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Research Article

A Comparative Study of Genetic Diversity Analysis through Molecular Markers and D^2 Statistic in Germplasm Accessions of Green Gram [*Vigna radiata* (L.)]

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ABSTRACT

*Genetic diversity analysis was carried out among fifty-six germplasm accessions of green gram through simple sequence repeats (SSR) markers and Mahalanobis D^2 statistic for quantitative traits. The D^2 statistic performed on 20 quantitative traits grouped all 56 genotypes into eight divergent clusters. Cluster V (8559.766**)** had maximum intra-cluster distance while inter-cluster distance was highest between clusters III and VI(329997.938) Cluster means indicated that germplasm lines falling in cluster III and V possessed higher values for quantitative traits such as plant height, plant diameter, number of pods per cluster, number of pods per plant, number of seeds per pod, pod yield per plant, pod length, threshing %, leaf area, specific leaf weight, leaf area ratio, seed yield per plant, seed yield per plot and biological yield. Molecular characterization was done with fifteen standardized SSR primers. All primers showed scorable polymorphism and the information was used to calculate Jackard's similarity matrix using NTSYS-pc version 2. The UPGMA dendrogram based on SSR results divided the 56 green gram genotypes into eight main clusters under which the cluster I was highly diverse compared to all other clusters and consisted of 15 genotypes, followed by cluster II with 9 genotypes.*

Keywords: *Green gram germplasm, Genetic diversity, Dendrogram, SSR markers, Mahalanobis D^2 statistic*

INTRODUCTION

Green gram [*Vigna radiate* (L.) Wilczek] also known as mung bean is an important short duration diploid (2n) food legume in the tropical and sub-tropical countries of the world. The crop belongs to the family Fabaceae. It is third most important pulse crop of India providing vegetable protein for people and grown primarily in intercropping with wheat, maize, potato, etc., during the monsoon season and as a monocrop in other seasons.

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India is the largest producer and consumer of pulses in the world. Green gram as a legume crop has the ability to fix atmospheric nitrogen via root rhizobial symbiosis leading to improved soil fertility and texture (Graham & Vance, 2003). Mung bean when inter-cropped in rice–rice and rice–wheat systems increases the yield of the subsequent cereal crop and reduces pest incidence (Yaqub et al., 2010 and Defaria, 1989). Being a short-duration legume crop it is ideal for catch cropping, intercropping, and relay cropping (Pooja et al., 2019). Pulses are the major source of dietary protein in vegetarian diets in most of the Asian countries. Green gram is principally grown for its high protein seeds that are used as human food, by cooking, fermenting, milling or sprouting, making soups, curries, bread, sweets, noodles, salads, papad etc. (Singh et al., 1988). Green gram has nitrogen fixing ability via symbiosis with nitrogen-fixing Rhizobium bacterium (Allito et al., 2015). Despite being an economically important pulse crop, overall production of mung bean in Indian subcontinent is low due to abiotic and biotic stresses (Bangar et al., 2018).

Genetic diversity present in the germplasm accessions is an important tool for any crop improvement program (Marilene et al.,2012). Multivariate analysis by means of Mahalanobis generalized distance (D^2) statistic is a powerful tool in quantifying the degree of divergence at the genotypic level and is an efficient tool in the quantitative estimation of genetic diversity (Mahalanobis, 1936). Success of plant hybridization followed by selection depends largely on the selection of parents with high genetic diversity for traits of interest (Murthy & Arunachalam 1966). The assessment of genetic diversity would provide us a correct picture of the extent of genetic variation, further helping us to improve the genotypes responses to biotic and abiotic stresses (Panigrahi & Baisakh, 2014). Molecular markers such as simple sequence repeats (SSRs) have been proven as powerful tools in studying the genetic diversity and population structure of the species (Yuliasti & Reflinur, 2015). Morphological markers used for diversity studies are not adequate, because these markers are subject to environmental influences unlike DNA based molecular markers (Nath et al., 2018) which are less affected by age, the physiological condition of samples, and environmental factors. The main objective of this study was to assess genetic diversity of germplasm accessions both at molecular level and also for quantitative traits so that a comparison study could be made.

