Original Research Article

Evaluation of Greengram (*Vigna radiata* L.) Germplasm for Seed Yield and Yield Related Traits Through Cluster Analysis

ABSTRACT

An investigation was conducted at the Agricultural Research Station, Madhira during the *rabi,* 2017-18 to study the genetic divergence and clustering pattern among 39 greengram germplasm accessions in Randomized Block Design (RBD) replicated twice for eight quantitative traits. Principal component analysis revealed that the first three principal components-PC I, PC II, and PC III—contributed about 83.95% of the variation for the understudied genotypes. According to a cluster analysis, genotypes can be divided into seven different groups, with cluster I being the largest with a maximum of 27 genotypes. Cluster III is second largest with 5 genotypes, and cluster II is third with 3 genotypes. The mono genotypic cluster indicated by genotypes IC-436634, IC-426458, IC-261272 and WGG-42 indicates the presence of diversity for generating variability through hybridization. The maximum distance inside cluster III was 54.11, while the greatest distance between clusters IV and VII was 507.95. Data on cluster means for various traits revealed that cluster VI had the highest mean value for the number of clusters per plant, the number of pods per plant, and the number of seeds produced per plant. The total genetic diversity was considerably influenced by both plant height and the quantity of pods per plant, Data on cluster means for various features revealed that cluster VI had the highest mean value for the number of clusters per plant, the number of pods per plant, and the number of seeds produced per plant. Plant height made the largest contribution (27.25%) and the number of pods per plant made the smallest contribution (20.23%) to the total genetic diversity. For the purpose of retrieving superior recombinants in segregating generations, the genotypes in the clusters with the greatest inter cluster distance may be included in the hybridization programme.

Key words: Greengram, germplasm, cluster analysis, genetic divergence and D^2 statistics

1. INTRODUCTION

One of the most widely cultivated short-duration grain legumes in India that complements all cropping systems is greengram (*Vigna radiata* (L.). Among the food legume crops grown in India, it is the third leading pulse crop of India next to chickpea and pigeon pea. It is a major source of protein for vegetarian diets and is grown during the *kharif* (both as a sole and intercrop), *rabi* and summer seasons because of its low water requirements and also suitable as a drought-tolerant crop. India is the major producer of green gram in the world and grown in almost all the States. It is grown in about 4.5 million hectares with the total production of 2.5 million tonnes with a productivity of 548 kg/ha and contributing 10 % to the

total pulse production. According to Government of India 3 rd advance estimates, greengram production in 2020-21 is at 2.64 million tones [www.indiastat.com]. The crop is well adapted to a variety of soils, and also enhances soil physical properties and fertility due to the presence of root nodules. Because of inadequate agronomic management, cultivars' intrinsic low yield potential and susceptibility to viral disease, the production of green gram has not grown significantly [25]. As green gram is highly self-pollinated, diversity within or across species or varieties is substantial [2, 5]. Creation of variability and selection of superior recombinants among the variants are the major objective of any plant breeding programme. One of the constraints listed for lack of breakthrough in green gram production has been the lack of genetic variability for high yield potential [26]. Lack of sufficient genetic diversity in the basic gene pool is one of the major factors in the greengram improvement programme [15, 8]. Plant breeders have a great potential to create novel, enhanced cultivars with desirable traits due to genetic diversity investigations [4]. For the purpose of genetic improvement or plant breeding, characterizing morphological and agronomical traits, as well as assessing phenotypic diversity, is crucial [24]. Breeders must study genetic diversity in plant genetic resources in order to establish suitable breeding techniques that take advantage of important variation, enhancing our knowledge of population genetics and evolutionary relationships and choose germplasm in a more methodical and effective manner [17]. Better cultivars could be created in a self-pollinated crop like greengram by using genetically diverse parents with the intention of combining suitable recombinants for specific trait improvement, followed by proper selection in segregating generations [25]. Multivariate statistics, a highly effective approach for measuring genetic variation, provide the most trustworthy evidence for the actual genetic distances between the tested genotypes. Principal component analysis and cluster analysis using Mahalanobis modified distance (D^2) statistics are the most used multivariate techniques for determining the best genotypes and estimating the variability of quantitative traits [34] and [14].

