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Assessment of the Genetic Diversity of Groundnut (*Arachis hypogaea* L.) Using SSR Markers

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ABSTRACT

The experiment was conducted during the *kharif*-2021 season (June–October, 2021) at the ICAR-Directorate of Groundnut Research, Junagadh, Gujarat, India, to assess genetic diversity in groundnut (*Arachis hypogaea* L.) using Simple Sequence Repeat (SSR) markers. The study revealed significant genetic variation, which was crucial for effective breeding and conservation strategies. Across markers, 2 to 10 alleles were identified, with an average of 4.63 alleles per marker, indicating a broad genetic base. Among the genotypes, DGR_D1 exhibited the highest genetic diversity (0.8425) and Shannon information index (1.983), making it valuable for enhancing variability in breeding programs. In contrast, DGR_D73 showed the lowest diversity (0.1017) but recorded the highest major allele frequency (0.9462), indicating limited variability. Heterozygosity ranged from 0 to 1, with DGR_D46 achieving the maximum observed heterozygosity (1.0), reflecting its genetic uniqueness.Regional analysis revealed significant patterns of genetic differentiation. Punjab and Haryana exhibited the greatest differentiation, suggesting high variability between these regions, while Maharashtra and Tamil Nadu populations were genetically similar, reflecting limited regional variation. These findings underscored the effectiveness of SSR markers in assessing genetic diversity and guiding breeding efforts. By identifying genetically diverse genotypes and understanding regional variations, the study provided a foundation for targeted breeding programs, conservation strategies, and the improvement of groundnut resilience, ultimately contributing to enhanced productivity and sustainable cultivation.

KEYWORDS: Genetic diversity, molecular marker, groundnut, population structure, heterozygous

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1. INTRODUCTION

Peanut (*Arachis hypogaea* L.) was a cleistogamous, self-pollinating, allotetraploid (4×=40) legume native to South America. It was extensively cultivated in tropical and subtropical regions for its dual purpose as a food source and oilseed crop. Recognized as the sixth most important oilseed globally, groundnut was valued for its high oil content (45–50%) and digestible protein levels (25–30%) (Namrata et al., 2016; Dhakar et al., 2017). The oil, rich in monounsaturated fatty acids (MUFA), such as oleic acid, and polyunsaturated fatty acids (PUFA), such as linoleic acid, was found in an optimal ratio that made it stable, nutritious, and deserving of its title, "King of Oilseed" (Rani, 2017; Gantait et al., 2017; Wang, 2018). Additionally, groundnut served as an excellent source of vitamins, minerals, and antioxidants, further enhancing its nutritional profile and economic importance in various countries.

Molecular markers had been widely used for plant genetic diversity and population genetics studies, which were essential for breeding and crop improvement, conservation, protection, introduction, and reintroduction of endangered and valuable plants (Tikendra et al., 2019; Hussain et al., 2020; Tuvesson et al., 2021). These markers enabled the identification of new plant varieties and detected genetic changes from known ones, providing valuable insights into the existing genetic variations within and between plant populations (Amom et al., 2023). Genetic diversity played a vital role in enabling plants to adapt and adjust to environmental changes (Pereira et al., 2019). The responses of plants and their adaptive abilities to climate change depended on their genetic diversity levels (Apana et al., 2021). Molecular markers offered essential insights into the variation in plant genetic composition and population structures, thereby playing vital roles in optimizing plant utilization and ensuring effective management (Gyani et al., 2020). These markers had become indispensable in modern plant breeding programs aimed at enhancing stress tolerance, disease resistance, and yield improvement.

A crop's evolutionary past and potential for future evolution were both reflected in its genetic structure (Minnaar-Ontong et al., 2021). Groundnut genetic characterization was essential for evaluating diversity, conserving germplasm, and enabling marker-assisted selection. Since molecular indicators were unaffected by gene interactions and environmental changes, they provided a more reliable approach than morphological markers (Zeinalzadeh-Tabrizi et al., 2018). In groundnut breeding, where precise paternal line identification was crucial for fruitful breeding results, molecular markers were just as significant (Tang et al., 2007; Hong et al., 2021). Marker-assisted selection (MAS) had proven particularly beneficial in selecting desirable traits such as high oil content, early maturity, and resistance to biotic and abiotic stresses.