MATERIALS AND METHODS

The experiment was conducted at experimental plot of College of Agriculture, Hassan, University of Agricultural Sciences, Bangalore. The experimental site is geographically located at Southern Transitional Zone (Zone-7) of Karnataka with an altitude of 827 m above Mean Sea Level (MSL) and at 33′ N latitude and 75^0 33′ to 76^0 E38′ longitude. The experiment was conducted in Replicated Augmented Design. Replicated treatments are tested in each block as in a RCBD. Fifty six (56) genotypes of green gram [*Vigna radiata* (L.) Wilczek] grown in 8 Blocks during *summer* 2017. The gross area of experiment was 302.5 m^2 and each block size was 3 x 3 m. The row spacing was 30 cm and inter plant distance was 10 cm. Observations were recorded on randomly chosen five competitive plants on 20 metric characters *viz*., days to 50% flowering, days to 50% maturity, plant height, plant diameter, number of primary branches per plant, number of clusters per plant, number of pods per cluster, number of pods per plant, pod length, number of seeds per pod, pod yield per plant, seed yield per plant, seed yield per plot, threshing percentage, biological yield, harvest index, 100 seed weight, leaf area, specific leaf weight and leaf area ratio.

2.1 Plant materials

The material used in the study comprised of 56 germplasm lines of green gram [*Vigna radiata* (L.) Wilczek] obtained from different Research Institutions and Agricultural Research Stations of India.

Table1: List of germplasm used in the study and their source

Sl. No.	Genotype	Source	Sl. No.	Genotype	Source
1	Selection- 4- Check	UAS, Raichur	29	LGG-572	RARS, Guntur
2	DGG-1- Check	ARS, Bidar	30	PM-110	RARS, Guntur
3	Barimung- Check	UAS, Raichur	31	LGG-577	RARS, Guntur
4	KKM-3	UAS, Banglore	32	IC-436624	IIPR, Kanpur
5	Harsha	UAS, Raichur	33	IC-436723	IIPR, Kanpur
6	VBN-1	Coimbatore	34	IC-413316	IIPR, Kanpur
7	BGS-9	ARS, Bidar	35	IC-436746	IIPR, Kanpur
8	KM13-16	ARS, Bidar	36	VGG10-010	Coimbatore
9	KM13-19	ARS, Bidar	37	VGG04-011	Coimbatore
10	KM13-39	ARS, Bidar	38	VGG04-007	Coimbatore
11	GG13-7	ARS, Bidar	39	COGG-93	Coimbatore
12	GG13-6	ARS, Bidar	40	VBNGG-2	Coimbatore
13	KM13-44	ARS, Bidar	41	TARM-2013	Coimbatore
14	GG13-10	ARS, Bidar	42	VGG04-005	Coimbatore
15	SML-668	ARS, Bidar	43	COGG-920	Coimbatore
16	KM13-9	ARS, Bidar	44	VGG07-003	Coimbatore
17	IPM99-125	ARS, Bidar	45	VGG10-002	Coimbatore
18	LGG-596	RARS, Guntur	46	VGG-112	Coimbatore
19	LGG-572	RARS, Guntur	47	IC-92048	NBPGR, Akola
20	LGG-450	RARS, Guntur	48	AKL-103	NBPGR, Akola
21	LGG-583	RARS, Guntur	49	AKL- 39	NBPGR, Akola
22	LGG-590	RARS, Guntur	50	AKL-106	NBPGR, Akola
23	LGG-588	RARS, Guntur	51	AKL-225	NBPGR, Akola
24	LGG-589	RARS, Guntur	52	AKL-95	NBPGR, Akola
25	LGG-579	RARS, Guntur	53	AKL-194	NBPGR, Akola
26	LGG-562	RARS, Guntur	54	AKL-212	NBPGR, Akola
27	LGG-582	RARS, Guntur	55	AKL-195	NBPGR, Akola
28	LGG-585	RARS, Guntur	56	AKL-211	NBPGR, Akola

2.2 Genomic DNA extraction and quantification

Total genomic DNA was isolated from 56 genotypes using acetyltrimethyl ammonium bromide (CTAB) extraction protocol (Doyle & Doyle, 1987) and was then quantified spectrophotometrically on a nano spectrophotometer (Implen, Germany).

