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Genetic Variability and Divergence of Morphological and Seed Quality Traits of Greengram (*Vigna radiata* L.) Genotypes

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Forty greengram genotypes were evaluated for their morphological traits and to find the extent of genetic variability. Analysis of variance revealed that the genotypes were highly significant for all the characters studied, indicating the existence of considerable magnitude of variability. High (>20%) phenotypic co-efficient of variation and high genotypic co-efficient of variation for seed yield (kg/4.05 m^2) in the present investigation was noticed and indicating the minimal influence of environment and presence of high genetic variability for the trait in the experimental material. Hence, selection based on phenotype in these genotypes can also be effective for improvement of seed yield. High heritability to plant height (cm), days to 50% flowering, days to maturity, pod length, 100 seed weight, protein estimation and medium heritability to seed yield (kg/4.05 m^2). High GAM to plant height and seed yield demonstrates the presence of additive gene effect indicating effectiveness of selection for improvement of these traits. Mahalanobis D^2 analysis suggested the maximum contribution of seed yield (74.87%) towards genetic diversity followed by Plant height (8.08%), Days to Maturity (7.69%), Pod length (4.36%), Days to 50% flowering (3.59%), Seedling Dry Weight (0.64%), Protein Estimation (0.64%), 100 seed weight (0.13%). All 40 genotypes were grouped into 12 clusters. The clustering pattern revealed that genetic diversity was associated with geographical diversity in the present research. Based on mean performances, the genotypes

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PUSA-9072, MLGG-21-2, IC-436557, MLGG-21-6, RMP-21-11, Gouri, MLGG-21-3, MGG-512, MGG-519 from these clusters can be directly used as parents in the hybridization programme. The output of this study is characterization of greengram genotypes for DUS characters and other traits. This study helps in identification of genotypes with suitable traits, helps in registration of lines with PPV and FRA and the material can be used in breeding programmes.

Keywords: Vigna radiate; variability; divergence; cluster; yield; protein; seed quality.

1. INTRODUCTION

Greengram [*Vigna radiata* (L.)] is an important food crop in developing countries of Africa, Asia and Latin America as it is an excellent source of high quality proteins (22-24%), essential aminoacids, vitamins and minerals [1]. It is relatively drought tolerant and well adapted to a varied soil conditions including light soils and can also thrive well even under limited irrigation [2]. The crop is also very well suited to crop rotation and fits well in various multiple and intercropping systems because of its short duration and helps in replenishing the soil as it can be incorporated as green manure after harvest. In India about 35.79 lakh ha area was covered under greengram during 2020-21 as against 30.75 lakh ha during the period in 2019-20 with the total production of 2.5 million tones and a productivity of 548 kg/ha. India contributes more than 70% of world's greengram production (Agricultural Market Intelligence Centre, PJTSAU, 2021).

Yield is a complex trait and highly influenced by the environment. The main cause of low yield in greengram is its indeterminate growth, non-synchronous maturity and losses due to pests and diseases. The breeding programmes in greengram mainly emphasize on yield improvement, hence knowledge of genetic variation and identifying agro-morphological traits associated with yield and their percent contribution will help in successful development of high yielding varieties.

Recombination breeding and trait manipulation are potential alternatives to develop high yielding varieties with determinate growth habit. In addition, variability estimation helps breeders to understand the genetic relationships among accessions and to select the superior accession in a more systemic and effective way [3]. Genetic diversity is important for crop improvement as well as its conservation, evaluation and utilization [4].

The highly self-pollinated nature of greengram reduces the natural variability which ultimately narrows down the effects of the selection process. But, the success of the selection programme in plant breeding depends on the magnitude of genetic variability in the population. Genetic variability and their quantification for qualitative and quantitative characters of economic importance are prerequisites for any crop improvement programme. Hence, the knowledge of variability, heritability and genetic advance become important for efficient breeding and it has great significance for the breeders in order to select the best genotypes for yield enhancement [5]. Exhaustive characterization of mungbean gene pool with utilization of superior lines in breeding programmes will help in development of superior varieties with desired characteristics like resistance to diseases and insect pests, pod shedding, synchronous maturity, larger seeds, higher seed quality and yield [6]. To increase the productivity, there is a need to evaluate a large set of genotypes for their effective performance to cope up with the abiotic stresses prevalent in this area. Thus, current research aims at studying the greengram varieties for its genetic variability and to evaluate the performance of different genotypes.