One benefit of using molecular biology techniques was that they could detect diversity at the gene level, which laid the groundwork for assessing the importance of preserving genetic resources within or between species. These techniques addressed the shortcomings of phenotypic-based evaluations of genetic diversity. SSR markers were crucial instruments for researching genetic diversity and creating linkage maps because of their wide genomic distribution, high polymorphism, and reproducibility when compared to other markers (Thanh et al., 2023).

Simple Sequence Repeats (SSRs) had emerged as a valuable molecular tool in addressing these challenges, owing to their co-dominant nature, reproducibility, and genome-wide distribution (Collard et al., 2005). However, these studies employed markers derived from related species in the *Vigna* genus, with limited markers specific to Bambara groundnuts. By expanding the repertoire of SSR markers, researchers gained deeper insights into the genetic structure of groundnut populations, improving breeding strategies and conservation efforts. The future of groundnut genetic studies lay in integrating next-generation sequencing technologies with marker-assisted selection, allowing for precise and efficient identification of desirable traits.

2. MATERIALS AND METHODS

The experiment was conducted during *kharif*-2021 (June-october-2021) at ICAR-directorate of groundnut research, Junagadh, Gujarat, India.

2.1. Plant material

A total of 96 groundnut cultivars, representing a broad geographic range and varying agronomic traits, were selected for this study at the ICAR-Directorate of groundnut research, Junagadh. The seeds of the genotypes were obtained from genetic resource section, DGR, Junagadh.

2.2. DNA extraction

Genomic DNA was extracted from leaves harvested from ten days old seedlings to each genotype by using the protocol described by Doyle and Doyle (1987). The quality and quantity of DNA were assessed using agarose gel electrophoresis and spectrophotometry.

2.3. SSR marker analysis

A total of 110 SSR markers were initially screened for polymorphism. Polymerase chain reaction (PCR) was performed in a 10 µL reaction volume containing 1.0 µl of genomic DNA, 2.0 µl 5X taq buffer, 1.0 µl MgCl2, 0.2 µl dNTPs, 1.0 µl of each primer, and 0.2 µl Taq DNA polymerase. Amplified products were analysed along with 50 bp DNA ladder (fermentas) on 6% non-denaturing

poly acrylamide gel (PAGE) running on 1x TBE buffer at constant power resistance of 225 volts for about 2.5–3.0 hr and stained with ethidium bromide (Benbouza et al., 2006). The gels were documented in automated gel documentation system (Fujifilm FLA-5000).

2.4. Data analysis

PopGene Version 1.32 software (Yeh et al., 2000) was used to estimate the various genetic diversity parameters. The principal co-ordinate analysis for microsatellite markers was performed by GenAlex software (Peakall and Smouse, 2006).

3. RESULTS AND DISCUSSION

3.1. Molecular diversity analysis in groundnut

The genetic diversity analysis using 110 SSR markers revealed significant polymorphism among the 96 groundnut genotypes. The number of alleles per locus ranged from 2 to 10, with an average of 4.63, indicating high variability within the germplasm. Marker DGR_D1 exhibited the highest gene diversity (0.8425) and Shannon's information index (1.983), whereas DGR_D61 had the lowest values (gene diversity 0.1017, Shannon's index 0.551), highlighting varying levels of genetic variation across loci (Table 1). The similar result was found by Daudi et al., 2021.

3.2. Population genetic studies on groundnut genotypes

Population structure analysis revealed a mean effective number of alleles of 2.94 and a mean observed number of alleles of 4.40, confirming the presence of significant genetic variation among the studied genotypes. Shannon's information index ranged from 0.551 to 1.983, with DGR_D1 showing the highest value, suggesting high genetic diversity within this locus (Table 2). In contrast, DGR_D61 exhibited the lowest genetic diversity, indicating conserved regions in certain genotypes. Similar trends were observed in previous studies assessing groundnut genetic diversity using SSR markers (Khan et al., 2023).