2.3 SSR-PCR amplification

Fifteen SSRs or microsatellite repeat primers were used to screen germplasm lines of green gram presented in Table 2. PCR amplification was carried out in a 20-µL reaction volume containing 200 µMdNTP mix, 1.5 mM MgCl2, 1 U of Taq polymerase,1X reaction buffer, 0.5 µM primer, double-distilled water, and 20 ng of genomic DNA. The amplification was performed with reaction conditions of pre-denaturation at 94 °C for 4 minutes followed by 30 cycles of denaturation at 94 °C for 1 minute, annealing at 48–56.7 °C for 1 minute, extension at 72 °C for 45 seconds and final extension was done for 10 minutes at 72 °C with a hold temperature of 4 °C. 3% metaphor

agarose (Sigma-Aldrich, India) was used for separation of amplification products by electrophoresis and gel images were captured.

Table 2: List of SSR Primers and their details

S.No	Seq Id.	Seq Name	Sequence $5^1 \rightarrow 3^1$	Length	Tm ^{0}C
1	1B28022	CEDG204	CCTTGGTTGGAGCAGCAGC	19	55.4
	IB28023	CEDG204	CACAGACACCCTCGCGATG	19	55.4
2	IB28030	CEDG092	TCTTTTGGTTGTAGCAGGATGAAC	24	54
	IB28031	CEDG092	TACAAGTGATATGCAACGGTTAGG	24	54
3	IB28036	CEDG275	CACACTTCAAGGAACCTCAAG	21	52.4
	IB28037	CEDG275	GTAGGCAACCTCCATTGAAC	20	51.8
4	IB28038	CEDG020	TATCCATACCCCAGCTCAAGG	20	51.8
	IB28039	CEDG020	GCCATACCAAGAAAGAGG	18	48
5	IB28040	CEDG264	GATTCCCTTCCTAGCTATGG	20	51.8
	IB28041	CEDG264	CTGCTGGACATGAAGATTCAG	21	52.4
6	IB28042	CEDG271	GCACTAAAGTTAGACGTGGTTC	22	53
	IB28043	CEDG271	CACTCCCACTGCCAAACAAGG	21	56.3
7	IB28046	CEDG056	TTCCATCTATAGGGGAAGGGAG	22	54.8
	IB28047	CEDG056	GCTATGATGGAAGAGGGCATGG	22	56.7
8	1B28054	CEDG016	TTAGTTCACTCCGCTTGGTC	20	51.8
	IB28055	CEDG017	CACGTCATCCTCTGTTAGAC	20	51.8
9	IB28058	CEDG022	AGGAATGTGAGATTTG	16	38.3
	IB28059	CEDG022	AATCGCYYCAAGGTCAAGCC	20	51.8
10	IB28062	CEDG198	CAAGGAAGATGGAGAGAATC	20	49.7
	IB28063	CEDG198	CCTTCTAAGAACAGTGACATG	21	50.5
11	IB28064	CEDG225	GAGGAAGTGTTGCAGCACC	19	53.2
	IB28065	CEDG225	GTAGACTCTGCAGAGGGATG	20	53.8
12	IB28070	CEDG112	GCAATATTCGCATTATTCATTCA	23	48.1
	IB28071	CEDG112	GTGTTTCAAAGCACTATACTTAA	23	48.1
13	IB28072	DMB-SSR182	TAGAGCCTTCTGGTTTTTCACA	22	51.1
	IB28073	DMB-SSR182	AGGAGGAGGATTTTGATGATGA	22	51.1
14	IB28076	DMB-SSR217	TCCTTGCCTTATGATTCTGTGA	22	51.1
	IB28077	DMB-SSR217	TTTGGCCACTTCCAAACTTTA	21	48.5
15	IB28080	LR738A	CGCAAAGAGAGAGAGAGAG	19	51.1
	IB28081	LR738A	CCCCCATCTGAAAGAAAGAG	20	51.8

