fifty nanograms of genomic DNA was restricted using the enzymes *Eco* RI and *Mse* I and ligated with adapters. The ligation products were pre amplified using an *Mse* I primer containing one selective nucleotide and *Eco* RI containing no selective nucleotide. The pre amplification products were diluted 1:1 with TE buffer and used for selective amplification. The selective amplifications was carried out with two primers each with three selective nucleotides (total 64 combinations), corresponding to the *Eco* RI and *Mse* I linkers supplied by Life Technologies Inc. Primer combinations were selected based on their ability to amplify different fragments, and having more than 10% polymorphism. Five selected primer pairs in the decreasing order of their polymorphism were selected and tested in four genotypes viz. TMV 7, Chandra, RS 1 and UF 70-103 for confirmation. These primers were again tested in another four genotypes viz. ICGS 1, ICGS 11, ICGS 44 and Kadiri 3 (all are selections from the single parent, Robut 33-1).
The amplification products were separated on an 8% denaturing polyacrylamide sequencing gel. Electrophoresis was done at 30W constant power for 3 hours. One common sample was run in all gels as reference to optimize band positions. The gels were stained using a modified silver staining technique of Bassam *et al* (1991).
Gels were scanned using Alphascan large format densitometer, scored, and analyzed using the software GelCompar *II*. The band positions were optimised and manually verified for correctness before analysis. Analysis of polymorphism and the similarity

matrix was based on Jaccard similarity coefficient (fuzzy logic). The overall percentage of polymorphism was calculated over the total number of bands in all the varieties used for comparison. Percentages of polymorphism for each primer pairs were also computed.

Results and discussion

The sixty-four primer pairs tested showed different banding patterns which ranged from no bands to 76 scorable bands. The primer pairs showing more than 10% of polymorphism, ranked in the decreasing order (The data on the screening of 64 combinations of primers not presented) and the five primer pairs from the top were *Eco* RI-AAC+*Mse* I-CAG (P3), *Eco* RI-ACG+*Mse* I-CAC (P50), *Eco* RI-AGC+*Mse* I-CAG (P51), *Eco* RI-AGC+*Mse* I-CAT (P52), and *Eco* RI-AGG+*Mse* I-CTG (P63).

Of the five primers tested on the cultivars TMV 7, Chandra, RS 1 and UF 70-103, P3 showed maximum similarity (62.6%) between TMV 7 and UF 70-103 (Fig 1). The minimum similarity was observed between TMV 7 and Chandra (30.02) with P50. The overall polymorphism observed was between 12.3 and 15.4%. The primer-wise polymorphism estimated ranged from 12.1 to 77.3% (Table 2).

Table 2. AFLP polymorphism in the four groundnut cultivars

Variety	Primer-wise polymorphism (%)				
	P3	**P50**	**P51**	**P52**	**P63**
Chandra	61.5	77.3	30.0	67.7	30.4
RS1	48.1	23.3	33.3	21.2	42.9
TMV 7	12.1	36.4	32.0	72.0	50.0
UF 70-103	34.8	50.0	40.0	46.4	--
Mean	*39.1*	*46.7*	*33.8*	*51.8*	*41.1*

Primer pair P52 showed maximum mean polymorphism across the four cultivars. However, the differences between primer pairs in polymorphism was not significant (P=0.386). This may be indicative of the difference in the genetic make up of the unrelated cultivars.

The same primer pairs could detect an overall polymorphism of 10.5 to 14% in the cultivars, ICGS1, ICGS11, ICGS 44 and Kadiri 3 (all selections from Robut 33-1).. The primer-wise polymorphism had a range from 24.5 to 65.5%. The difference in polymorphism between primers were significant (P=0.005). The maximum number of bands was resolved between 329 and 61 bp (Fig 2). For primer pairs, in the decreasing order of polymorphism was P65>P3>P52>P50>P51 (Table 3).The similarity between the cultivars was 45.8 to 74.5% and was higher than that was observed in the previous set of cultivars but for the primer pair P3. The higher similarity and lower level of polymorphism between the cultivars indicates the near relatedness of the cultivars being selections from a common parent.