3.3. Heterozygosity and genetic identity

Heterozygosity analysis revealed an average observed heterozygosity of 0.3946 and an expected heterozygosity of 0.6018. Marker DGR_D1 had the highest expected heterozygosity (0.845) but lacked observed heterozygotes, suggesting inbreeding or selection pressures. Conversely, marker DGR_D73 exhibited the lowest expected heterozygosity (0.102) with minimal observed heterozygosity (0.021). Observed (*Ho*) and expected (*He*) heterozygosity of 0.01 and 0.5 respectively obtained Molosiwa et al., 2015. These findings suggest a need for breeding strategies to maintain heterozygosity and prevent genetic erosion in specific groundnut populations (Table 3).

Table 1: Genetic parameter including allele frequency, number of alleles locus^{-1}, gene diversity, expected heterozygosity, polymorphic information content (PIC) for SSR marker analyzed in groundnut

Marker	Major. Allele. Freq.	Allele No.	Gene diversity	Hetero zygosity	PIC
DGR_D1	0.2169	10	0.8425	0.0000	0.8232
DGR_D2	0.4045	7	0.7645	0.0000	0.7373
DGR_D4	0.4945	7	0.6989	0.0000	0.6691
DGR_D5	0.5000	3	0.5302	0.0000	0.4198
DGR_D8	0.6220	3	0.5024	0.0000	0.4161
DGR_D9	0.4198	5	0.6917	0.0000	0.6401
DGR_D15	0.3167	6	0.7622	0.8778	0.7239
DGR_D16	0.5260	2	0.4986	0.9479	0.3743
DGR_D22	0.3253	8	0.7444	0.0241	0.7024
DGR_D23	0.2184	9	0.8395	0.0000	0.8195
DGR_D29	0.6237	3	0.4734	0.0108	0.3663
DGR_D31	0.3696	5	0.7060	0.0000	0.6553
DGR_D33	0.3059	9	0.7983	0.0000	0.7706
DGR_D37	0.5106	4	0.6066	0.0000	0.5335
DGR_D39	0.3407	4	0.7129	0.0330	0.6574
DGR_D40	0.4839	6	0.6461	0.9785	0.5843
DGR_D44	0.5604	5	0.6224	0.2527	0.5802
DGR_D45	0.5380	3	0.5820	0.9241	0.5040
DGR_D46	0.5000	2	0.5000	1.0000	0.3750
DGR_D47	0.5054	2	0.4999	0.9892	0.3750
DGR_D49	0.5813	5	0.5420	0.7925	0.4604
DGR_D50	0.5476	3	0.5008	0.8810	0.3812
DGR_D61	0.8105	3	0.3142	0.0000	0.2764
DGR_D62	0.3404	7	0.7770	0.0213	0.7445
DGR_D68	0.3514	4	0.6973	0.7582	0.6382
DGR_D72	0.5330	3	0.5470	0.0215	0.4498
DGR_D73	0.9462	2	0.1017	0.0000	0.0966
DGR_D74	0.5349	4	0.6239	0.0375	0.5681
DGR_D75	0.5375	3	0.5132	0.8118	0.3997
DGR_D77	0.5118	2	0.4997	0.3115	0.3749

3.4. Genetic distance and population differentiation

Nei's genetic identity and distance analyses revealed significant variability among populations. The highest genetic identity (0.941) was observed between Maharashtra and Tamil Nadu, while the greatest genetic distance (1.154) was recorded between Punjab and Haryana (Table 4). A dendrogram based on genetic distance data confirmed that