1. STATISTICAL ANALYSIS

The data was subject to genetic divergence analysis using Mahalanobis D^2 statistic (Mahalanobis 1936) as suggested by Rao (1952). All the genotypes were grouped into respective clusters on the basis of D^2 values following Tocher's method. Twenty morphometric characters were evaluated for plant specimens from 56 germplasm lines.

3.1 Mahalanobis D^2 Analysis

Mahalanobis (1936) D^2 analysis was used for assessing the genetic divergence among the test entries involving quantitative characters. The generalized distance between any two populations is given by the formula.

$\mathbf{D^2} = \sum\sum \lambda ij\ \sigma ai\ \sigma aj$

Where,

$\mathbf{D^2}$= Square of generalized distance

λij = Reciprocal of the common dispersal matrix

$\sigma\ ai = (\mu i1 - \mu i2)$

$\sigma aj = (\mu j1 - \mu j2)$

μ=General mean

b) Cluster of $\mathbf{D^2}$ values

All n (n-1)/2 $\mathbf{D^2}$ values were clustered using Tocher's method described by Rao (1952).

c) Intra cluster distance

Square of the intra cluster distance = $\dfrac{\sum \mathbf{D^2\ i}}{N}$

Where $\sum \mathbf{D^2\ i}$ is the sum of distance between all possible combinations of the entries included in a cluster.

n = Number of all possible combinations

d) Inter cluster distance

Square of the inter cluster distance = $\dfrac{\sum \mathbf{D^2\ i}}{ni\ nj}$

Where,

$\sum \mathbf{D^2\ i}$ is the sum of distances between all possible combinations (ninj) of the entries included in the clusters study.

ni = Number of entries in cluster i

nj =Number of entries in cluster j

1.2 SSR Marker Data Analysis

DNA bands generated from SSR-PCR amplification were scored using binary system where a scoring of 1 was given for presence of band and 0 for absence of bands for each primer and were used to calculate Jackard's similarity matrix using NTSYS-pc version 2.1. Cluster analysis was performed on molecular data. Similarity matrix were compiled for all pairs of accessions using Jackard's similarity coefficient and dendrogram for genetic diversity was constructed using un-weighted pair-group method with arithmetic mean (UPGMA) analysis.

RESULTS AND DISCUSSION

4.1 Mahalanobis D^2

Analysis of variance revealed that a wide range of variability existed for all the traits studied indicating the presence of significant variation among the genotypes. Based on the D^2 analysis, germplasm accessions were grouped into eight different clusters as presented in the Table 3. Cluster II was the largest with fifteen genotypes followed by cluster I with fourteen, cluster IV with eleven, cluster III with six, cluster V with five, cluster VI with three and cluster VII and VIII were solitary clusters consisting only one genotype each. The mode of distribution of genotypes from different geographical regions into various clusters was at random indicating that the genotypes originating from different agro-climatic / geographical regions grouped together into different clusters showing no parallelism between genetic diversity and geographical distribution. Our results are on par with findings of Raje and Rao et al. (2001), Venkateswarlu (2001), Dasgupta et al., (2005), Makeen et al. (2007), Tabasum et al.,(2010) and Gunjeet Kaur et al. (2015).

