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Research Article

DNA fingerprinting of groundnut (*Arachis hypogaea* L.) varieties of Tirupati using SSR markers

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Abstract
Unambiguous identification of varieties is important for registration and certification of newly released varieties. Molecular markers are powerful tools, which help in differentiating plant varieties at the DNA level and have been widely used for fingerprinting in a number of crop varieties. In the present study, a set of 12 groundnut varieties released from Regional Agricultural Research Station, Tirupati were fingerprinted employing SSR markers. A total of 300 SSR were screened and fifteen potential markers were employed for fingerprinting of groundnut varieties. The SSR markers generated alleles ranging from 2 to 7 with an average of four per locus. The polymorphism information content (PIC) values ranged from 0 to 0.85. The genotypic data from all the loci provided unique SSR allelic fingerprints which helped in varietal identification of groundnut. Core set of highly informative primers viz., PM 377, TC1A02, TC5A06 and GM1489 identified in this study has the potential to identify most of the groundnut varieties. Cluster analysis using SSR marker grouped 12 groundnut varieties into two major clusters. Finger printing of the groundnut genotypes provide information about phylogenetic relationships and assists groundnut breeders in varietal registration and protection of intellectual property rights.

Key words
Groundnut, SSR markers, DNA fingerprinting, Polymorphism information content, Cluster analysis.

Introduction

Groundnut or peanut (Arachis hypogaea L.) is an important legume and oilseed crop cultivated globally in 20.9 million hectares with a production of 34.7 million tonnes (FAOSTAT, 2012). About one-third of the groundnut produced globally is eaten and two-thirds are crushed for oil. India, China and the USA are the major groundnut producing countries in the world and India has been contributing a significant share occupying first place in area and second place next to China in production (Madhan Mohan and Nigam, 2013.). In India, 70-75% of the groundnut area (4.19 m ha) and production (5.62 m tonnes) is concentrated in four states i.e., Gujarat, Andhra Pradesh, Tamil Nadu and Karnataka ((Madhusudhana, 2013). The genetic variability in groundnut is low due to origin of the crop through a single hybridization event between two diploid species followed by chromosome doubling (amphidiploid) and crossing barriers with wild diploid species (Kochart *et al*., 1996).

Ever increasing demand of high performance varieties restricted breeders to recurrently employ limited elite lines as parents to develop high yielding varieties suitable for diverse agroclimatic zones and consumer preference (oil or direct seed consumption). Unambiguous identification of varieties is important for registration and certification of varieties to curb supply of spurious seed and avoid selling same variety in different names by seed production agencies (Korir *et al*., 2012). In groundnut, substantial amount of diversity exists for morphological, physiological and agronomic traits which are being used to identify cultivars. Traditionally, variety identification involves DUS (Distinctness, Uniformity and Stability) testing system based on morphology and traditional phenotypic evaluation following crop specific descriptors (IPGRI, 1991). As more and more number of cultivars are being developed from few elite parents, it becomes more difficult to distinguish varieties solely based on morphological characters. This necessitated the development of an authentic method for varietal identification viz., DNA fingerprinting to protect plant varieties by appropriate characterization and documentation.

Compared with other crops, groundnut is lagged behind in molecular characterization and the reason being meagre polymorphism detected by protein or DNA based markers like RAPD and AFLP (Paik-Ro *et al*., 1992; Stalker *et al*., 1994). The analysis of simple sequence repeat (SSR) loci is the current method of choice for fingerprinting groundnut as the allelic diversities are extremely high in SSR markers (He *et al*., 2003). SSRs are ideal markers for fingerprinting in groundnut as they are hyper variable, multi-allelic, distributed throughout the genome in high abundance (Ahmed *et al*., 2013). These markers have been shown to be highly informative because of their variability, ease of use,

accessibility, reproducibility and co-dominant with greater informativeness, (Jannati *et al*., 2009). SSR markers have been widely employed for varietal finger printing in diverse crops like Cereals (Bryan *et al*., 1997; Rahman *et al*., 2009), oil seeds (Poljuha *et al*., 2008; Qu *et al*., 2012), fiber crops (Ahmed *et al*., 2013), fruits (Guilford *et al*., 1997; Huang *et al*., 1998; Korkovelas *et al*., 2008; Zhang *et al*., 2012) and vegetables (Arens *et al*., 1995; Becher *et al*., 2000).

The major objective of the study work was the identification of highly informative SSR markers which can be employed for fingerprinting groundnut. In the present study groundnut varieties released from Regional Agricultural Research Station, Tirupati of Acharya N. G. Ranga Agricultural University and SSR primers were used.

Material and methods

Plant material: A total of twelve varieties released over a period of 25 years from Regional Agricultural Research Station, Tirupati (Table 1) were used for developing DNA fingerprints with SSR markers (Vasanthi et al., 2012). Ten seeds from each variety were sown in pots and were grown in glass house. Tender leaf samples from ten days old seedlings were collected for genomic DNA isolation and stored at -20ºC.