Table 2: Summary of genic variation statistics for all loci

Primer	Sample size	Na	Ne	I
DGR_D1	166	10	6.280	1.983
DGR_D2	178	7	4.248	1.677
DGR_D4	182	7	3.322	1.515
DGR_D5	124	3	2.060	0.763
DGR_D8	164	3	2.010	0.808
DGR_D9	162	5	3.243	1.342
DGR_D15	180	6	4.114	1.540
DGR_D16	192	2	1.994	0.691
DGR_D22	166	8	3.961	1.555
DGR_D23	172	8	6.092	1.899
DGR_D29	186	2	1.884	0.662
DGR_D31	184	5	3.401	1.366
DGR_D33	170	8	4.826	1.733
DGR_D37	94	3	2.507	0.993
DGR_D39	182	4	3.483	1.296
DGR_D40	186	6	2.776	1.173
DGR_D44	182	5	2.689	1.220
DGR_D45	158	3	2.392	0.961
DGR_D46	188	2	2.000	0.693
DGR_D47	186	2	1.999	0.693
DGR_D49	160	4	2.023	0.834
DGR_D50	168	2	1.977	0.687
DGR_D61	190	3	1.458	0.551
DGR_D62	188	6	4.344	1.586
DGR_D68	148	4	3.303	1.257
DGR_D72	182	3	2.213	0.872
DGR_D73	186	2	1.113	0.209
DGR_D74	172	4	2.658	1.131
DGR_D75	160	3	2.056	0.768
DGR_D77	170	2	1.998	0.692

Na: observed numbers of alleles; Ne: effective numbers of allele; I-Shannon's information index

populations with high genetic identity exhibited low genetic distance and vice versa. In khan et al., 2023 observed nei genetic distance is 0.023 and genetic identity is 0.977 These patterns reflect regional differences in genetic diversity and divergence. A dendrogram constructed from genetic distance data (Figure 1) indicated that populations with a high genetic identity typically exhibit low genetic distance, and vice versa.

3.5. Principal coordinate analysis (PCoA)

Principal coordinate analysis (PCoA) revealed that the first

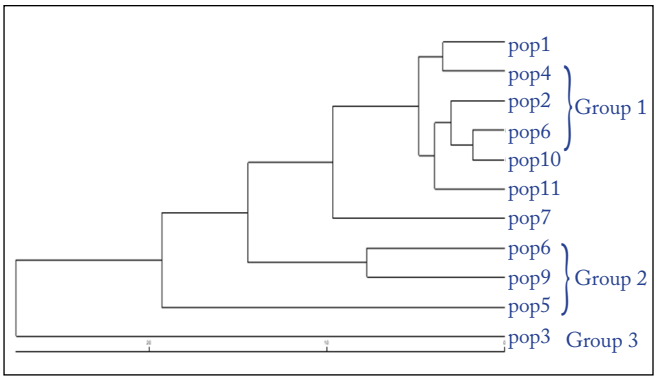

Figure 1: Dendrogram of genetic identity and genetic distance of 11 population; pop1: Andhra Pradesh; pop 2: Gujarat; pop 3: Haryana; pop 4: Karnataka; pop 5: Madhya Pradesh, pop 6: Maharastra, pop7: Orissa, pop 8: Punjab, pop9: Rajasthan, pop 10: Tamil Nadu, pop11: Telangana

Table 3: Summary of heterozygosity statistics for all loci

Primer	S size	Obs_ Hom	Obs_ Het	Exp_ hom	Exp_ het	Nei'	Ave_ het
DGR_ D1	166	1.000	0.000	0.154	0.845	0.840	0.570
DGR_ D2	178	0.988	0.011	0.231	0.768	0.764	0.535
DGR_ D4	182	1.000	0.000	0.297	0.702	0.698	0.428
DGR_ D5	124	1.000	0.000	0.481	0.518	0.514	0.374
DGR_ D8	164	1.000	0.000	0.494	0.505	0.502	0.358
DGR_ D9	162	0.133	0.000	0.304	0.696	0.691	0.429
DGR_ D15	180	0.052	0.866	0.239	0.760	0.756	0.659
DGR_ D16	192	1.000	0.947	0.498	0.501	0.498	0.497
DGR_ D22	166	1.000	0.000	0.247	0.752	0.747	0.513
DGR_ D23	172	1.000	0.000	0.159	0.840	0.835	0.588
DGR_ D29	186	1.000	0.000	0.528	0.472	0.469	0.309
DGR_ D31	184	1.000	0.000	0.290	0.709	0.706	0.458
DGR_ D33	170	1.000	0.000	0.202	0.797	0.792	0.530
DGR_ D37	94	1.000	0.000	0.392	0.607	0.601	0.293

Table 3: Continue...