2. MATERIALS AND METHODS

2.1 Description of the Study Area

An experiment was carried out to study genotypic variability and divergence in greengram using 40 genotypes during Summer, 2022 at Research Farm, Seed Research and Technology Centre, Professor Jayashankar Telangana State Agricultural University, Rajendranagar, Hyderabad. Sixteen genotypes under study were received from Agricultural Research Station, Madhira and the remaining from Regional Agricultural Research Station, Warangal. Each genotype was sown in three rows of 3 m length at a spacing of 45 x 15 cm. The crop was raised by following all the crop management practices for effective varietal characterization of genotypes which was recommended for greengram.

2.2 Research Design

The field experiment was laid out in Randomized Block Design (RBD) with three replications and forty treatment combinations. The average plant population was maintained as per DUS guidelines. Five healthy plants of each genotype were randomly selected in each replication and were labelled. Field observations were taken at different crop growth stages such as cotyledons Unfolded stage, 50% flowering stage, premature pod development stage, fully developed green pods stage, harvest maturity stage. Twenty four DUS descriptors were recorded like Hypocotyl pigmentation, time of flowering, plant growth habit, Plant habit, stem colour, stem pubescence, leaflet lobes, leaf shape, leaf colour, leaf vein colour, petiole colour, leaf size, flower colour of petal, premature pod colour, pod pubescence, pod position, plant height, pod colour, pod curvature of mature pod, pod length, seed colour, seed lusture, seed shape, seed size etc [7,8].

Plant morphological characters such as days to 50% flowering, days to maturity, plant height (cm), Pod length (cm), number of clusters per plant, number of pods per cluster, number of seeds per pod, 100 seed weight (g), seed yield per plot (kg) etc. were recorded.

Seed physiological traits namely germination %, field emergence, seedling length, seedling dry weight(mg), seedling vigour indices (I & II) and biochemical traits i.e., protein estimation were determined for all the forty genotypes.
The percentages of germination, seedling vigour index-I, II (No.s) and field emergence percentages are as follows:

Germination (%) = Number of normal seedlings / Number of total seeds kept for germination test X 100

Seedling Vigour Index-I= Germination (%) x (seedling length) (cm)
Seedling Vigour Index-II = Germination (%) x seedling dry weight (g)

Field emergence % = Number of seedlings emerged on eighth day / Number of total seeds sown x 100

Total nitrogen is estimated by the micro- Kjeldahl method as per procedure suggested by AOAC (1995).

The micro-Kjeldahl method consists of the three steps;

1. Digestion
2. Distillation and
3. Titration.

Reagents:

1. Concentrated sulphuric acid (H_2SO_4).
2. Catalyst mixture: Mix with 250 g potassium sulphate (K_2SO_4), 50 g cupric sulphate ($CuSO_4$. 5 H_2O) and 5 g metallic selenium powder in the ratio of 50:10:1.
3. 40 % sodium hydroxide (NaOH).
4. 4 % boric acid containing 20 - 25 ml mixed indicator /litre.
5. Mixed indicator: 0.066 g methyl red + 0.099 g bromocresol green dissolve in 100 ml of 95 % alcohol.
6. 0.02 N sulphuric acid (H_2SO_4).

2.3 Data Collection Procedures

1. Digestion:

Weigh 0.5 g of prepared plant sample and transfer it to the digestion tube. Add 10 ml of concentrated sulphuric acid and 5 g of catalyst mixture to the sample. Load the digestion tubes into the digester and heat the digestion block. Switch on the digestion unit and set the initial temperature 100℃ till frothing is over. Then block temperature is raised to 400℃. The effective digestion starts only at 360℃ and beyond 410℃. The sample turns light green colour or colourless at the end of the digestion process.

2. Distillation:

After cooling the digestion tube, load the tube in distillation unit and other side of those keep 20 ml of 4% boric acid with mixed indicator in 250 ml conical flask. 40 ml NaOH (40%) is automatically added by distillation programme. The digestion sample is heated by passing steam at a steady rate and the liberated ammonia absorbed in 20 ml of 4% boric acid containing mixed indicator solution kept in a 250 ml conical flask. With the absorption of ammonia, the pinkish colour turns to green. Nearly 150 ml of distillate is collected in about 8 minutes. Simultaneously, blank sample (without plant) is to be run.

