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Short Communication

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Genetic Diversity of Groundnut (*Arachis hypogaea* L.) Revealed by RAPD and ISSR Markers

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Abstract: The genetic diversity of twenty-six genotypes of groundnut was evaluated using RAPD (Random amplified polymorphic DNA) and ISSR (Inter-simple sequence repeat) markers. Fifteen RAPD primers produced 58 bands out of which 44 were polymorphic. The average percentage of polymorphism and the polymorphic information content (PIC) for RAPD was calculated as 73.11 and 0.691, respectively while Jaccard's similarity percentage ranged between 63.0-97.0. Thirty-one ISSR primers amplified 98 polymorphic bands out of total 146 bands obtained. In ISSR analysis, average values for fragments per primer, percentage of polymorphism and PIC were 89.87, 65.05 and 0.728, respectively. Jaccard's similarity percentage range for ISSR data was from 66.0-93.0.

Key words: Groundnut, *Arachis hypogaea*, RAPD, ISSR, molecular markers.

Groundnut (*Arachis hypogaea* L.) is a highly self-pollinated, annual legume crop belonging to Family *Fabaceae*, originating from Bolivian region of South America (Kraovickas and Gregory, 1994; Gregory and Gregory, 1976; Belamkar *et al*., 2011). There are about 70 species of groundnut that are grown in more than 80 different countries of Asia, America and Africa and have substantial variability in morphology, geographic distribution and cross ability (Smartt, 1990). Groundnut seeds are also economically important for industries due to their valuable content of essential minerals, vitamins, phytosterol and other phytochemicals (Vyas *et al*., 2014).

Economic importance and narrow genetic base of groundnut enforce scientists to characterize available germplasm against current abiotic and biotic stress based upon its morphological characters and molecular markers. Morphological characters are environmental dependent, are being used regularly since the early days of research. Molecular markers like random amplified polymorphic DNA (RAPD) and inter-simple sequence repeat (ISSR) are simple, speedy, and low-cost polymerase chain reaction (PCR) based techniques for genetic diversity study (Tomar *et al*., 2010). Assessment of genetic diversity in groundnut through RAPD (Pramanik *et al*., 2019; Subramanian *et al*., 2000; Mallikarjuna *et al*., 2005; Mondal *et al*., 2005; Lang and Hang, 2007) and ISSR (Raina *et al*. 2001; Azzam *et al*., 2007; Sutar *et al*., 2009; Tshilenge-Lukanda *et al*., 2012) has been done performed by many researchers. These markers have also been used in other crops like castor (Tomar *et al*., 2014b; Dhingani *et al*., 2012), fenugreek (Tomar *et al*., 2014c), cowpea (Saxena and Tomar, 2020a), coriander (Tomar *et al*., 2014), etc to study the genetic diversity.

The main purpose of the present study is to evaluate available groundnut germplasm through molecular markers for determining their genetic diversity which can be helpful for future planning of groundnut breeding programs.

Materials and Methods

Twenty-six groundnut genotypes were obtained from Main Oilseed Research Station, Junagadh Agricultural University, Junagadh and were utilized for molecular characterization. The details of the genotypes selected and the source of these genotypes have been described in Table 1.

DNA extraction

Two to three young fresh leaves were collected in ice-cooled conditions for obtaining 100 mg samples to isolate total genomic DNA. Total genomic DNA was isolated with the modified Doyle and Doyle (1990) method and stored at 4°C for further use. The quantity and quality of DNA was analyzed spectrophotometrically

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Table 1. List of groundnut genotypes studied for RAPD and ISSR markers

Genotype	Origin	Genotype	Origin
ICGV-91114	ICRISAT, Hyderabad	Dh-101	UAS, Dharwad
R-2001-2	UAS, Raichur	GG-3	JAU, Junagadh
R-2001-3	UAS, Raichur	GG-5	JAU, Junagadh
JL-24	ICRISAT, Hyderabad	GG-7	JAU, Junagadh
Pratap Mungphali-1 (PM-1)	MPAU&T, Udaipur	GG-8	JAU, Junagadh
Pratap Mungphali-2 (PM-2)	MPAU&T, Udaipur	GJG-9	JAU, Junagadh
VG-5	TNAU, Coimbatore	Kadiri-4	ANGRAU, Kadari
AK-159	PKV, Akola	Kadiri-6	ANGRAU, Kadari
TMV-13	TNAU, Coimbatore	Kadiri-9	ANGRAU, Kadari
TPG-41	BARC, Mumbai	Kadiri Harithandhra	ANGRAU, Kadari
TG-37A	BARC, Mumbai	Narayani	ANGRAU, Tirupati
GPBD-4	UAS, Dharwad	Greesma	ANGRAU, Tirupati
GPBD-5	UAS, Dharwad	Abhaya	ANGRAU, Tirupati