2. MATERIAL AND METHODS

During *rabi* season (October to December) of 2017–18, an experiment was conducted at PJTSAU, Agricultural Research Station, Madhira. The farm is situated at an altitude of 189 m AMSL and at coordinates 17° 58 'North Latitude, 78° -44' East Longitude. The experimental material was comprised of 39 different germplasm accessions that were kept at the research site. Randomized block design with two replications was used to evaluate the germplasm lines. Each entry was planted in two rows measuring each of 4 meters long, with a 30 cm and 10 cm space between row to row and plant to plant respectively. Recommended fertilizer dose of 16:50 kg ha-1 of N:P and timely plant protection measures were taken to

grow a healthy crop. Following standard procedures, observations were made on five randomly chosen plants per replication for each of the eight quantitative traits viz., days to 50% flowering, days to maturity, plant height (cm), number of clusters/plant, number of pods/plant, 100 seed weight (g), seed yield/plant (g) and seed yield (kg/ha). On a plot-by-plot basis, data on days to 50% flowering and days to maturity were recorded and the results were statistically analyzed.

2.1.Statistical analysis

2.1.1 Estimates of genetic divergence

Using the Generes statistical programme, the Mahalanobis D^2 statistic was used to determine the degree of genetic divergence and the Tocher's approach was used to classify the germplasm accessions into different clusters. Data recorded on characters were used for Mahalanobis D^2 statistics (Mahalanobis' [18]) as as described by CR Rao [27] to group the genotypes into different clusters. The variables were divided into various clusters using Tocher's approach based on the D^2 values obtained (CR Rao [27]).

Mahalanobis D^2 analysis between two genotypes estimated on the basis of the 'p' characters is given by the equation:

D2ij= (Yit-Yjt) 2

Where,

Yit = uncorrelated mean value of the ith genotype for t character

Yjt = uncorrelated mean value of the jth genotype for t character

D2ij = D2 between ith and jth genotype

In the present study 8 characters (p = 1 to 8) were used to perform the above analysis.

By placing each character according to transformed uncorrelated values, the percentage contribution of each trait to the overall divergence has been computed. When all of the characters' ranks added up to 100, the percent contribution for each trait was calculated. The recorded data has been analyzed using WINDOSTAT software.

2.1.2 Principal component analysis

Principal component analysis for yield and yield related traits was done by using WINDOSTAT software. The criteria followed for selecting the principal components to be included in further analysis was based on Eigen values of principal components. Multivariate statistical techniques which simultaneously analyze multiple measurements on each individual under investigation are widely used in analysis of genetic diversity irrespective of the data set. It was taken that Eigen values above unity indicated that the evaluated principal component weight is reliable.

3. RESULTS AND DISCUSSION

3.1 Analysis of variance

The analysis of variance revealed highly significant variation in all of the examined features between the germplasm accessions, indicating that the experimental materials were highly variable (Table-1). Garg et al. [10], Shwetha et al. [31] and Ravi et al. [28] also reported similar findings.

Table : 1 Analysis of Variance for yield and yield component traits of greengram

Source of Variation	DF	Mean Sum of Squares							
		Days to 50% flowering	Days to maturity	Plant height (cm)	Number of clusters/ plant	Number of pods/ plant	100 seed weight (g)	Seed yield/ plant (g)	Seed yield (kg/ ha)
Replications	1	0.01	1.28	5.13	2.41	12.72	0.13	3.53	225293.10
Genotypes	38	22.18**	22.02**	484.87**	9.71**	91.80**	0.65**	15.14**	946381.21**
Error	38	0.75	0.99	7.27	1.21	11.98	0.05	1.60	100945.00

***P=0.01 Probability level, DF: Degrees of freedom*

3.2 Principal component analysis

The significant contribution of the key contributors to the total variance along each axis of distinction is indicated using principal component analysis (PCA) as suggested by Sharma, [30]. While each appropriate vector coefficient demonstrates the extent to which each original variable contributed to each principal component with which it was correlated, it also assesses the relevance and contribution of each element to the total variance. According to the partitioning of overall variance using PCA (Figure 1), for the analyzed germplasm lines, three principal components (PC I, PC II, and PC III) accounted for about 83.95% of the overall variation. These three PCs, PC I, PC II, and PC III, explained 48.13, 23.83, and 11.98% of the total variance, respectively (Table 2). The original eight variables can be examined using these five principal component scores in any subsequent research. These findings concurred with those of Divyaramakrishnan and Savithramma, [7], Kumar et al. [16] and Nainu et al. [23]. Cluster analysis with UPGMC was used to further support the PCA results (Unweighted Paired Group Method using Centroids).