3. Titration:

The green colour distillate is titrating with 0.02 N sulphuric acid and the colour changes to original shade (pinkish colour). Note the blank & sample titer reading (ml) and calculate the total nitrogen content present in plant samples.

Calculations:

Nitrogen content in plant (%) = R (Sample titer-blank titer) x Normality of acid x Atomic weight of nitrogen x 100 / Sample weight (g) x 1000

= R x 0.1 x 14 x 100 / 0.5 x 100

Factor = R x 0.28

Crude protein content:

The total nitrogen is estimated by micro-Kjeldahl method as per procedure suggested by AOAC (1995) and the crude protein is calculated by the following formula:

Crude protein content (%) = micro-Kjeldahl nitrogen content (%) x 6.25 (based on the assumptions that nitrogen constitutes 16 % of protein).

2.4 Data Analysis

The data collected for the above mentioned characters were analyzed by using statistical software (SAS).

3. RESULTS AND DISCUSSION

Analysis of variance (Table 1) revealed that all the quantitative traits *viz.,* days to 50% flowering, plant height (cm), days to maturity, pod length (cm), number of clusters per plant, number of pods per cluster, number of seeds per pod, seed yield per plot (kg), 100 seed weight (g), germination (%), field emergence (%), seedling vigour index-I, seedling vigour index-II and protein estimation showed significant variation among the forty genotypes. The results of present study are in line with those of Ramyashree et al. [9], Arshad et al. [10], Zafar et al. [11], Reni and Rao [12] in Soybean who observed a wide range of variability for traits under study.

Information on mean phenotypic coefficient of variation, genotypic coefficient of variation, heritability and genetic advance for these traits is furnished in Table 2.

Under variability study, it was observed that the estimates of PCV was higher than GCV for all the parameters.

This indicating the presence of environmental influence on expression of all the characters (Table 2) as earlier reported by Tyagi and Khan [13] in Lentil.

High (>20%) PCV and high GCV for seed yield (kg/4.05 m^2) in the present investigation was noticed.

This indicating the presence of high genetic variability for the trait and minimal influence of environment in the experimental material. Hence, selection based on phenotype in these genotypes can also be effective for improvement of seed yield. Similar results were reported earlier by Mishra and Verma [14] and Hasib *et al.* [15] in Rice.

Table 1. Analysis of variance for yield and laboratory parameters in greengram

Sl.No.	Character	Mean Sum of Squares		
		Replicates (d.f=2)	Treatments (d.f=39)	Error (d.f=78)
1	Days to 50% flowering	1.075	42.735 ***	1.195
2	Days to maturity	2.158	43.596 ***	0.731
3	Plant height (cm)	4.322	152.421 ***	2.747
4	Pod length (cm)	0.959	1.172 ***	0.494
5	No.of clusters	1.913	2.966 ***	0.938
6	No.of pods per cluster	0.408	4.074 ***	1.203
7	No.of seeds per pod	1.570	2.542 ***	0.937
8	Seed yield (kg/4.05 m^2)	0.026	0.051 ***	0.009
9	100 Seed Weight (g)	0.131	0.701 ***	0.114
10	Germination	9.608	5.196*	3.334
11	Seedling dry weight (mg)	0.001	0.003 ***	0.001
12	Vigour Index-I	1118225.83	72360.949	101739.944
13	Vigour Index-II	16.249	26.453 ***	6.005
14	Field emergence	13.108	6.680	4.758
15	Protein estimation	1.131	16.987 ***	1.413