at 260 nm and 280 nm. Purity of DNA was also checked by using 1% agarose gel, stained with ethidium bromide, visualized under a UV transilluminator and photographed using the Gel documentation system. The concentration of DNA was calculated by using the formula:

DNA concentration (µg ml^{-1}) = OD at 260 nm x dilution factor x 50 µg ml^{-1}.

The ratio of absorbance at 260 and 280 nm indicates DNA purity. The OD of DNA should be in the range of 1.7-1.8. The samples with the variation in OD range of 1.7-1.8 were treated with either protease K or RNase to remove protein and RNA contamination respectively.

Molecular markers

Twenty six genotypes of groundnut were characterized using random primer. Twenty five RAPD primers (decamer oligonucleotides) were used to check the fidelity of amplification and those that gave the best performance in terms of the number of bands per reaction were selected for further analysis. The list of 15 primers which were used for RAPD based diversity estimation are listed in Table 2. The PCR reaction for RAPD fragments generated from random decamer primers in a 25 µL total reaction volume contained 10× reaction buffer (100 mM Tris-HCl pH 9.0, 15 mM $MgCl_2$ and 500 mM KCl), 0.6 µl of 10mM dNTPs, 1 U of Taq DNA polymerase, 2 µl of 10 pmol primer and 50 ng of genomic DNA. PCR reactions were performed in a thermo cycler with initial denaturation at 94°C for 5 min, followed by 35 cycles of 1 min at 94°C, 45 sec at 44 to 52°C (varied according to primer Tm value), 2 min at 72°C and a final 10 min extension at 72°C.

Table 2. Size, number of amplified bands, percentage of polymorphism and PIC obtained by RAPD primers

RAPD primer	Allele/ Band size	Total no. of Alleles/ Bands (A)	No. of polymorphic Bands (B)	No. of monomorphic Bands (C)	Total no of Amplicons	% of polymorphism (B/A)	PIC value
OPR-09	690-1568	3	2	1	60	66.66	0.657
OPR-10	163-1597	4	3	1	90	75.00	0.741
OPS-08	433-707	2	1	1	44	50.00	0.494
OPT-02	451-2528	4	2	2	36	50.00	0.709
OPT-04	606-3155	3	2	1	41	66.66	0.643
OPT-08	117-879	4	3	1	79	75.00	0.697
OPT-09	491-3474	4	2	2	65	50.00	0.707
OPU-03	431-2014	4	3	1	56	75.00	0.728
OPU-07	294-2274	2	1	1	42	50.00	0.444
OPU-08	276-2214	5	4	1	89	80.00	0.791
OPY-01	273-2556	4	4	0	74	100.00	0.720
OPY-02	422-2286	4	3	1	78	75.00	0.737
OPY-05	227-2560	6	5	1	90	83.33	0.796
OPY-06	280-1880	5	5	0	85	100.00	0.793
OPY-07	300-3066	4	4	0	49	100.00	0.709
Mean		3.86	2.93	0.93	65.20	73.11	0.691
Total		58.00	44.00	14.00	978.00		

Table 3. Size, number of amplified bands, percentage of polymorphism and PIC obtained by ISSR primers