Genomic DNA isolation and quantification: Genomic DNA was isolated by CTAB method of Doyle and Doyle (1990) with minor modifications. Five grams of leaf tissue was ground to very fine powder in liquid nitrogen and dispensed in pre warmed DNA extraction buffer (100mM Tris-Cl; 20mM EDTA; 1.4 M NaCl, 2% CTAB, 1% poly vinyl pyrolidone and 0.1% β- mercaptoethanol; pH 8.0) incubated at 60ºC for one hour with intermittent swirling. The supernatant was emulsified with equal volumes of phenol: chloroform (1:1 v/v). Following centrifugation, the supernatant was extracted twice with chloroform: isoamyl alcohol (24:1 v/v). DNA was precipitated by adding 0.6 volumes of chilled isopropanol and 0.1 volumes of 3M sodium acetate and incubated at 4ºC for 1 hour. The DNA pellet was washed with 70% ethanol twice, air died and dissolved in appropriate volume of TE (10mM Tris and 1mM EDTA) buffer. RNA was removed by RNase treatment at 37ºC for 1 hour. The quality of the DNA was checked on 0.8% agarose gels after staining with ethidium bromide and quantified by nanodrop spectrophotometer (Thermoscientific, ND1000). DNA was diluted with autoclaved milliQ water to a working concentration 20 ng/µl and was subsequently used for SSR analysis.

DNA amplification and visualization of DNA profiles: PCR amplification was carried out in a reaction volume of 10 µl containing 1 µl of 10x assay buffer, 2mM of MgCl2, 60ng of template DNA, 0.2 mM of each dNTPs, 10 ρm each of forward and reverse SSR primers, 0.3U of Taq polymerase and suitable amount of sterile deionized water. All PCR amplifications were performed in a thermal cycler (Eppendorf Vapo. Protect) with the thermal profile of initial denaturation at 94ºC for 3 min followed by 35 cycles of denaturation at 94ºC for 15 sec, primer annealing at specific annealing temperature of each primer for 30 sec and extension at 72ºC for 45 sec and a final extension at 72ºC for 10 min. Prior to electrophoresis, the density of PCR products was increased by adding loading dye (0.25% xylene cynol, 0.25% bromophenol blue and 30% glycerol) which assists in proper placement of the contents in the wells and helps in monitoring the progress of electrophoresis. The PCR amplification products were separated on 9% polyacylamide gels containing 19:1 Acrylamide: Bis-acrylamide; 1.5X TBE buffer (89 mM Tris, 89mM Borate and 2mM EDTA); 10% Ammonium per sulphate and 130 µl TEMED. Electrophoresis (Genei) was carried out in 1x TBE buffer at 120V for 3 hours using the power supply (Major Science MP-300V) and stopped after the bromophenol blue dye reached the bottom of the gel. Gels were stained with ethidium bromide as per the protocol of Amaravathi et al. (2008) and DNA profiles were captured by exposing gels to UV light (Alphainnotech).

SSR markers selection for fingerprinting, allele scoring and data analysis: Initially, 300 SSR markers available in the public database were selected and analyzed *insilico* by BLAST search of the EST for the primer binding sites and PCR product size and thereby the expected allele size (Amaravathi et al., 2014). These markers were subsequently screened with two varieties viz., Narayani and Tirupati-3 and the size of the intensely amplified band was determined based on the relative migration of DNA relative to the known molecular weight size marker (100bp ladder). Finally 15 potential SSR markers were selected for fingerprinting based on the PCR amplification and correspondence of allele size estimated *insilico* (Table 4). The size of the alleles for each marker was estimated using the software alphaimager analytical tool. For data analysis, each band was defined as a single character. The alleles were scored and converted into '1' and '0' matrix of which '1' indicated the presence and '0' indicated absence of the allele and thereby developed a binary digit format for 15 SSR markers included in this study. Polymorphism information content (PIC) of each SSR marker was

calculated using the formula (Anderson et al., 1993)

$$\text{PIC} = 1 - \sum_{i=1}^{k} \text{pi}^2$$

where k is the total number of alleles (bands) detected for one SSR locus and p is the proportion of the cultivars or genotypes containing the allele (band) in all the samples analysed. The genetic distance for all pair wise combinations of groundnut varieties were calculated using Jaccad's similarity coefficient (Jaccard, 1908). The cluster analysis was based on the average linkage between groups and dendrogram was constructed using software SPSS (ver. 20, IBM software 2009; Norusis, 2004).