Primer	S size	Obs_ Hom	Obs_ Het	Exp_ hom	Exp_ het	Nei'	Ave_ het
DGR_ D39	182	0.967	0.033	0.283	0.716	0.712	0.490
DGR_ D40	186	0.021	0.978	0.356	0.643	0.639	0.594
DGR_ D44	182	0.736	0.263	0.368	0.631	0.628	0.460
DGR_ D45	158	0.075	0.924	0.414	0.585	0.582	0.551
DGR_ D46	188	0.000	1.000	0.497	0.502	0.500	0.500
DGR_ D47	186	0.010	0.989	0.497	0.502	0.499	0.497
DGR_ D49	160	0.275	0.725	0.491	0.508	0.505	0.484
DGR_ D50	168	0.131	0.889	0.502	0.497	0.494	0.444
DGR_ D61	190	1.000	0.000	0.684	0.315	0.314	0.255
DGR_ D62	188	1.000	0.000	0.226	0.773	0.769	0.497
DGR_ D68	148	0.986	0.013	0.297	0.702	0.697	0.505
DGR_ D72	182	0.230	0.769	0.448	0.551	0.548	0.479
DGR_ D73	186	0.978	0.021	0.897	0.102	0.101	0.053
DGR_ D74	172	1.000	0.000	0.372	0.627	0.623	0.324
DGR_ D75	160	0.962	0.037	0.482	0.517	0.513	0.288
DGR_ D77	170	0.188	0.811	0.497	0.502	0.499	0.484

Exp_Het.: Heterozygosity; Exp_Hom: Expected homozygosity; Obs_Het: Observed heterozygosity; Obs_ Hom: Observed homozygosity

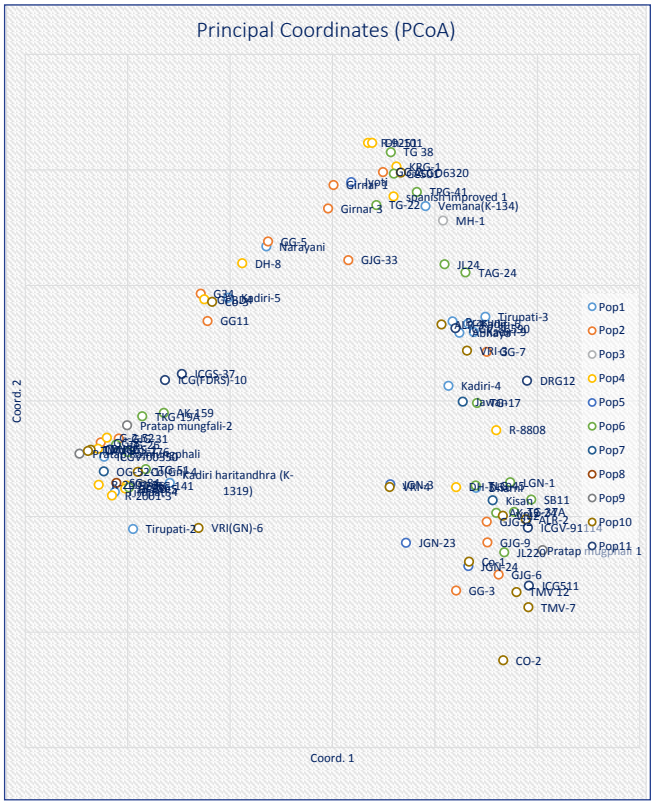

Figure 2: Biplot of principle coordinate analysis in 11 populations of groundnut

three principal axes explained 35.74% of the total genetic variation among the genotypes. Axis one accounted for 19.8% of the variation, axis two explained 10.06%, and axis three explained 6.50% of the variation (Table 5). The PCoA plot clustered populations into three distinct groups, with Haryana forming a separate cluster due to its genetic uniqueness. This confirms the presence of significant genetic differentiation among the studied genotypes, aligning with findings in other groundnut diversity studies (Gandhadmath et al., 2024). The spatial distribution of genotypes in the PCoA plot (Figure 2) aligned with the dendrogram, grouping populations into three distinct clusters, with Haryana forming a separate cluster due to its genetic uniqueness. The corresponding Eigenvalues for each