significant at 1% level* **significant at 5% level* * significant at 10 % level*

Table 2. Genetic parameters for quantitative traits among greengram genotypes

S.No.	Character	Mean	Range		Std. Error (+)	PCV (%)	GCV (%)	h^2 (b) (%)	GA (%)	GAM (%)
			Min.	Max.						
1	Plant height(cm)	42.37	32.23	58.42	0.95	17.12	16.67	94.78	14.16	33.43
2	Pod length (cm)	7.85	6.64	9.68	0.40	8.16	7.86	92.92	1.22	15.61
3	No.of clusters (No.s)	8.01	5.93	9.46	0.55	15.85	10.26	41.90	1.09	13.68
4	No.of pods per cluster (No.s)	8.23	6.13	10.96	0.63	17.84	11.87	44.30	1.34	16.28
5	No.of seeds per pod (No.s)	10.41	8.40	13.00	0.55	12.74	6.11	23.05	0.63	6.05
6	Days to 50% flowering (days)	47.00	39.66	54.00	0.63	8.25	7.91	92.06	7.35	15.64
7	Days to maturity (days)	69.35	63.00	76.00	0.49	5.58	5.45	95.13	7.59	10.95
8	Germination (%)	96.18	93.00	98.33	1.05	2.20	0.58	7.00	0.30	0.31
9	Seedling dry weight(mg)	0.22	0.18	0.33	0.01	16.27	11.95	53.98	0.04	18.09
10	Vigour index-I	2238.59	1943.50	2575.13	184.15	10.987	3.44	9.81	49.71	2.22
11	Vigour index-II	22.06	18.16	32.13	1.41	16.22	11.83	53.16	3.92	17.77
12	Field emergence (%)	91.53	88.33	94.00	1.25	2.53	0.87	11.87	0.56	0.62
13	100 seed weight (g)	4.09	3.59	5.54	0.19	13.27	11.01	68.79	0.76	18.81
14	Protein estimation (%)	23.92	19.72	28.93	0.68	10.41	9.70	86.82	4.45	18.62
15	Yield (kgs)	0.40	0.17	0.76	0.05	37.26	28.87	60.00	0.24	59.05

PCV : Phenotypic coefficient of variation, GCV: Genotypic coefficient of variation, GA: Genetic Advance
GAM: Genetic advancement as percent of mean, h^2 (b) : Heritability in broad sense

Table 3. Distribution of greengram genotypes in different clusters based on analysis of morphological data

Cluster number	No.of genotypes	Name of the genotype
I	17	Janakalyani, RMP-20-10, RMP-20-14, RMP-21-9, RMP-20-11, RMP-20-12, RMP-21-7, RMP-21-8, Mdr-Local, PLM-858, IC-282136, TJM-03, RMP-21-4, RMP-21-5, RMP-20-5, MLGG-21-1, MGG-453.
II	2	MGG-512, MGG-519
III	1	RMP-21-11
IV	10	TARM-18, RMP-20-18, RMP-21-2, RMP-21-3, KM-17-129, RMP-20-1, RMP-21-17, MLGG-21-4, MGG-474, RMP-20-8.
V	1	Gouri
VI	1	MLGG-21-3
VII	1	MLGG-21-2
VIII	1	MH-521
IX	1	RMP-20-2
X	2	IC-436557, MLGG-21-6
XI	2	MGG-389, MLGG-21-9
XII	1	PUSA-9072