Intra and inter-cluster distances are presented in the Table 4. Average intra cluster distance ranged from 0.00 to 8559.77. The maximum intra-cluster (D^2) distance was recorded for Cluster V (8559.77) followed by Cluster II (2960.06), Cluster I (2395.56), Cluster III (1395.90), Cluster VI (1027), Cluster IV (909.99), cluster VII(0.00) and VIII

(0.00).The highest intra-cluster distance recorded by cluster V indicates the presence of wide genetic diversity among the 5 genotypes viz., LGG-582, VBNGG-2, VGG04-007, LGG-577 and TARM-2013. Within the cluster, the maximum inter cluster D^2 distance value was found between cluster III and VI (329997.90) followed by cluster II and III (242988.80), cluster V and VI (187971.10), cluster III and IV (169053.60), cluster VI and VIII (133744.00) and cluster VII and VI (120870.70). These results suggest that the genotypes grouped in different clusters may be used as potential parental lines for hybridization programmes to develop desirable genotypes as genetic diversity can be best exploited and chances of getting best transgressive segregants are more.

D^2 analysis is very much useful in assessment of green gram diversity and also to develop core collection of germplasm to be utilised in crop improvement programmes (Muthusamy et al., 2008; Arpita et al., 2010; Ghulam et al., 2010; Singh et al., 2013; Vyas et al., 2018). Breeding strategies to improve traits for yield, biotic and abiotic stress are largely dependent upon presence of genetic variability among parental lines. Hence assessment and characterization of genetic diversity among germplasm accessions is of great importance. In this study, we have successfully assessed the levels of genetic diversity, intra and inter cluster diversity and genetic relatedness that existed among germplasm accessions of green gram representing different eco-geographical / agro-climatic zone.

4.2 Genetic diversity as revealed by SSR markers

Molecular characterization of 56 green gram genotypes was attempted with 15 standardized primers. All of the primers showed scorable polymorphism by presence and absence of bands. Jaccard's similarity coefficient values for SSR primers ranged from 0.35 to 1.00 with an average of 0.85. Based on the dendrogram generated through the UPGMA genotypes were divided into eight main clusters as presented in the Table 5. Cluster I consisted of 15genotypes, followed by cluster II with 9 genotypes, cluster VI with seven genotypes, cluster IV and V with six genotypes, cluster III with five genotypes, and cluster VII and VIII with four genotypes each. A minimum similarity coefficient of 0.35 was observed between genotypes LGG-585 and LGG-573 and maximum was between KKM-3 and TM-962 (1.00) exhibiting minimum genetic divergence.Absence and presence of bands represented as monomorphic and polymorphic in Table 6. The genotype AKL-211 showed presence of polymorphic band at 100bp for the primer CEDG-092. CEDG-225 primer amplified 250bp polymorphic band in the genotype VGG04-005 (Figure 2). The primer CEDG-275 showed presence of polymorphic band at 250bp and 200bp, for the genotypes KM13-9 and LGG-582 respectively. The genotype KM13-9 showed presence of 300bp polymorphic band for the primer DMB-SSR-182.

The cluster I was the biggest, comprising 15 genotypes, and was subdivided into I-A and I-B. Sub-cluster I-A comprised 8 genotypes namely; KKM-3, KM13-16, LGG-572, LGG-450, AKL-103, BGS-9, 1C-92048 and AKL-212. Within this sub-cluster, KKM-3, KM13-16 and LGG-572 showed highest similarity value of 1.00 followed by genotypes LGG-450, AKL-103, BGS-9 and 1C-92048 (0.95). The genotypeAKL-212 had lowest similarity value of 0.90. Sub-cluster I-B comprised 7 genotypes; AKL-39, AKL-106, AKL-225, AKL-95, AKL-194, AKL-195 and AKL-211. The cluster II, comprised 9 genotypes, and was subdivided into II-A and II-B. Sub-cluster II-A was comprised of 5 genotypes namely; Harsha, LGG-596, IPM99-125, LGG-583 and PM110. Within this sub-cluster, genotype IPM99-125 and LGG-583 registered similarity value of 0.95 and genotype Harsha and LGG-596 recorded similarity value of 0.90 and the genotype PM110 was somewhat distinct from other genotypes with a similarity value of 0.75. Sub-cluster II-B consisted4 genotypes;VGG10-010, VGG04-011, VGG04-007 and Barimung. Within this sub-cluster, genotype VGG10-010