Results and discussion

Identification of informative SSR markers for fingerprinting of groundnut varieties: Fifteen SSR markers were used for fingerprinting, out of which four were monomorphic and the remaining 11 markers generated 44 polymorphic loci (Table 2). Representative fingerprint pattern of twelve groundnut varieties with SSR markers IPAHM 23 and PM377 were shown in Figure 1. The polymorphic primers generated alleles ranging from 2 to 7 with an average of four per locus. Highest number of seven alleles were detected with TC1A02 while it was lowest of one allele with Seq14H6-1. Mondal and Badigannavar (2009) characterized groundnut genotypes with 20 polymorphic SSR markers with an average of 4 alleles per locus. In contrast, the average number of polymorphic bands for the SSR markers was high when few highly polymorphic SSRs were employed for groundnut characterization (Guohao *et al.,* 2003; Molla *et al.,* 2010). When one primer would not distinguish individual variety from others, additional markers needed to be tested to get unique profiles. All the 12 genotypes were successfully discriminated among themselves with 11 SSR markers. The genotypic data from all the loci provided unique SSR allelic fingerprints which help in varietal identification of groundnut (Fig. 2). The banding pattern corresponding to groundnut genotypes greatly helps to recognize the variety under test by simply comparing the banding pattern produced with corresponding SSR marker. The groundnut fingerprint data can unambiguously distinguish one individual from another with much higher probability than DUS characters alone. Recently, varietal fingerprinting is mandate for variety registration which helps in avoiding misappropriation and for protection of plant breeder's and farmers' rights.

In the present study we found eight unique alleles with markers Seq16G8, Seq5D5, GM 1489, PM377, PM36 and TC5A06 specific to six groundnut varieties viz., Bheema, Tirupati-3, Tirupati-4, Narayani, Prasuna and Tirupati-1 (Fig. 2). The SSR markers Seq16G8 produced unique alleles for Tirupati 3 (195bp) and Prasuna (230bp). Similarly, GM 1489 amplified unique allele for Bheema (520bp). In contrast, the fingerprint pattern with SSR markers Seq5D5 and PM3 were unique in Dharani with respect to allele at 267bp and 225 bp respectively. In Tirupati 2, an allele at 230 bp with SSR marker PM3 and in Tirupati 4, an allele at 240 bp with SSR marker PM36 were absent. If these alleles were confirmed as unique over a broader range of groundnut genotypes, then these cultivar specific unique alleles can be used for identification of these varieties directly. This study also supported the fact that genotypes with most distinct DNA profiles were likely to contain greater number of novel alleles.

Polymorphism information content of SSR markers: Polymorphism information content (PIC) provides an estimate of the discriminatory power of the marker taking in to account both the number and relative frequency of the alleles (Smith et al., 1998) and the values ranged from 0 (monomorphic) to 1 (Highly polymorphic). In this study, PIC values ranged from 0 to 0.85. PIC values were 0 for four primers (TC2E11, PM15, PM45 and Seq14H6-1) which are monomorphic in all groundnut varieties analyzed. PIC values in polymorphic markers ranged from 0.49 (PM384) to 0.85 (TC1A02) with 0.67 as average (Table 2). PIC values in the present study were lower than the previous reports of Guohao *et al.* (2003); Molla *et al.* (2010). This might due to limited number of genotypes screened which mostly have one of the parents in common in their parentage, varietal difference and varying climatic conditions which trigger genetic variations at DNA level. The polymorphic markers with high PIC (>0.5) have been regarded as highly informative markers (Zhang *et al.*, 2012). Out of 15 SSR markers, 10 of them displayed PIC values above 0.5 (Table 2). PIC information along with number of alleles provides reliable information on highly informative SSR markers for universal fingerprinting and estimation of genetic diversity (Ahmed *et al.,* 2013). Therefore, only 15 SSR markers were employed for DNA profiling which were found polymorphic in the initial screening with two varieties. Based on the level of polymorphism detected by individual marker, four most informative SSR markers (PM 377, TC1A02, TC5A06 and GM1489) were identified which displayed very high PIC values (Table 2). This set of markers had the enormous potential to identify most of the groundnut genotypes. Minimum number of informative SSR markers was exploited for discriminating different crop varieties by many researchers (Molla *et al.,* 2010; Hameed *et al.,*

2012; Ahmed *et al.,* 2013). The core set of SSR markers identified in the present study are highly useful in discrimination of varieties bred from Tirupati can be employed for genotyping varieties bred in other stations and thereby the efficiency of the markers can be further verified.

Genetic diversity analysis of groundnut genotypes: The genotypic data was converted to binary digit code format and genetic similarity was estimated which ranged from 0.89 to 0.45 (Table 3). Determination and estimation of genetic similarity was eased by pair wise comparison. Highest similarity was found between Rohini and Tirupati 4 (0.89) which corresponds well with the pedigree information (Table 1) as Tirupati 4 is the female parent of Rohini. Maximum diversity was evident between Tirupati 3 and Prasuna displaying genetic similarity of only 0.45. Cluster analysis based on similarity values classified groundnut genotypes in to two major clusters (Fig. 3). The first major group consisted of eight varieties where as three varieties were included in the second group. The first major group was further sub-clustered in to two groups i.e., IA and IB with five and three genotypes respectively. The rest three were grouped in II cluster whereas Tirupati 3, a Virginia bunch variety, was most divergent and did not group with any of the other varieties. Hence it can be concluded that the cluster of genotypes as per the dendrogram constructed using SSR markers data was in close agreement with the pedigree relationship of the varieties.