Table: 2 Principal component analysis for yield and yield component traits in greengram genotypes

		1 Vector	2 Vector	3 Vector
	Eigene value (Root)	882.534	436.901	219.583
	% Var. Exp.	48.143	23.833	11.978
	Cum. Var. Exp.	48.143	71.976	83.955
1	Days to 50% flowering	0.203	0.720	0.069
2	Days to maturity	0.160	0.535	-0.342
3	Plant height (cm)	-0.856	0.170	-0.294
4	Number of clusters plant^{-1}	0.100	0.097	-0.022
5	Number of pods plant^{-1}	0.060	0.242	0.487
6	100 seed weight (g)	-0.183	-0.053	0.656
7	Seed yield plant^{-1} (g)	0.368	-0.265	-0.218
8	Seed yield (kg ha^{-1})	0.137	-0.163	-0.276

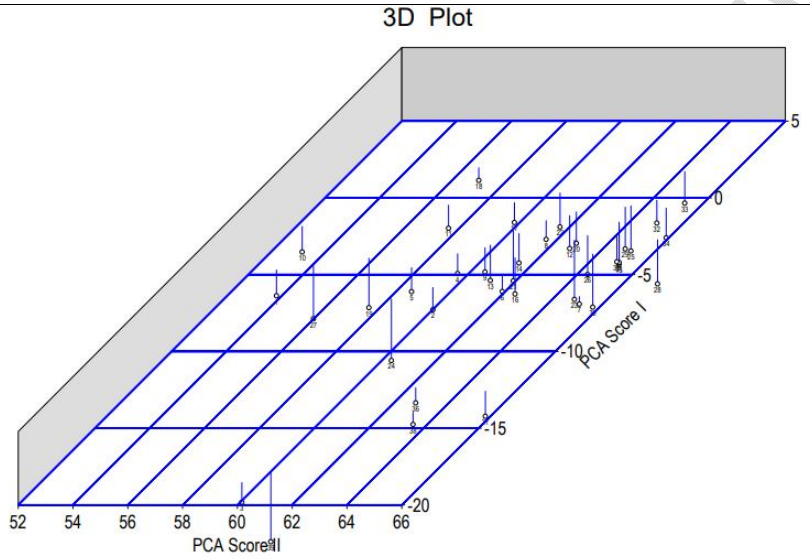

Figure 1: Principal component analysis diagram for greengram germplasm

3.3 Grouping of genotypes into different clusters

The 39 greengram germplasm accessions were separated into seven distinct groups (Table 3 and Figure 2). Cluster II includes three genotypes, Cluster III has five and Cluster I has a maximum of 27 genotypes, suggesting that the genotypes included in the study had a wide range of diversity. The genotypes, IC-436634, IC-426458, IC-261272 and WGG-42 may have completely different genetic make-ups, leading to the establishment of independent clusters that are also designated to be more varied than other clusters. Clusters IV, V, VI, and VII were solitary clusters or mono-genotypic clusters (Figure 2). Similar findings were also documented by Chaudhary et al. [6], Garg et al. [10] and Jakhar and Kumar [12]. This

D^2 analysis result shows that the examined greengram accessions exhibit high genetic diversity and it offers an excellent opportunity to choose parents for the improvement programme from these varied clusters.

Table 3: Distribution of greengram genotypes into different clusters.

Cluster	No. of accessions	Genotypes
I	27	IC-436563, IC-436570, IC-436531, IC-282070, IC-282083, IC-249570, IC-282115, IC-436526, IC-436630, IC-436548, IC-436611, IC-436555, IC-436668, IC-436648, IC-436715, IC-436762, IC-436810, IC-436712, IC-436658, IC-436908, IC-436824, IC-436844, IC-436573, IC-436673, IC-436775, IC-436738, IC-436945
II	3	IPM-02-14, MGG-347, MGG-295
III	5	IC-249567, IC-436542, IC-436637, IC-436731, IC-436700
IV	1	IC-436634
V	1	IC-426458
VI	1	IC-261272
VII	1	WGG-42