and VGG04-011 had a similarity value of 1.00. The cluster III, comprised 5 genotypes, and was subdivided into III-A and III-B. Sub-cluster III-A comprised 3 genotypes; GG13-6, KM13-44 and GG13-7. Within this sub-cluster, genotype GG13-6 and KM13-44 exhibited similarity value of 0.90 and the genotype GG13-7 was distinct from other genotypes with a similarity value of 0.80. Sub-cluster III-B comprised2 genotypes; LGG-585 and LGG-573 showing similarity value of 0.70.The cluster IV, comprised 6 genotypes, and was subdivided into IV-A and IV-B. Sub-cluster IV-A comprised 3 genotypes; VBN-1, VBNGG-2 and DGG-1. Within this sub-cluster, genotype VBN-1 and VBNGG-2 recorded similarity value of 0.95. Sub-cluster IV-B comprised 3 genotypes; LGG-577, COGG-93 and IC-413316. Within this sub-cluster, genotype LGG-577 and COGG-93 recorded similarity value of 0.90 and the genotypes IC-413316 was distinct from other genotypes with a similarity value of 0.83.The cluster V, comprised 6 genotypes, and was subdivided into V-A and V-B. Sub-cluster V-A comprised 3 genotypes; TARM-2013, VGG04-005 and VGG-112. Within this sub-cluster, genotype TARM-2013 and VGG04-005 registered similarity value of 0.94. Sub-cluster V-B comprised 3 genotypes; COGG-920, VGG07-003 and VGG10-002. Within this sub-cluster, genotype COGG-920 and VGG07-003 exhibited similarity value of 1.00. The cluster VI, consisted of 7 genotypes, and was subdivided into VI-A and VI-B. Sub-cluster VI-A comprised of 5 genotypes; KM13-39, KM13-9, KM13-19, LGG-590, LGG-582, GG13-10 and SML-668.

Within this sub-cluster, genotype KM13-9 and KM13-19 and genotype LGG-590 and LGG-582 had similarity value of 0.95. Sub-cluster VI-B comprised of 3 genotypes; COGG-920, VGG07-003 and VGG10-002 exhibiting similarity value of 0.85.The cluster VII, comprised 4 genotypes; IC-436624, IC-436723, Selection-4 and IC-436746. The genotype IC-436624 and IC-436723 had similarity value of 1.00. The cluster VIII, comprised of 4 genotypes, and was subdivided into VIII-A and VIII-B. Sub-cluster VIII-A consisted of 2 genotypes; LGG-588 and LGG-579 showing similarity value of 0.93. Sub-cluster VIII-B comprised 2 genotypes; LGG-589 and TM-962 with a similarity value of 0.88.

The SSR markers showed a high level of polymorphism. Similar finding is reported by Singh et al. (2013) and Chattopadhyay et al. (2008) who assessed polymorphism in green gram with combined RAPD, ISSR and SSR markers.

Table 3: List of different cluster formed from 56 mungbean genotypes

Cluster number	Number of genotypes	Name of the genotypes
I	14	LGG-450, VGG04-011, LGG-583, PM-110, LGG-572, VGG10-002, VGG04-005, LGG-573, IC-413316, KKM-3, VGG07-003, LGG-596, LGG-588, Selection-4
II	15	KM13-16, KM13-19, AKL-195, BGS-9, GG13-7, IPM99-125, AKL-212, KM13-39, VGG-112, AKL-194, KM13-9, AKL-225, AKL-95, AKL-103, IC-436624
III	6	TM-962, COGG-93, LGG-589, VGG10-010, LGG-585, LGG-579
1V	11	KM13-44, IC-92048, COGG-920, GG13-6, VBN-1, Barimung, Harsha, DGG-1, LGG-590, SML-668, AKL-106
V	5	LGG-582, VBNGG-2, VGG04-007, LGG-577, TARM-2013
VI	3	GG13-10, AKL-211, AKL-39
VII	1	IC-436746
VIII	1	IC-436723