Based on the foregoing discussion, it may be concluded that, a set of informative SSR markers were identified which can help in the identification of groundnut. The present study also clearly demonstrated the usefulness of SSR markers for DNA finger printing.

References

Ahmed, M.M., Guo, H., Huang, C., Zhang, X. And Lin, Z. 2013. Selection of core SSR markers for fingerprinting upland cotton cultivars and hybrids. Australian J. Crop Sci., **7**(12): 1912-1920.

Amaravathi, Y. Singh, R., Singh, A.K., Singh,V.P., Mohapatra, T., Sharma, T.R. and Singh, N.K. 2008. Mapping of Quantitative trait locifor basmati grain quality traits in rice (*Oryza sativa* L.). *Mol. Breed*., **21**(1): 49-65.

Amaravathi, Y. Sai Sruthi V and Vasanthi R.P. 2014. Application of Bioinformatics in groundnut genomics. In 5th National seminar on Bioinformatics.

Anderson, J.A., Churchill, G.A., Sutrique, J.E., Tanksley, S.D. and Sorrells, M.E. 1993. Optimizing parental selection for genetic linkage maps. *Genome*, **36**: 181-186.

Arens, P., Bredemeijer, G., Smulders, M. and Vosman, B. 1995. Identification of tomato varieties using microsatellites. *Acta. Hort*., **412**: 49–57.

Becher, S.A., Steinmetz, K., Weising, K., Boury, S., Peltier, D., Renou, J.P., Kahl, G. and Wolff, K. 2000. Microsatellites for cultivar identification in pelargonium. *Theor. Appl. Genet.*, **101**: 643–651.

Bryan, G.J., Collins, A.J., Stephenson, P., Orry, A., Smith, J.B. and Gale, M.D. 1997. Isolation and characterisation of microsatellites from hexaploid bread wheat. *Theor. Appl. Genet.,* **94**:557–563.

Doyle, J.J. and Doyle, J.L. 1990 Isolation of plant DNA from fresh leaf tissue. *Focus*, **12**: 13-15.

FAOSTAT. 2012. Statistical databases. (http://www.fao.org).

Guilford, P., Prakash, S., Zhu, J.M., Rikkerink, E., Gardiner, S., Bassett, H. And Forster, R. 1997. Microsatellites in *Malus x domestica* (apple): abundance, polymorphism and cultivar identification. *Theor. Appl. Genet*., **94**: 249–254.

Guohao, H., Meng, R., Newman, M., Gao, G., Pitman, R. and Prakash, C.S. 2003 Microsatellites as DNA markers in cultivafte peanut (*Arachis hypogaea* L.). *BMC Pl. Biol.,* **3** (3): 1-6.

Hameed, U., Pan, Y.B., Muhammad, K., Afghan, S. and Iqbal, J. 2012. Use of simple sequence repeat markers for DNA fingerprinting and diversity analysis of sugarcane (*Saccharum* spp) cultivars resistant and susceptible to red rot. *Genet. Mol. Res.,* **11**:1195-1204.

He, G., Meng, R., Newman, M., Gao, G., Pittman, RN. And Prakask, C.S. 2003. Microsatellites as DNA markers in cultivated peanut (*Arachis hypogaea* L.). *BMC plant boil.,* http://www.biomedcentral.com/1471-2229/3/3.

Huang, W.G., Cipriani, G., Morgante, M. and Testolin, R. 1998. Microsatellite DNA in Actinidia chinensis: isolation, characterisation, and homology in related species. *Theor. Appl. Genet.,* **97**: 1269–1287.

Jaccard, P. 1908 Nouvelles researchers sur la distribution florale Société. *Vaudoise des Sci. Naturelles*, **44**:22-27.

Jannati, M., Fotouhi, R., Pourjan, A.and Salehi, A.Z. 2009. Genetic diversity analysis of Iranian citrus varieties using microsatellite (SSR) based markers. *J. Hortic. For.*, **1**: 120–125.

Kochart, G., Stalker, H.T., Gimenes, M., Galgaro, L., Lopes, C.R., and Moore, K. 1996. RFLP and cytogenetic evidence on the origin and evolution of allotetraploid domesticated peanut *Arachis hypogaea* (Leguminosae). *Am. J. Bot.,* **83**: 1282-1291.

Korir, N.K., Han, J., Shangguan, L., Wang, C., Kayesh, E., Zhang, Y. And Fang, J. 2012. Plant variety and cultivar identification: advances and prospects. Critical reviews in biotech. Early online:1-15 DOI: 10.3109/07388551.2012.675314.

Madhan Mohan M and Nigam SN. 2013. Principles and practices for groundnut seed production in India. *Information Bull.,* **94**: 64 pp. ISBN 978-92-9066-556-4.

Madhusudhana, B. 2013. A Survey on Area, Production and Productivity of Groundnut Crop in India. *IOSR J. Economics and Finance*, **1**(3): 1-7 www.iosrjournals.org.