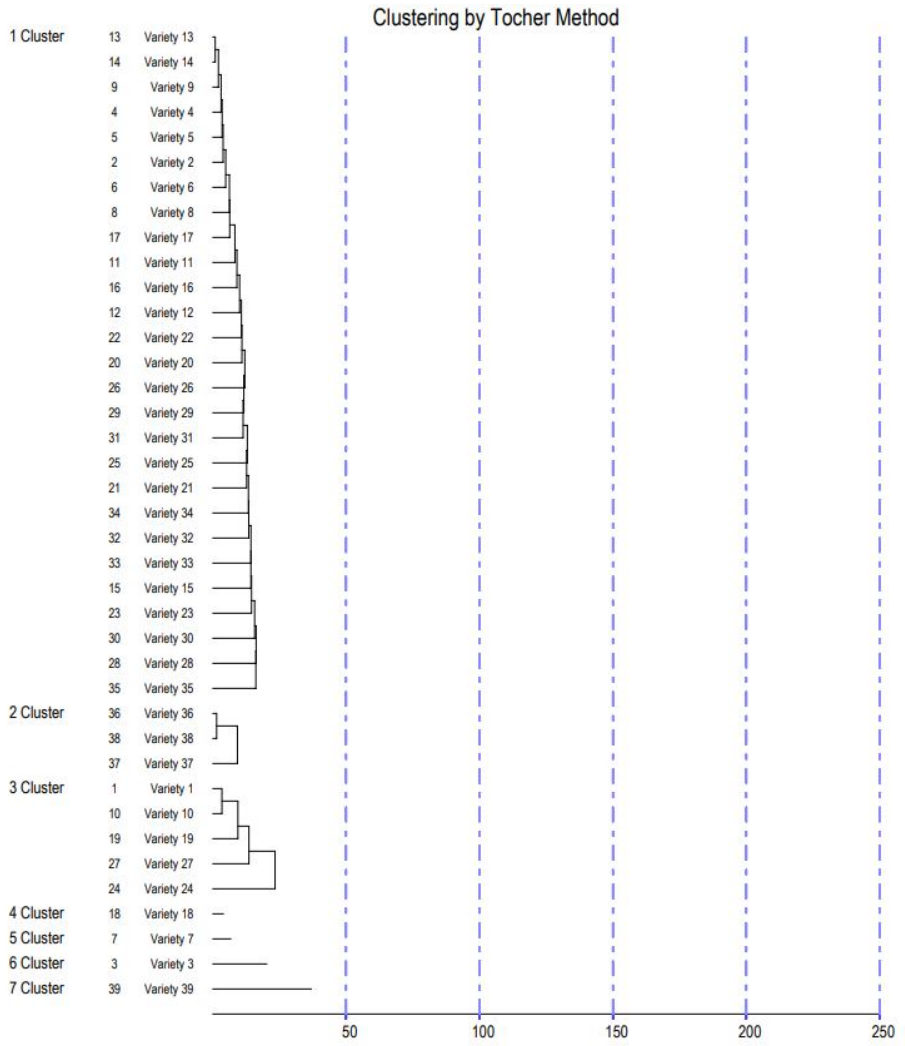

Figure 2: Dendrogram showing clustering of greengram germplasm

3.4. Cluster distances and cluster means

Table 4 displays the genetic divergence among the greengram genotypes as determined by intra and inter-cluster distances for seven different clusters (Figure 3). The D2 values for the 39 genotypes inside and between clusters clearly demonstrate that there is restricted genetic variation within a cluster when inter-cluster distances are greater than intra-cluster distances. Cluster III had the greatest intra-cluster distance (54.11), followed by Cluster I (35.2) and Cluster II (26). Clusters VI, V, VI and VII were mono genotypic and displayed no intra-cluster distance. As a result, they were less heterogeneous, suggesting that the genetic architecture of the different genotypes found in these clusters would differ. The genotypes appeared to have arisen from a shared gene pool if they were grouped together in clusters with the smallest intra-cluster distance. Earlier, Patel et al. [25], Divyaramakrishnan and