ISSR Primer	Allele/Band size	Total no. of Bands (A)	No. of polymorphic Bands (B)	No. of monomorphic Bands (C)	Total no. of Bands	% of polymorphism (B/A)	PIC value
UBC-809	149-1227	5	4	1	95	80.00	0.791
UBC-810	396-1965	6	5	1	109	83.33	0.811
UBC-811	374-5000	8	6	2	91	75.00	0.814
UBC-812	162-760	5	3	2	103	60.00	0.781
UBC-822	649-4401	5	4	1	75	80.00	0.738
UBC-823	1376-2027	3	1	2	71	33.33	0.660
UBC-825	255-1880	5	3	2	83	60.00	0.592
UBC-826	503-3366	6	2	4	118	33.33	0.819
UBC-836	438-3102	5	4	1	104	80.00	0.735
UBC-840	341-3197	4	4	0	58	100.00	0.725
UBC-841	273-1405	4	3	1	71	75.00	0.727
UBC-843	464-3280	4	3	1	72	75.00	0.696
UBC-845	200-1176	6	5	1	144	83.33	0.832
UBC-846	548-3471	5	3	2	102	60.00	0.781
UBC-847	174-4286	8	6	2	178	75.00	0.873
UBC-853	118-1828	4	2	2	98	50.00	0.749
UBC-855	674-3254	2	1	1	49	50.00	0.498
UBC-857	524-2286	5	4	1	100	80.00	0.735
UBC-859	180-1981	6	3	3	135	50.00	0.827
UBC-864	197-2307	3	1	2	70	33.33	0.657
UBC-873	421-1256	2	1	1	41	50.00	0.464
UBC-884	436-1282	4	3	1	63	75.00	0.710
UBC-885	459-4080	6	5	1	103	83.33	0.799
UBC-886	464-1951	5	4	1	76	80.00	0.742
UBC-887	373-2536	5	3	2	90	60.00	0.765
UBC-890	432-1683	6	4	2	110	66.66	0.814
UBC-891	231-1496	5	3	2	102	60.00	0.789
UBC-895	506-4767	4	3	1	57	75.00	0.682
UBC-896	626-2308	4	2	2	89	50.00	0.742
ISSR-5	379-754	2	1	1	44	50.00	0.483
ISSR-10	225-1597	4	2	2	85	50.00	0.735
Mean		4.71	3.16	1.54	89.87	65.05	0.728
Total		146.00	98.00	48.00	2786.00		

Eighty three ISSR primers of UBC series (The University of British Columbia, Vancouver, Canada) were screened and out of them 31 primers which gave a greater number of polymorphic bands were taken for analysis (Table 3). The PCR reaction for ISSR was carried out in 20 µl volume containing 1µl DNA (40 ng), 10× reaction buffer (100 mM Tris–HCl pH 9.0, 15 mM $MgCl_2$ and 500 mM

KCl), 0.6 µl of 10 mM dNTPs and 1 unit of Taq DNA polymerase. The PCR reaction consisted of an initial denaturation step at 94°C for 5 min, followed by 35 cycles of 1 min at 94°C (denaturation), 1 min at a specific annealing temperature based on the Tm value of the primes and 2 min at 72°C (extension) followed by a final extension step at 72°C for 5 min.

The complete amplified reaction was loaded on 1.4 and 1.6% agarose gel for RAPD and ISSR respectively, containing ethidium bromide (0.5 µg ml^{-1}) and 50 bp DNA ladder. Gel was run at 120V for 2 hrs to separate amplified products which were visualized and documented using gel documentation system (Syngene).

Data analysis

The RAPD and ISSR banding pattern were scored; for present it was marked 1 and for absence 0 was noted to obtain binary data format for analysis by NTSYS-pc (Numerical Taxonomy and Multivariate Analysis for Personal Computer, Exeter Software) (Rohlf, 1992). Jaccard's similarity coefficient was used to estimate genetic similarity between genotypes (Jaccard, 1908) while UPGMA (Unweighted pair-group method with arithmetic average) was utilized for RAPD and ISSR based dendrogram construction (Sneath and Sokal, 1973).

Results and Discussion

Fifteen RADP primers amplified band size ranging from 117-3474 bp (Table 2). RAPD primers produced 978 amplified fragments for 58 bands out of which 44 were polymorphic. The average number of amplicons, number of alleles per band and number of polymorphic bands were 65.2, 3.86 and 2.93, respectively. Average percentage of polymorphism and polymorphic information content (PIC) was calculated as 73.11 and 0.691, respectively. The highest number of bands were found for RAPD primer OPY-05 (5) while OPS-08 and OPU-07 (2) gave the lowest number of bands. RAPD primer, OPY-05 and OPY-06 produced maximum polymorphic bands while OPS-08 and OPU-07 produced minimum polymorphic bands. RAPD primer, OPR-10 and OPY-05 generated 90 fragments which was highest while OPT-02 gave the lowest (36). In the case of the percentage of polymorphism, RAPD primer OPY-01, OPY-06 and OPY-07 gave 100% polymorphism; whereas 50% polymorphism was found for RAPD primer OPS-08, OPT-02, OPT-09 and OPU-07. RAPD primer OPY-05 was estimated with highest PIC value of 0.796 while primer OPU-07 showed least PIC of 0.444. Kumari *et al*. (2009), Arulbalachandran *et al*. (2009) and Vyas *et al*. (2014) depicted less than 60.00 % of polymorphism rate using RAPD primers, in the present study it was 73.11% showing the efficacy of RAPD primers for depicting the genetic divergence in groundnut.