Molla, M.R., Islam, M.N., Rohman M.M. and Rahman L. 2010. Microsatellite allele size profiling to determine varietal identity and genetic diversity among groundnut varieties in Bangladesh. *Nature and Sci.,* **8**(12): 123-129. http://www.sciencepub.net.

Mondal, S.and Badigannavar, A.M. 2009. Molecular diversity and association of SSR markers to rust and late spot resistance in cultivated groundnut (*Arachis hypogaea* L.). *Plant Breed.,* (online), doi:10.1111/j.1439-0523.2009.01635.

Norusis, M. 2004. *SPSS 13.0 Statistical Procedures Companion.* Upper Saddle-River, N.J.: Prentice Hall, Inc.

Paik-Ro, O.G., Smith, R.L. and Knauft, D.A. 1992. Restriction fragment length polymorphism evaluation of six peanut species within the *Arachis* section. *Theor. Appl. Genet.,* **84**:201-208.

Poljuha, D., Sladonja, B., Setic, E., Milotic, A., Bandelj, D., Jakse, J. and Javornik, B. 2008. DNA fingerprinting of olive varieties in Istria (croatia) by microsatellite markers. *Scientia Hortic.*, **115**:223-230.

Qu. C., Hasan, M., Lu, K., Liu, L., Liu, X., Xie, J., Wang, M., Lu, J., Odat, N., Wang, R., Chen, L., Tang, Z. and Li, J. 2012. Genetic diversity and relationship analysis of the Brassica napus germplasm using simple sequence repeat (SSR) markers. *Afr. J. Biotechnol*., **11**:6923-6933.

Rahman, M.S., Molla, M.R., Alam M.S. and Rahman, L. 2009. DNA fingerprinting of rice (*Oryza sativa* L.) cultivars using microsatellite markers. *Australian J. Crop Sci.,* **3**(3): 122-128.

Smith, J.S.C. and Register, J.C. 1998 Genetic purity and testing technologies for seed quality: a company perspective. *Seed Sci. Res*., **8**: 285–293.

Stalker, H.T., Phillips, T.G., Murphy, J.P. and Jones, T.M. 1994. Diversity of isozyme patterns in *Arachis* species. *Theor. Appl. Genet*., **87**:746-755.

Vasanthi, R.P., John, K., Mohan Vishnuvardhan, K., Purushotham, M., Sirisha, K., Thejaswi, Y. And Giridhar Krishna T. 2012. In: Groundnut varieties developed/ Released by RARS, Tirupati (AP). Pp.17-26.

Zhang, J., Zhu, W.Y., Xuan, C., Sun, H. and Li, Y. 2012. Molecular fingerprinting and relative relationship of apricot cultivars in China by simple sequence repeat (SSR) markers. *Afr. J. Biotechnol*., **11**:2631-2641.

Table 1. Groundnut varieties from Regional Agricultural Research Station, Tirupati used for DNA fingerprinting

S. No.	Variety	Pedigree	Year of release	Duration (Days)	Season recommended	Salient features
1.	Tirupati 1	Selection from E.C-106983/3	1989	100	Rainy and Post rainy seasons	Spanish bunch variety, Early maturing, drought tolerant
2.	Tirupati 2	GAUG-1 **X** Nc.Ac.FLA.14	1989	100-105	Post rainy season	Spanish bunch variety, Moderately tolerant Kalahasthi malady, suitable for black soils
3.	Tirupati 3	Selection from TMV-10	1991	125-130	Rainy and Post rainy seasons	Virginia bunch variety, red testa colour and resistant to Kalahasthi malady
4.	Tirupati 4	JL-24 **X** Ah316/S	1995	105	Rainy and Post rainy seasons	Spanish bunch Early maturing variety light rose testa, high yielding, tolerant to midseason moisture stress,
5.	Narayani	JL-24/Ah316/S	2002	100	Rainy and Post rainy seasons	Spanish bunch variety with red testa colour, early maturing and drought tolerant
6.	Kalahasti	TCG1709/ TCG1518	2002	105-110	Post rainy season	Spanish bunch variety with red testa colour, early maturing and resistant to Kalahasthi malady
7.	Prasuna	TCG1717/ TCG1518	2006	105-110	Early Rainy and Post rainy seasons	Spanish bunch variety, medium bold seeds and rose testa, tolerant Kalahasthi malady,
8.	Abhaya	K-134 **X** TAG-24	2006	105-110	Early Rainy and Post rainy seasons	Spanish bunch Drought tolerant variety with fresh seed dormancy and late leaf spot tolerant(LLS)
9.	Greeshma	TIR46 **X** JUG37	2009	95-100	Early Rainy and Post rainy seasons	Spanish bunch early maturing variety, LLS tolerant and water use efficient
10.	Rohini	Tirupati4 **X** TIR45	2010	90-95	Early and post rainy seasons	Spanish bunch variety, ultra early and LLS tolerant
11.	Bheema	TAG24 **X** TG19	2010	110-115	Post rainy season	Bold seeded Spanish bunch variety with low oil and high sucrose suitable for table purpose
12.	Dharani	VRI-2 **X** TCGP-6	2012	100-105	Earl, rainy and post rainy seasons	Spanish bunch variety, drought resistant, stem rot tolerant

Table 2. Allele size, frequency and PIC of polymorphic SSR markers in 12 groundnut varieties.