Savithramma [7] and Suhel Mehandi et al. [36] and others documented similar types of results. The inter cluster distance varied from 63.68 between clusters I and V (the smallest value) to 507.95 between clusters IV and VII (the largest value). Other inter cluster distances with higher values include, 443.1 between cluster IV and cluster VI followed by 325.26 between cluster I and cluster VII, 308.84 between cluster I and cluster VI, 296.31 between cluster III and cluster VII, 290.11 between cluster II and cluster IV, 285.15 between cluster III and cluster VI and 274.03 between cluster V and cluster VII. The examination of means in table 3 showed that inter-cluster distances were greater than intra-cluster distances, indicating that there was greater genotypic diversity present in these clusters and that it might be advantageous for members of these clusters to hybridize, which could result in a heterotic response. Similar findings have been reported by researchers like Mehandi et al. [21], Singh et al. [32] (2015), Jayamani and Sathya [13] (2015) and Ravi et al. [28]. The importance of genetic diversity was emphasised by Gadakh et al. [10] , Garje et al. [9], Abna et al. [1] and others. High genetic variation and genetic gain under selection must result from this. Genetically more divergent genotypes can be found in clusters with a maximum intra-cluster distance and hybridization between divergent clusters is likely to produce desirable segregants with a wide range of variability. Crosses with parents from clusters IV and VII, which are the two most divergent clusters, are expected to result in the largest amount of heterosis, followed by crosses with parents from clusters IV and VI. These findings concur with past studies by Mahalingam et al., [19,] Sneha et al., [34] and Sridhar et al. [35]. Wide variability is anticipated in the progeny of these crossings, increasing the opportunity to identify transgressive segregants in subsequent generations and aiding in the selection of appropriate genotypes to increase seed yield of greengram.

The cluster mean computed for eight characters (Table 5) showed that the days to 50% flowering and days to maturity cluster means ranged from 34.8 and 58.7 days in cluster III to 43 and 69.5 days in cluster V with an overall mean of 38.28 and 63.65 days correspondingly in cluster III and V. Clusters VI and IV had higher and lower plant heights (cm) of 76 and 11, respectively. With respect to the number of clusters per plant, number of pods per plant and seed yield per plant, Cluster VI had the greatest mean values of 15.75, 47.25 and 17.2 (g), respectively with corresponding overall means of 8.45, 25.72, and 9.38 (g). This showed that none of the clusters had genotypes that had all the desirable traits that could be chosen and used. The findings of the current study thus imply that inter-cluster distance must be taken into account when choosing parents for hybridization, since, it may provide a wide spectrum of diversity in the segregating generations (Sneha et al., [34], Sen and De [29], Mohan et al., [22] and Shweta [31].

Table 4: Intra (bold) and inter cluster average distances among clusters of greengram genotypes.

Cluster	I	II	III	IV	V	VI	VII
I	**35.2**	163.34	102.11	71.22	63.68	308.84	325.26
II		**26**	201.01	290.11	66.04	84.92	166.19
III			**54.11**	95.52	171.22	285.15	296.31
IV				**0**	141.38	443.1	507.95
V					**0**	194.66	274.03
VI						**0**	98.76
VII							**0**