Though DAF studies of He and Prakash (1997) reported a polymorphism to the level of 37.5%, in AFLP studies a maximum of only 9.3% polymorphic loci was reported. The higher level of polymorphism observed in our study may be partially due to elimination of less prominent bands from analysis by applying band filters.

In both sets of cultivars, the polymorphism detected by the primer pair P3 was relatively less. Though each primer pairs could produce polymorphic banding patterns, the similarity values above 30% even in far related cultivars necessitates the use of a set of primer pairs for differentiating cultivars.

Table 3. AFLP polymorphism in the four selections from Robut 33-1

Variety	Primer-wise polymorphism (%)				
	P3	**P50**	**P51**	**P52**	**P63**
ICGS 1	42.1	37.2	36.4	42.9	51.3
ICGS 11	52.2	32.5	35.4	36.8	46.3
ICGS 44	35.3	44.9	33.3	38.5	46.7
Kadiri 3	38.9	34.1	24.5	41.5	62.5
Mean	*42.1*	*37.2*	*32.4*	*39.9*	*51.7*

From the polymorphism obtained in the related cultivars using the set of five primer pairs, it could be concluded that to obtain clear cut distinction between cultivars, the selected primers were sufficient and can be used in varietal identification and finger printing, and to tag genes of economic importance in breeding.