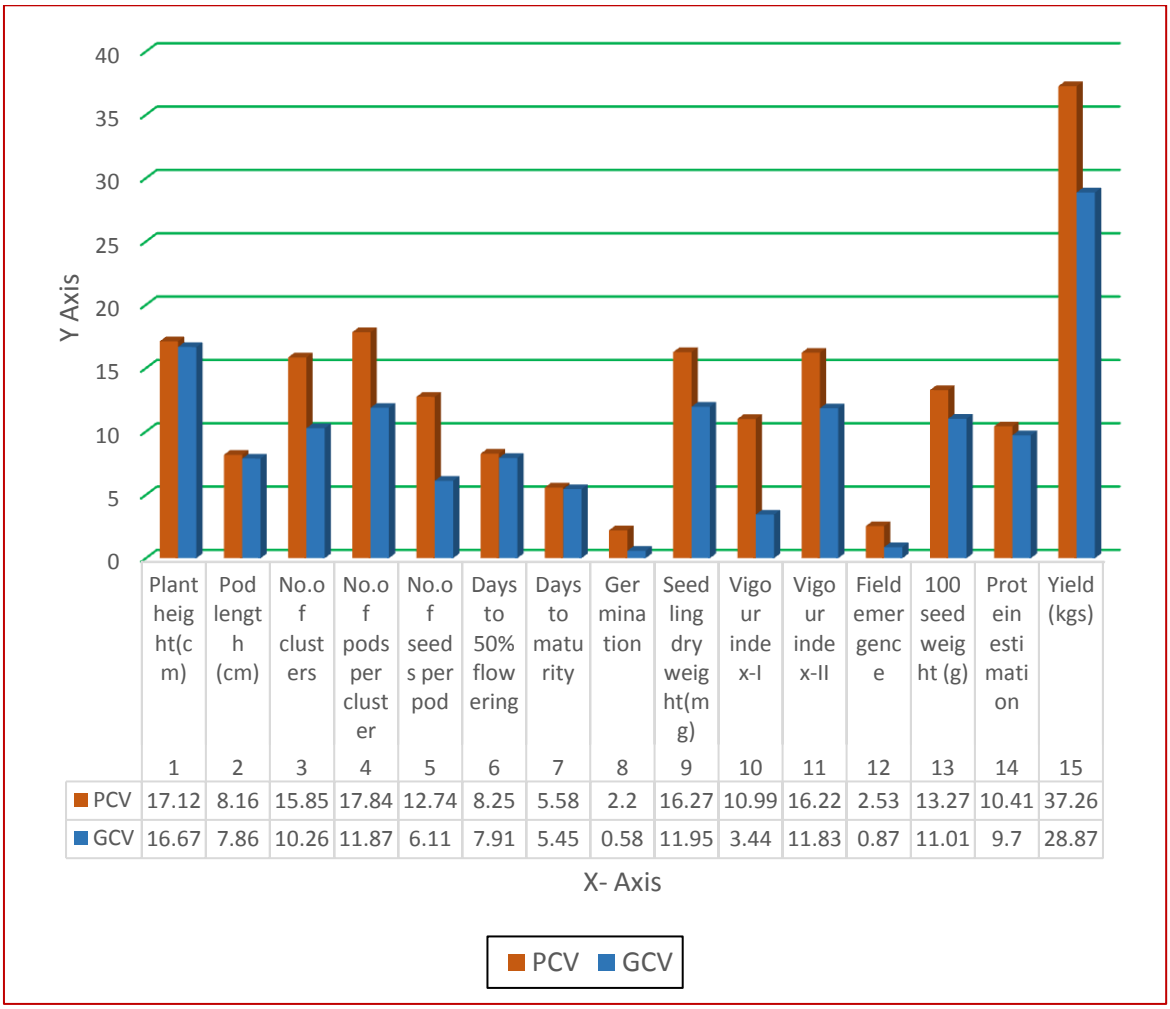
Table 4. Contribution of the different characters towards genetic divergence among forty greengram genotypes

S.No.	Characters	Times ranked first	Contribution (%)	Cumulative contribution (%)
1	Plant height (cm)	63	8.08	8.08
2	Pod length (cm)	34	4.36	12.44
3	No. of Clusters	0	0.00	12.44
4	No. of Pods per cluster	0	0.00	12.44
5	No. of seeds per pod	0	0.00	12.44
6	Days to 50% flowering	28	3.59	16.03
7	Days to Maturity	60	7.69	23.72
8	Germination %	0	0.00	23.72
9	Seedling Dry Weight (mg)	5	0.64	24.36
10	Vigour Index-I	0	0.00	24.36
11	vigour Index-II	0	0.00	24.36
12	Field Emergence	0	0.00	24.36
13	100 seed weight (g)	1	0.13	24.49
14	Protein Estimation (%)	5	0.64	25.13
15	Yield (kg/4.05 m^2)	584	74.87	100.00

The difference between PCV and GCV was very small for plant height, pod length, days to 50% flowering, days to maturity.

This indicating the greater role of genetic factors in expression of these characters and lower influence of environment indicating rapid progress from selection for these traits. These results are in accordance with Aditya et al. [16] in Soybean.

Plant height (cm), number of clusters per plant, seedling dry weight (mg), vigour index-II, 100 seed weight (g) showed moderate magnitudes of PCV and GCV. This suggests that there is a scope to enrich the variation for these characters as they possess high heritability coupled with GAM. High heritability to plant height (cm), days to 50% flowering, days to maturity, pod length, 100 seed weight, protein estimation and medium heritability to seed yield (kg/4.05 m^2). High GAM to plant height (cm) and seed yield (kg/4.05 m^2). This specifies the presence of additive gene effect representing effectiveness of selection for improvement of these traits. Praveen kumar *et al*. (2005) recorded similar results for plant height, 100 seed weight and seed yield per plant in Soybean. High heritability accompanied by



	Plant height(cm) 1	Pod length (cm) 2	No.of clusters 3	No.of pods per cluster 4	No.of seeds per pod 5	Days to 50% flowering 6	Days to maturity 7	Germination 8	Seedling dry weight(mg) 9	Vigour index-I 10	Vigour index-II 11	Field emergence 12	100 seed weight (g) 13	Protein estimation 14	Yield (kgs) 15
PCV	17.12	8.16	15.85	17.84	12.74	8.25	5.58	2.2	16.27	10.99	16.22	2.53	13.27	10.41	37.26
GCV	16.67	7.86	10.26	11.87	6.11	7.91	5.45	0.58	11.95	3.44	11.83	0.87	11.01	9.7	28.87