Table 4: Nei's original measures of genetic identity and genetic distance

Pop. ID	1	2	3	4	5	6	7	8	9	10	11
1		0.888	0.625	0.909	0.654	0.914	0.803	0.653	0.811	0.891	0.882
2	0.118		0.635	0.889	0.739	0.923	0.735	0.610	0.787	0.913	0.856
3	0.469	0.453		0.617	0.522	0.713	0.541	0.315	0.458	0.599	0.559
4	0.094	0.117	0.481		0.631	0.903	0.753	0.747	0.878	0.862	0.826
5	0.424	0.301	0.648	0.459		0.729	0.583	0.483	0.614	0.746	0.672
6	0.089	0.079	0.337	0.101	0.314		0.795	0.598	0.787	0.941	0.903
7	0.218	0.307	0.614	0.282	0.539	0.229		0.591	0.784	0.807	0.787

Table 4: Continue...

Pop. ID	1	2	3	4	5	6	7	8	9	10	11
8	0.426	0.493	1.154	0.290	0.726	0.512	0.525		0.799	0.605	0.576
9	0.208	0.239	0.779	0.129	0.487	0.238	0.242	0.223		0.820	0.796
10	0.115	0.090	0.511	0.147	0.292	0.060	0.214	0.501	0.197		0.905
11	0.125	0.154	0.580	0.191	0.396	0.101	0.238	0.551	0.227	0.099	

Population 1: Andhra Pradesh; Population 2: Gujarat; Population 3: Haryana; Population 4: Karnataka; Population 5: Madhya Pradesh; Population 6: Maharastra; Population 7: Orissa; Population 8: Punjab; Population 9: Rajasthan; Population 10: Tamil Nadu; Population 11: Telangana

Table 5: Percentage variation explained by the first three axis

Axis	1	2	3
%	19.18	10.06	6.50
Cum%	19.18	29.24	35.74

axis, along with the sample Eigenvectors, are presented in Table 6. These findings demonstrate dsubstantial genetic diversity in groundnut, crucial for breeding programs. High-diversity genotypes like DGR_D1 are valuable for developing improved cultivars with enhanced traits. The

Table 6: Eigen Values by Axis and Sample Eigen Vectors

Axis No.	1	2	3	Axis No.	1	2	3
Eigen value	57.926	30.365	19.639	GG-8	-1.132	-0.180	0.124
Dharni	0.701	-0.376	-0.532	GJG-31	-1.044	-0.162	-0.497
Kadiri-4	0.566	0.066	0.815	MH-1	0.539	0.781	-0.102
Kadiri-9	0.690	0.299	0.932	DH-3-30	0.603	-0.373	0.328
Prasuna	0.586	0.345	0.762	R-8808	0.800	-0.126	0.342
Tirupati-3	0.746	0.365	0.678	KRG-1	0.312	1.015	-0.773
Kadiri-6	0.662	0.335	0.951	Spanish improved	0.298	0.885	-0.647
Abhaya	0.619	0.295	0.339	R-9251	0.173	1.117	-0.489
Vemana (K-134)	0.454	0.843	0.364	Dh-101	0.193	1.117	-0.449
Narayani	-0.323	0.669	-0.071	DH-8	-0.441	0.596	-0.062
Kadiri-5	-0.509	0.447	-0.055	GPBD 4	-0.626	0.440	0.239
ICGV-00350	-1.115	-0.241	0.473	G-2-52	-1.102	-0.159	0.440
Kadiri haritandhra	-0.794	-0.354	-0.182	S206	-1.006	-0.366	0.284
Tirupati -2	-0.974	-0.554	-0.249	R-2001-2	-1.141	-0.363	0.310
Tirupati-4	-1.063	-0.394	-0.199	GPBD-5	-1.012	-0.378	-0.004
GJG-32	0.753	-0.522	-0.439	R-2001-3	-1.076	-0.409	-0.267
GJG-9	0.756	-0.612	-0.752	DH86	-1.145	-0.206	-0.015
GJG-6	0.811	-0.751	-0.476	JGN-3	0.282	-0.306	0.172
GG-3	0.603	-0.820	-0.262	JGN-24	0.359	-0.613	-0.390
GG-7	0.753	0.213	0.377	Jyoti	0.663	-0.714	0.537
GG -2	0.247	0.991	-0.440	TLG-45	0.092	0.947	-0.388
Girnar 3	-0.022	0.833	-0.607	LGN-1	0.699	-0.365	-0.257
Girnar 1	0.004	0.934	-0.309	JL220	0.868	-0.351	0.885
GJG-33	0.077	0.609	-0.137	TG-37A	0.838	-0.655	0.137
GG-5	-0.315	0.691	-0.315	SB11	0.886	-0.480	-0.206
GG11	-0.610	0.346	0.041	AK-12-24	0.970	-0.426	0.172
G34	-0.644	0.464	0.253	TG-17	0.800	-0.484	0.033