S. No.	SSR marker	No. of Alleles	Size of allele (bp)	Allele frequency	PIC
1	PM3	3	195	0.31	0.67
			215	0.34	
			230	0.34	
2	PM36	3	210	0.48	0.57
			240	0.44	
			255	0.08	
3	PM375	3	100	0.42	0.57
			105	0.08	
			115	0.5	
4	PM377	6	155	0.13	0.80
			170	0.31	
			180	0.23	
			195	0.08	
			230	0.13	
			240	0.13	
5	PM384	2	115	0.58	0.49
			125	0.42	
6	Seq5D5	4	231	0.25	0.67
			245	0.13	
			267	0.46	
			298	0.17	
7	Seq16G8	4	195	0.06	0.60
			206	0.5	
			221	0.37	
			230	0.06	
8	IPAHM23	4	130	0.17	0.71
			145	0.35	
			175	0.13	
			180	0.35	
9	GM1489	4	325	0.34	0.73
			450	0.2	
			520	0.17	
			540	0.28	
10	TC1A02	7	270	0.19	0.82
			275	0.10	
			294	0.19	
			300	0.09	
			334	0.17	
			346	0.14	
			415	0.12	
11	TC5AO6	4	197	0.36	0.72
			213	0.14	
			256	0.23	
			420	0.27	

Table 3. Summary of Jaccard's similarity coefficient values between 12 groundnut (*Arachis hypogaea* L.) varieties for all SSR loci

Groundnut variety	Abhaya	Kalahasti	Dharani	Greeshma	Prasuna	Bhema	Narayani	TPT4	Rohini	TPT3	TPT1	TPT2
Abhaya	1.000											
Kalahasti	.696	1.000										
Dharani	.733	.696	1.000									
Greeshma	.689	.617	.520	1.000								
Prasuna	.810	.689	.652	.644	1.000							
Bhema	.609	.644	.644	.532	.565	1.000						
Narayani	.744	.630	.705	.553	.738	.614	1.000					
TPT4	.682	.574	.609	.636	.714	.522	.775	1.000				
Rohini	.721	.609	.609	.714	.714	.556	.775	.892	1.000			
TPT3	.490	.587	.490	.578	.449	.605	.489	.468	.468	1.000		
TPT1	.609	.574	.480	.714	.674	.458	.614	.707	.750	.568	1.000	
TPT2	.689	.490	.551	.682	.609	.469	.698	.714	.714	.543	.714	1.000

Table 4.Potential SSR markers selected for fingerprinting based on the PCR amplification and correspondence of allele size estimated *insilico*

S.No.	SSR marker	EST	Allele size (insilico) bp	Alleles scored in vivo (by PCR in bp)
1	GM1489	EZ743640	380	325, 450, 520, 540
2	IPAHM23	ER974415	150	130, 145, 175, 180
3	IPAHM356	ER974515	109	122,145, 175
4	PM3	AY237738	190	195, 215, 230
5	PM15	AY237742	101	195, 260
6	PM36	AY237749	200	210, 240, 255
7	PM45	AY237754	90	85, 100
8	PM375	AY310556	102	100, 105, 115
9	PM377	AY310558	164	155, 170, 180, 195, 230, 240
10	PM384	AY310559	105	115, 125
11	Seq5D5	No EST available	-	231, 245, 267, 298
12	Seq14H6	BZ999738	285	285
13	Seq16G08	BZ999888	220	195, 206, 221,230
14	TC1A02	DQ099163	255	270, 275, 294, 300, 334, 346, 415
15	TC5A06	DQ099203	171	197, 213, 256, 420