Fig. 1. Graph showing the values of Phenotypic and Genotypic coefficients of variation for fifteen components in greengram

moderate genetic advance as percent of mean was reported for pod length, days to 50% flowering, days to maturity, 100 seed weight, protein estimation. This indicating the role of both additive and non-additive gene effects for control of the characters. The results are in conformity with the reports of Seyoum *et al.* (2012) for plant height in Rice. High heritability accompanied by low genetic advance was reported for pod length, days to 50% flowering, days to maturity, 100 seed weight, protein estimation and seed yield (kg/4.05 m^2).This suggesting the role of non additive gene action for the expression of these characters which are agree with the results of Aditya et al*.* [16] in soybean. In the present study, high PCV coupled with high genetic advance as percent of mean were observed for seed yield (kg/4.05 m^2). This indicating the pre-ponderance of additive gene action and therefore scope for the improvement of the trait through selection. Similar results were reported earlier by Mohana Krishna et al. [17] in Rice. The diversity studies based on the Mahalanobis D^2 values resulted considerable genetic diversity and grouped all the genotypes into twelve clusters. Cluster I was very large containing seventeen genotypes followed by cluster IV with ten genotypes. Clusters II, X and XI with two genotypes each and clusters II, III, V, VI, VII, VIII, IX and XII having single genotype each. The clustering pattern revealed that genetic diversity was associated with geographical diversity in the present research. Similar results were reported by Lavanya et al. (2014), Patel et al. [18], Kingsly et al. [19], Divyaramakrishnan and Savithramma [20] in greengram, Ramyshree *et al.* (2016) in Soybean. Among all the cluster means, Cluster II recorded highest mean values for seed yield. Cluster III was recorded highest mean values for number of pods per cluster, germination. Cluster

V was recorded highest mean values for seedling dry weight, seedling vigour index-II and field emergence. Seedling vigour index-I and hundred seed weight were recorded highest mean values in cluster VI. Cluster VII is having highest mean values for plant height and days to 50 percent flowering. Cluster X recorded highest mean values for number of clusters, number of seeds per pod, seedling vigour index-I. Cluster XII recorded highest mean values for pod length and protein estimation.

Out of the fifteen characters studied genotypes from Seed yield was recorded highest in cluster II (MGG-512, MGG-519), highest number of clusters and highest number of seeds per pod was recorded in cluster X (IC-436557, MLGG-21-6), highest number of pods per cluster were recorded in cluster-III (RMP-21-11), Cluster XII (PUSA-9072) was recorded maximum protein estimation (28.66).

The above results revealed that selection of genotypes having high values for particular character may be useful for the hybridization programme for the improvement of the trait. It is understandable that no other cluster consisted at least one genotype with all the desirable characters, there will be possibility of selecting directly one genotype for immediate use. Therefore, hybridization among the selected genotypes with the divergent clusters are important to obtain all the desired characters.

Hence the genotypes PUSA-9072, MLGG-21-2, IC-436557, MLGG-21-6, RMP-21-11, Gouri, MLGG-21-3, MGG-512, MGG-519 from these clusters can be directly used as parents in the hybridization programme.