Dendrogram constructed using RAPD data showed Jaccard's similarity percent range from 63.0 to 97.0 (Fig. 1). Genotype AK-159 and TG-37A indicated highest similarity of 97.0% while TMV-13 and Dh-101 were least similar (72.00%). Kumari *et al*. (2009), Arulbalachandran *et al*. (2009) and Vyas *et al*. (2014) reported 88.00-98.00%, 62.10-78.50% and 76.00-94.00% genetic similarity range, respectively which is narrow

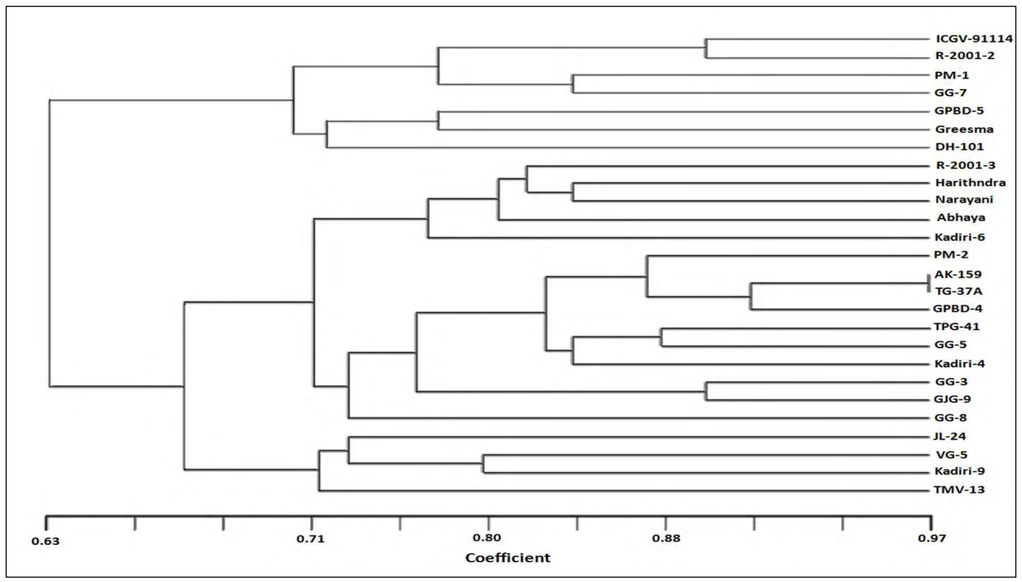

Fig. 1. Dendrogram depicting the genetic relationship among 26 groundnut genotypes based on the RAPD marker.

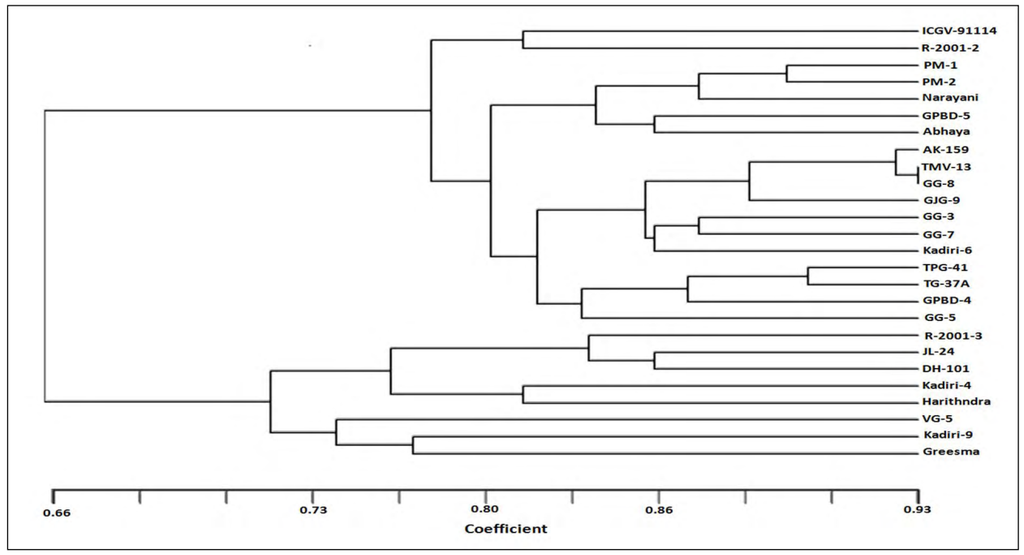

Fig. 2. Dendrogram depicting the genetic relationship among 26 groundnut genotypes based on the ISSR marker.