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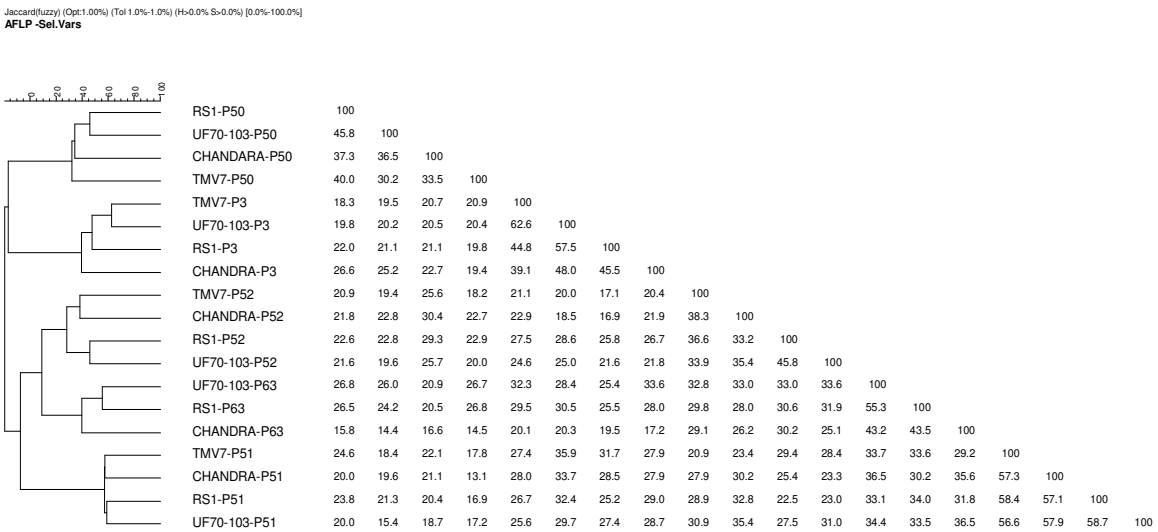
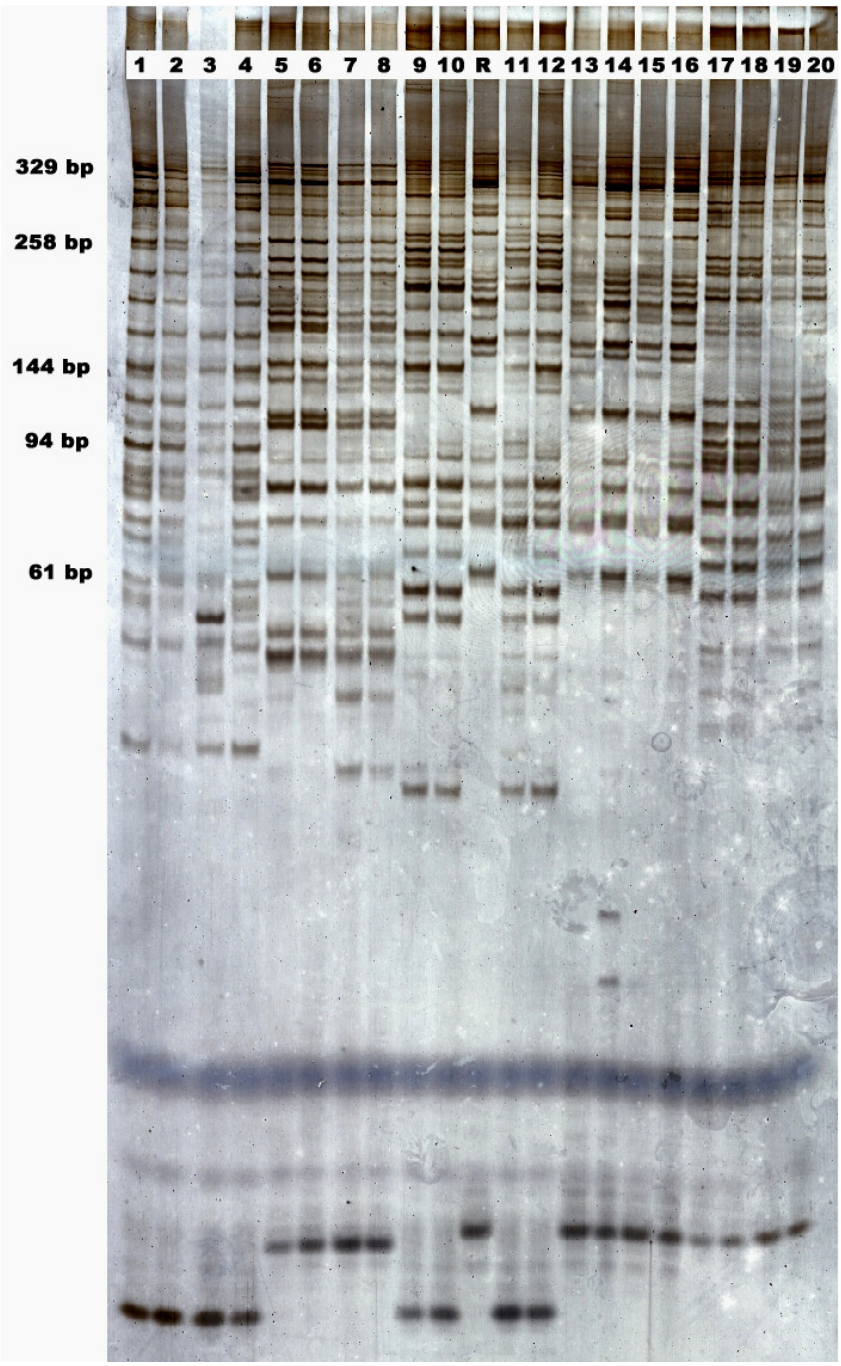


Fig 1. Similarity matrix for four different groundnut cultivars with five selected primers



.**AFLP Gel showing DNA polymorphism. 1=ICGS1 P3, 2= ICGS1 P50, 3= ICGS1 P51, 4= ICGS1 P52, 5= ICGS1 P63, 6=Kadiri3 P3, 7= Kadiri3 P50, 8= Kadiri3 P51, 9= Kadiri3 P52, R= Reference, 10= Kadiri3 P63, 11=ICGS 44 P3, 12= ICGS44 P50, 13= ICGS44 P51, 14= ICGS44 P52, 15= ICGS44 P63, 16=ICGS11 P3, 17= ICGS11 P50, 18= ICGS11 P51, 19= ICGS11 P52, 20= ICGS1 P63**