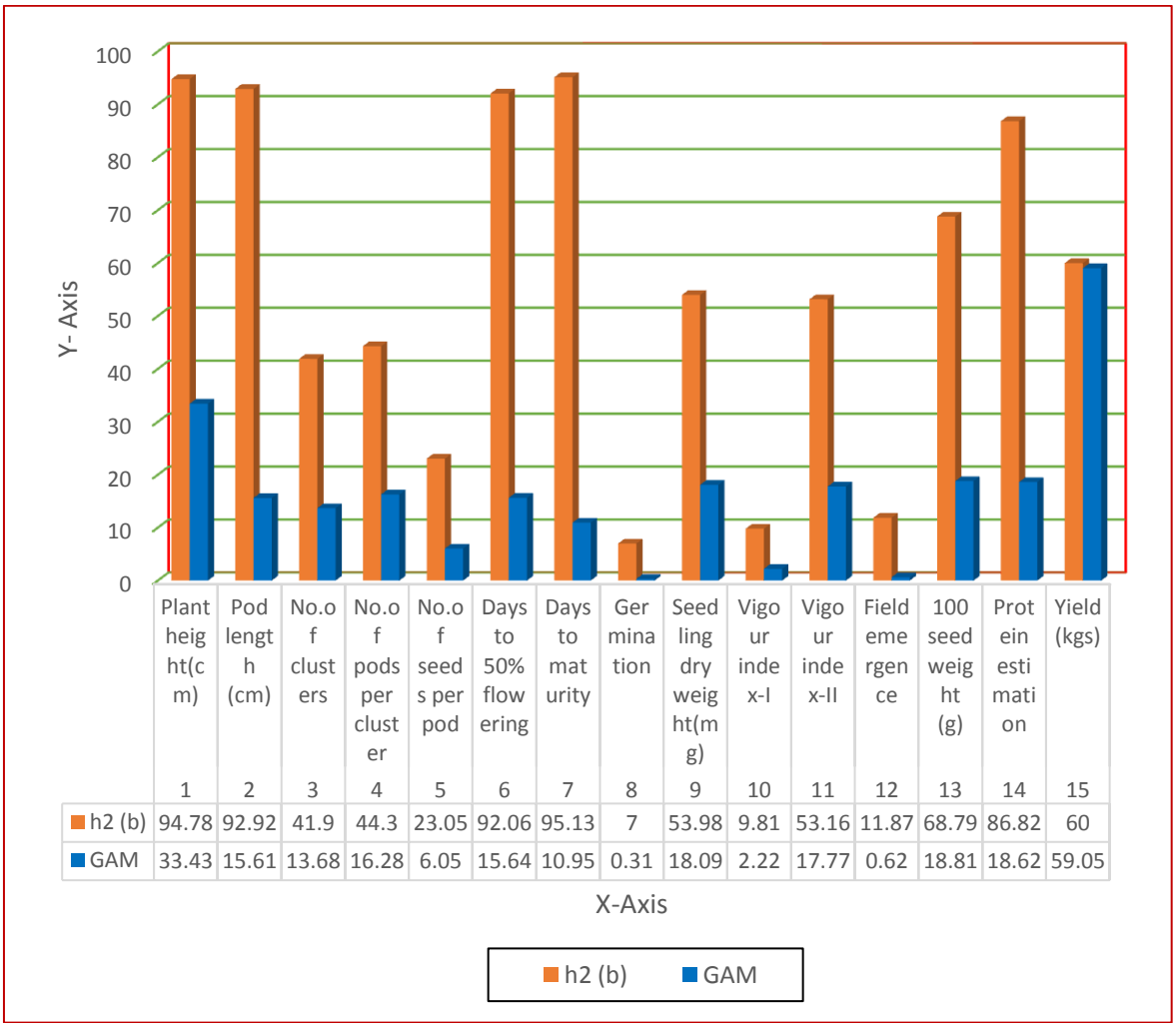


Fig. 2. Graph showing the values of heritability (h^2, broad sense) and genetic advance as percent of mean (GAM) for fifteen components of greengram

The percentage contribution of each trait towards genetic divergence among the greengram genotypes was estimated and presented in Table 4. Among the characters, seed yield showed maximum contribution (74.87%) towards genetic diversity followed by Plant height (8.08%), Days to Maturity (7.69%), Pod length (4.36%), Days to 50% flowering (3.59%), Seedling Dry Weight (0.64%), Protein Estimation (0.64%), 100 seed weight (0.13%). Remaining quantitative characters such as Number of Clusters, Number of Pods per cluster, Number of seeds per pod, Germination, Vigour Index-I, vigour Index-II, Field Emergence have not shown any contribution towards diversity.

4. CONCLUSION

It was concluded that the greengram germplasm under study exhibited a wide range of variability for most of the traits. Some genotypes possessed desirable genes for more than one character and hence could be utilized directly or included in hybridization programme for variety development suitable for southern zone of Telangana. The genotypes PUSA-9072, MLGG-21-2, IC-436557, MLGG-21-6, RMP-21-11, Gouri, MLGG-21-3, MGG-512, MGG-519 from these clusters can be directly used as parents in the hybridization programme. Morpho-physiological and biochemical characterization of the mungbean germplasm will help to identify trait specific germplasm for crop improvement programme which will also be helpful for plant variety protection and the advanced germplasm material can be used in the future breeding programmes.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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