Table 6: Continue...

Axis No.	1	2	3
JL24	0.705	-0.008	0.833
TAG-24	0.547	0.592	0.960
TPG-41	0.649	0.557	-0.268
GL501	0.412	0.905	-0.288
TG38	0.299	0.984	-0.562
TG-22	0.284	1.077	-0.516
AK-159	-0.824	0.848	-0.516
TKG-19A	-0.929	-0.051	0.322
TG-26	-1.057	-0.066	0.279
JL286	-0.944	-0.128	0.447
JL776	-1.000	-0.374	0.012
TG-51	-0.912	-0.221	-0.370
Kisan	0.722	-0.295	-0.329
Jawan	0.636	-0.429	0.179
OG-52-1	-1.116	-0.003	1.040
SG-84	-1.055	-0.304	0.256
Pratap mungphali 1	1.026	-0.354	-0.300
Pratap Raj mungphali	-1.235	-0.645	-0.095
Pratap mungphali-2	-1.003	-0.228	0.379
RG-141	-0.926	-0.105	0.072
VRI-4	0.229	-0.364	-0.437
Co-1	0.279	-0.372	0.038
TMV-7	0.666	-0.695	-0.249
TMV 12	0.956	-0.892	-0.301
CO-2	0.898	-0.827	-0.541
VRI2	0.833	-1.122	-0.354
ALR-2	0.832	-0.496	0.207
VRI-3	0.944	-0.512	-0.171
ALR-3	0.532	0.331	0.063
ALGO-06-320	0.334	0.988	-0.545
CO-3	-0.588	0.429	-0.039
CO(Gn)-4	-0.950	-0.306	0.113
TMV-2	-1.181	-0.211	0.215
VRI(GN)-6	-1.194	-0.216	0.197
ICGV511	-0.654	-0.549	-0.234
ICGV-91114	0.959	-0.799	-0.351
DRG12	0.954	-0.548	0.248
ICGV-86590	0.949	0.088	0.851
ICGV(FDRS)-10	0.600	0.315	0.945
ICGV-37	-0.818	0.091	0.249
ICGS-1	-0.736	0.117	0.479

observed genetic divergence supports hybridization efforts to exploit heterosis. Conservation strategies should prioritize genetically diverse populations to mitigate genetic erosion and ensure sustainable germplasm utilization.

4. CONCLUSION

This study evaluated the genetic diversity of 96 groundnut genotypes using SSR markers, revealing significant variability. Key genetic metrics, such as allele frequency, gene diversity, and heterozygosity, highlighted the diversity, with DGR_D1 showing the highest and DGR_D73 the lowest diversity. Unique genetic groups were identified, aiding parent selection for breeding. PCoA and dendrogram analyses confirmed these findings.

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