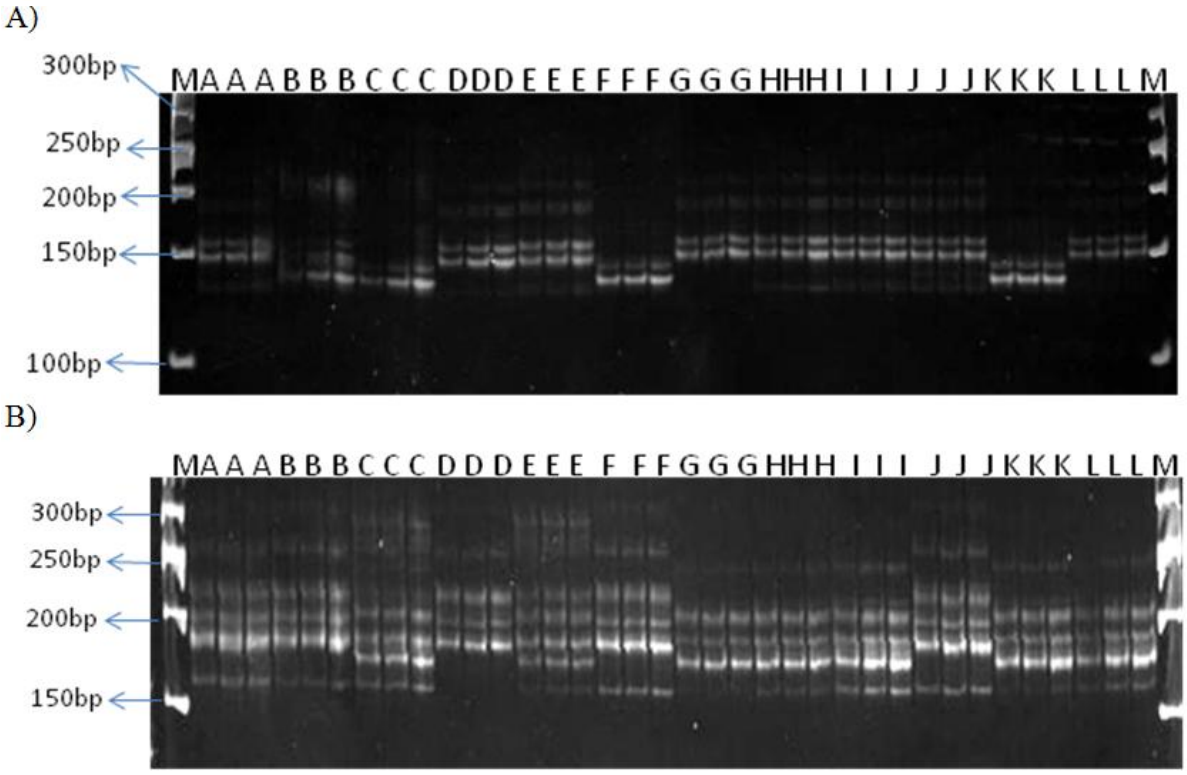

Figure 1: DNA profiles of groundnut cultivars with SSR markers A) IPAHM 23 and B) PM377 where M: 50bp ladder; A: Abhaya; B: Kalahasthi; C: Dharani; D: Greeshma; E: Prasuna; F: Bheema; G: Narayani H: Tirupati-4; I: Rohini; J: Tirupati-3; K: Tirupati-1 and L: Tirupati-2

SSRs	A	B	C	D	E	F	G	H	I	J	K	L
GM1489-2	■	■	■	■	□	□	■	■	■	□	□	□
GM1489-3	□	□	□	□	□	■	□	□	□	□	□	□
GM1489-4	■	■	■	■	■	□	■	■	■	■	■	■
IPAHM23-1	□	■	■	□	□	■	□	□	□	■	□	□
IPAHM23-2	■	□	□	■	■	□	■	■	■	□	■	■
IPAHM23-3	□	□	■	□	□	■	□	□	□	■	□	□
IPAHM23-4	■	□	□	■	■	□	■	■	■	□	■	■
PM3-1	□	■	■	□	□	■	■	□	□	■	□	□
PM3-2	■	■	■	■	■	□	■	■	■	□	■	□
PM3-3	■	■	□	■	■	■	□	■	■	■	■	□
PM15-4	■	■	■	□	□	□	□	■	□	□	■	■
PM15-5	■	■	■	■	■	■	■	■	■	□	■	■
PM36-2	■	■	■	■	■	■	■	□	■	■	■	■
PM36-3	□	□	■	□	□	□	□	■	□	□	□	□
PM377-1	■	■	■	■	■	■	□	■	■	■	■	■
PM377-2	■	■	■	■	■	■	■	■	■	□	□	□
PM377-3	■	■	■	■	■	□	□	■	□	□	□	□
PM377-4	□	□	■	□	□	□	■	■	■	□	■	■
PM377-5	■	■	■	■	□	□	□	■	■	■	□	□
PM377-6	■	■	□	■	■	■	□	□	□	□	□	□
PM375-1	■	□	■	■	■	■	■	■	■	□	■	■
PM375-2	□	■	□	□	□	□	□	□	□	■	□	□
PM384-1	■	□	□	■	■	□	□	■	■	□	■	■
PM384-2	□	■	■	□	□	■	■	□	□	■	□	□
Seq5D5-1	□	□	■	□	□	■	■	■	■	□	□	■
Seq5D5-2	□	■	□	□	■	□	□	□	□	□	■	□
Seq5D5-3	■	■	□	■	■	■	■	■	■	■	■	■
Seq5D5-4	□	■	□	□	■	□	□	□	□	■	■	□
Seq16G8-1	□	□	□	□	□	□	□	□	□	■	□	□
Seq16G8-2	□	■	■	■	■	□	■	■	■	□	■	□
Seq16G8-3	■	■	■	■	□	□	□	□	■	□	■	□
Seq16G8-4	□	□	□	□	■	□	□	□	□	□	□	□
TC1A02-1	■	■	■	□	■	■	■	■	■	□	□	□
TC1A02-2	□	□	□	■	□	□	□	□	□	■	■	■
TC1A02-3	■	■	■	□	■	■	■	■	■	□	□	□
TC1A02-4	□	□	□	■	□	□	□	□	□	■	■	■
TC1A02-5	■	■	■	□	■	■	■	■	■	□	□	□
TC1A02-6	□	□	□	■	□	□	□	■	■	■	■	■
TC1A02-7	■	□	■	□	□	■	■	□	□	□	□	■
TC2E11-3	■	□	■	■	□	□	□	□	□	■	□	■
TC5A06-2	■	■	■	□	■	□	□	■	□	■	■	■
TC5A06-3	□	■	□	■	□	□	□	□	□	□	□	■
TC5A06-4	■	□	■	□	■	□	■	□	□	□	□	■
TC5A06-5	■	■	■	■	■	■	□	□	□	□	□	□
TC5A06-6	■	■	■	■	■	■	■	■	■	■	□	■