Table 4: Intra and Inter cluster distance of 56 genotypes using Mahalonobis D^2 analysis

	Cluster I	**Cluster II**	**Cluster III**	**Cluster IV**	**Cluster V**	**Cluster VI**	**Cluster VII**	**Cluster VIII**
Cluster I	**2395.557**	40370.133	90405.266	14325.948	27689.197	78636.539	7312.957	11729.597
Cluster II		**2960.056**	242988.844	8463.021	124285.242	9458.265	70811.992	79202.133
Cluster III			**1395.901**	169053.625	25584.635	329997.938	54153.438	53738.402
Cluster IV				**909.989**	73759.844	28312.922	34126.000	41053.754
Cluster V					**8559.766**	187971.141	11732.245	12340.687
Cluster VI						**1027.017**	120870.719	133743.953
Cluster VII							**0.000**	5134.390
Cluster VIII								**0.000**

Table 5: List of different clusters formed from 56 genotypes using SSR markers

Cluster number	**Number of genotypes**	**Name of genotypes**
Cluster I	15	KKM-3, KK13-16, LGG-572, LGG-450, AKL-103, BGS- 9, IC-92048, AKL-212, AKL-39, AKL-106, AKL-225, AKL-95, AKL-194, AKL-195, AKL-211
Cluster II	9	Harsha, LGG-596, IPM99-125, LGG-583, PM-110, VGG10-010, VGG01-011, VGG04-007, Barimung
Cluster III	5	GG13-6, KM13-44, GG13-7, LGG-585, LGG-573
Cluster IV	6	VBN-1, VBNGG-2, DGG-1, LGG-577, COGG-93, IC-413316
Cluster V	6	TARM-2013, VGG04-005, VGG-112, COGG-920, VGG07-003, VGG10-002
Cluster VI	7	KM13-39, KM13-9, KM13-19, LGG-590, LGG-582, GG13-10, SML-668
Cluster VII	4	IC-436624, IC-436723, Selection-4, IC-436746
Cluster VIII	4	LGG-588, LGG-579, LGG-589, TM-962

Table 6: List of number of monomorphic and polymorphic green gram genotypes

Primers	**No. of Monomorphic Genotypes (Presence of band)**	**No. of Polymorphic genotypes (Absence of band)**	**No. of Polymorphic genotypes (Presence of band at different length)**
CEDG204	38	18	-
CEDG092	14	41	1 (AKL-211 at 100bp)
CEDG275	50	4	2 (KM13-9 at 250bp) & LGG (582 at 200bp)
CEDG020	33	23	-
CEDG264	40	16	-
CEDG271	44	12	-
CEDG056	44	12	-
CEDG016 & 17	25	31	-
CEDG022	33	23	-
CEDG198	53	3	-
CEDG225	44	11	1 (VGG04-005 at 100bp)
CEDG112	49	7	-
DMB-SSR182	43	12	1 (KM-13 at 300bp)
DMB-SSR217	52	4	-
LR738A	40	16	-