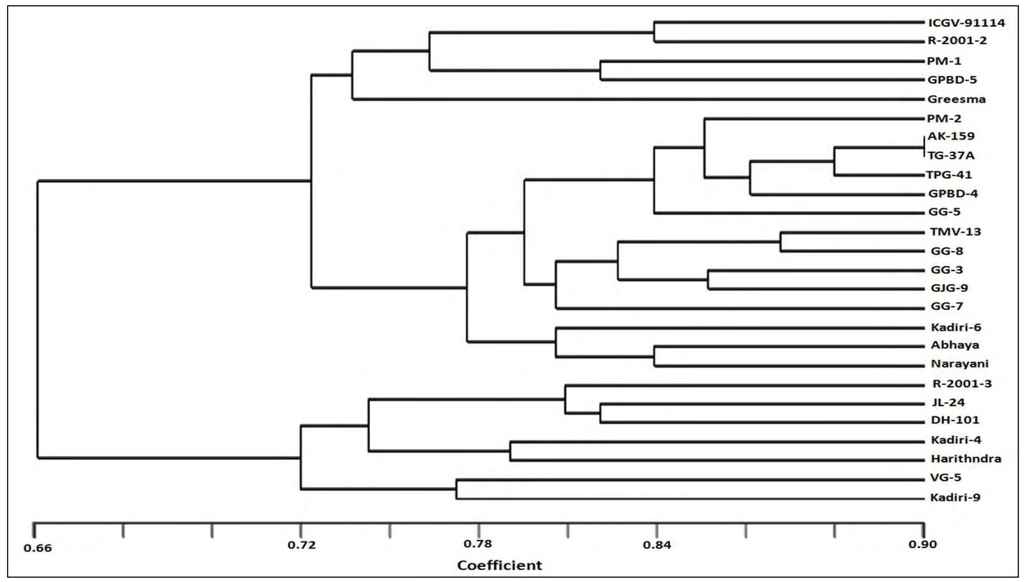

Fig. 3. Dendrogram depicting the genetic relationship among 26 groundnut genotypes based RAPD and ISSR marker.

as compared to present findings of similarity range in the crop.

Thirty-one ISSR primers gave variable band size ranging from 118-5000 bp with 98 polymorphic bands out of the 148 bands obtained (Table 3). Total numbers of amplified product were 2786 with an average of 89.87 fragments per primer. The average values for percentage of polymorphism and PIC was 65.05 and 0.728, respectively. ISSR primers, UBC-811 and UBC-847 generated highest number of total bands (8) as well as polymorphic bands (6) while UBC-855, UBC-873 and ISSR-5 produced lowest number of bands (2) and polymorphic bands (1). ISSR primer, UBC-823 and UBC-864 also gave lowest number of polymorphic bands, while the lowest percentage of polymorphism (33.33%) was with UBC-826. Primer, UBC-840 showed 100% polymorphism. Maximum PIC value was calculated for primer UBC-847 (0.873) while was minimum for UBC-873 (0.464).

Two main clusters were obtained during dendrogram construction from ISSR data, having Jaccard's similarity per cent ranging from 66.0-93.0 (Fig. 2). TMV-31 and GG-8 showed maximum similarity (93.00%) while least was calculated for VG-5 (85.00%). When pooled molecular marker data (RAPD and ISSR) were utilized to construct the dendrogram, the Jaccard's similarity per cent ranged from 66.0 to 90.0 (Fig. 3). The highest similarity percentage was observed between genotype AK-159 and TG-37A (93.0). Azzam *et al*. (2007) found almost similar range for genetic similarity among the groundnut genotypes.

Groundnut germplasm can be molecularly characterized using molecular markers. RAPD and ISSR are dominant markers, easy to use and reproducible. Genetic differences among genotypes can help in identification of diverse genotype for recombination breeding.

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