Figure 2: Bar-code-like DNA fingerprint pattern generated with 11polymorphic SSR markers after electrophoretic separation of DNA fragments. ■ Presence of allele □ Absence of allele
A: Abhaya; B: Kalahasthi; C: Dharani; D: Greeshma; E: Prasuna; F: Bheema; G: Narayani H: Tirupati-4; I: Rohini; J: Tirupati-3; K: Tirupati-1 and L: Tirupati-2

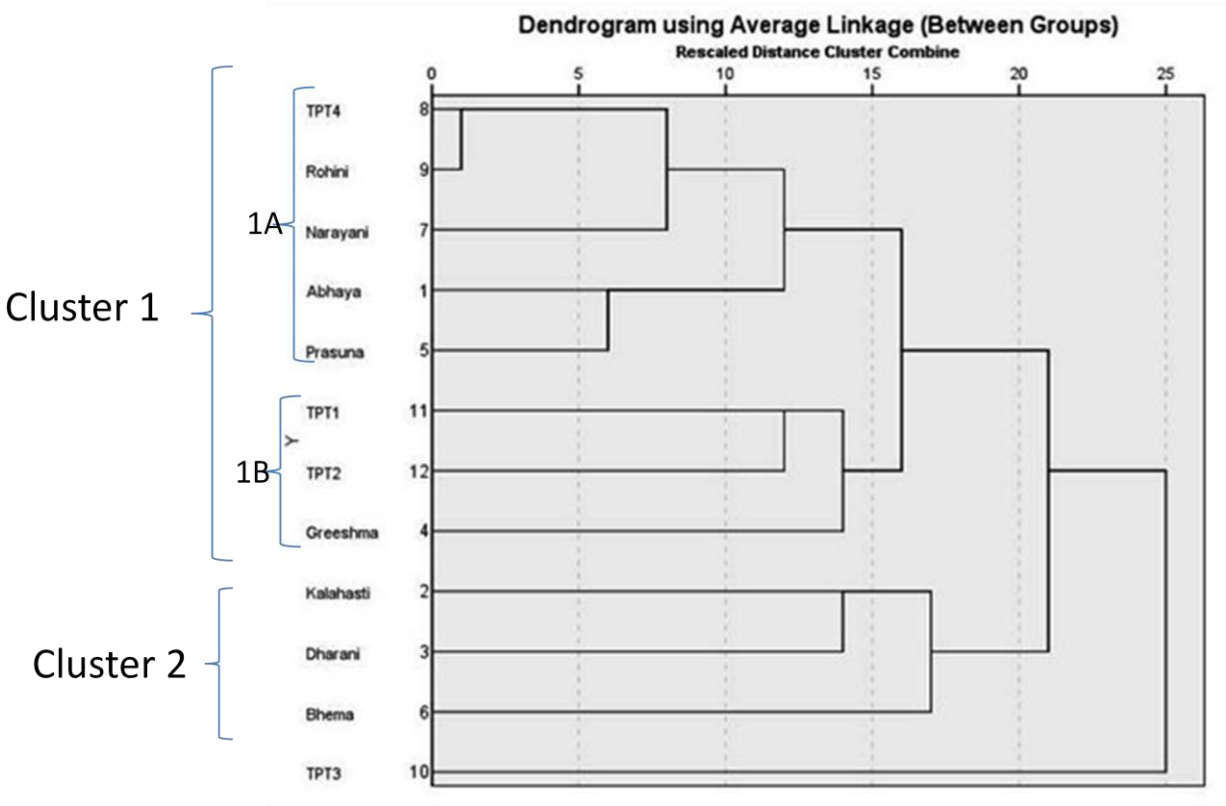

Figure 3: Dendrogram of groundnut cultivars constructed using SPSS software based on 15 SSR markers. The major clusters are indicated in left margin.