Jaccard's genetic similarity values of SSR markers were found in the ranges of 0.35 to 1.00 (average: 0.85) revealing low level of diversity observed through SSR within green gram clusters. Similar finding is reported by Wang et al. (2018). This moderate level of genetic diversity within the self-pollinated members of green gram genotypes from the genus *Vigna* suggests its moderate genetic base, which is possibly due to accumulation of novel gene combinations in response to dynamic pressures of natural selection (Kaur et al., 2016). Comparative studies in *Vigna* species involving RAPD, AFLP, ISSR, and SSR marker systems were successfully used and reported by researchers (Souframanien and Gopalakrishna, 2004; Gillaspie et al., 2005; Dikshit et al., 2007; Muthusamy et al., 2008; Lestari et al., 2014; Zia et al., 2014; Changyou et al., 2017; Wang et al., 2017; Kanimoli et al.,2018); however, the reports on green gram are very limited. The genotypes from different clades show genetic proximity because of genome conservation depending on their phylogenetic relationships and such orthologous or conserved regions play a pivotal role in exploiting the genomic resources (Souframanien & Dhanasekar, 2014). The potentiality of SSRs in green gram diversity analysis has also been reported by many earlier workers (Dikshit et al., 2007; Gupta et al., 2013; Saiful et al., 2014; Sanghani et al., 2015; Kaur et al., 2018. Ranade and Gopalakrishna, 2009 revealed that using of more sensitive techniques for DNA fragment size analysis like PAGE or capillary electrophoresis (Dutta et al., 2011) may give better results.

4.3 Comparison of genetic diversity revealed by D^2 statistic and SSR markers (Jaccard's similarity coefficient)

Both the methods, D^2 statistic and Jaccard's similarity coefficient generated 8 clusters, but there is lot of difference with respect to the number and genotypes carried by each of the cluster, for example cluster I of D^2 statistic contained 14 genotypes and dendrogram generated through the UPGMA comprised 15 genotypes in cluster I and also these clusters comprised different genotypes for example cluster I of D^2 statistic comprised genotypes LGG-450, VGG04-011, LGG-583, PM-110, LGG-572, VGG10-002, VGG04-005, LGG-573, IC-413316, KKM-3, VGG07-003, LGG-596, LGG-588, and Selection-4 whereas cluster I created by Jaccard's similarity coefficient contained the genotypes KKM-3, KK13-16, LGG-572, LGG-450, AKL-103, BGS- 9, IC-92048, AKL-212, AKL-39, AKL-106, AKL-225, AKL-95, AKL-194, AKL-195, AKL-211. There are only three genotypes namely; LGG 450, LGG-572 and KKM-3which are common to both the clusters and all other genotypes are different. This trend is observed in all other clusters. D^2 statistic indicated presence of wide genetic diversity among the 5 genotypes viz., LGG-582, VBNGG-2, VGG04-007, LGG-577 and TARM-2013 whereas Jaccard's similarity coefficient identified PM110, LGG-585 and LGG-573 genotypes as genetically most divergent. Through D^2 statistic it was possible to identify genotypes possessing maximum and minimum values for different quantitative traits; for example genotypes in Cluster VII were early flowering (53.00 days) whereas genotypes in cluster VIII were late flowering (58.00 days) but Jaccard's similarity coefficient will not generate such results since the input data used for analysis is SSR marker data. This data is obtained by scorable polymorphic DNA bands. Most of the times, DNA bands / SSR-PCR amplifications are generated from un- transcribed genomic region which does not represent any functional gene. This is because SSR markers are mostly confined to heterochromatic region of chromosome and hence the primers of SSR markers will usually amplify non-coding region of DNA. But D^2 statistic uses the data generated on quantitative traits for analysis and these data points come from coding region of DNA hence it was possible to identify a cluster possessing either maximum or minimum values for the quantitative traits. This is the reason why genetic diversity revealed by D^2 statistic is not matching / comparable with Jaccard's similarity coefficient.

Choice of selecting suitable statistic for genetic diversity analysis depends on the purpose of study. If researcher is interested to get heterotic groups or clusters of genotypes possessing extreme expressions for quantitative traits then D^2 statistic is useful. This information will also help plant breeder to identify suitable parents to be involved in hybridization programmes representing different heterotic groups. If objective of the study is molecular characterization and also to study ancestral relationship or genetic relatedness among germplasm accessions, then one should opt for Jaccard's similarity coefficient.

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