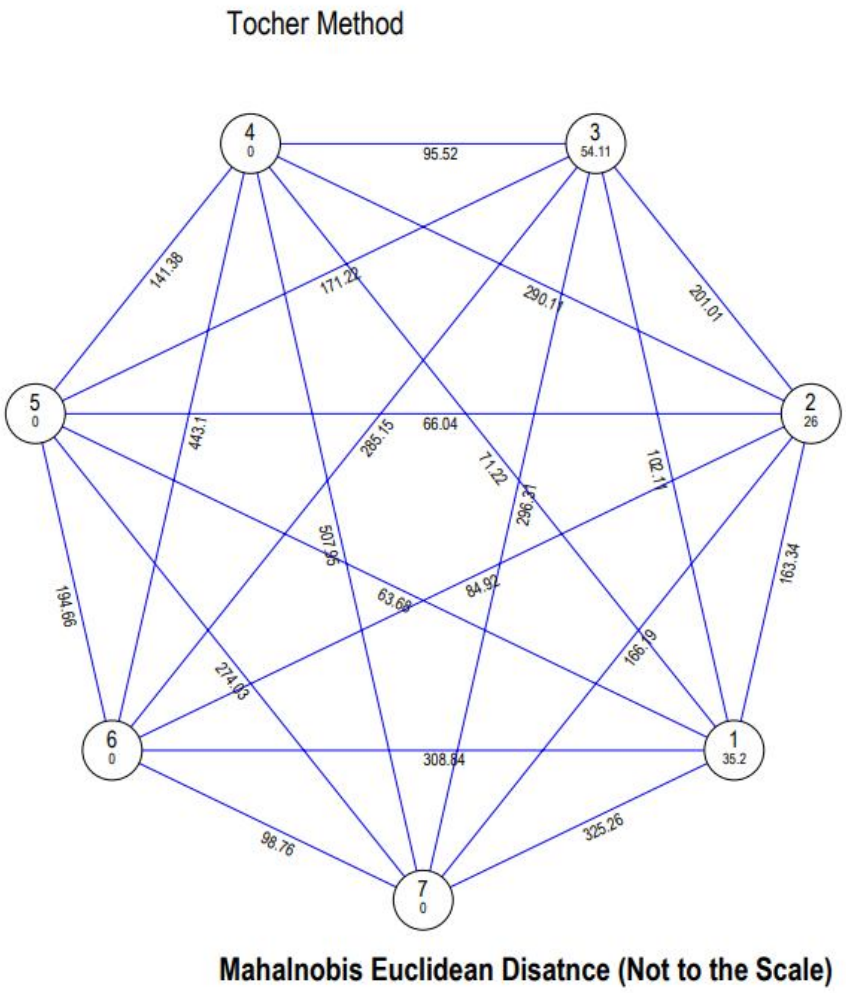

Figure 3: Mahalnobis Euclidean distances for greengram germplasm

Table 5: Cluster mean values for different component traits in greengram.

Cluster	Days to 50% flowering	Days to maturity	Plant height (cm)	Number of clusters plant^{-1}	Number of pods plant^{-1}	100 seed weight (g)	Seed yield plant^{-1} (g)	Seed yield (kg ha^{-1})
I	41.5	66.2	22.78	6.41	20.02	3.63	7.66	1913
II	39.67	66.17	64	8.72	26.93	3.21	9.18	2301
III	34.8	58.7	24.34	6.74	21.35	3.86	8.58	2147
IV	37.5	64.5	11	5.4	16.2	2.75	4.8	1202
V	43	69.5	43.65	7.25	21.75	2.8	6.6	1653

VI	35	61.5	76	15.75	47.25	3.32	17.2	4302
VII	36.5	59	56.5	8.85	26.55	5.36	11.65	2913
Over all mean	**38.28**	**63.65**	**42.61**	**8.45**	**25.72**	**3.56**	**9.38**	**2347**

3.5. Percent contribution towards genetic divergence

Table 6 illustrates how several traits included in the current study contributed differently to genetic divergence. The major contribution was made by plant height (27.25%), followed by the number of pods per plant (20.23%), days to 50% flowering (19.83%), days to maturity (10.39%), seed yield per plant (8.36%), 100 seed weight (7.69%), seed yield (kg/ha) (4.45%) and number of clusters per plant (1.75%). Plant height, number of pods per plant, the days till 50% flowering and the seed yield per plant all made the largest contribution to the total divergence. Since each trait contributes a certain percentage to the cluster mean, genotypes from clusters VI and VII would be promising candidates for use as breeding stock in a hybridization programme. These findings concur with prior studies by Sneha et al. [34], Malli and Lavanya, [20], Singh et al. [33] and Basnet et al. [3].

Table 6: Percent contribution of different traits to genetic divergence in greengram.

Character	Times ranked 1st	Contribution %
Days to 50% flowering	147	19.83
Days to maturity	77	10.39
Plant height (cm)	202	27.25
Number of clusters plant^{-1}	13	1.75
Number of pods plant^{-1}	150	20.23
100 seed weight (g)	57	7.69
Seed yield plant^{-1} (g)	62	8.36
Seed yield (kg ha^{-1})	33	4.45

4. CONCLUSION

While selecting parents for hybridization inter-cluster distance must be taken into consideration that may provide a wide spectrum of variation in the segregating generations. The average inter-cluster D^2 values among the 39 genotypes were highest between clusters IV and VII, then between clusters IV and VI. In order to obtain superior transgressive segregants, the genotypes from clusters IV (IC-436634), I (27 genotypes) and WGG-42 from cluster VII, which has the largest inter cluster distance, can be employed in hybridization. For

further enhancement of seed yield and its components, the cluster means for various morphological and yield characters, including number of clusters/plant, number of pods/plant, seed yield/plant and equally 100 seed weight should be given emphasis.

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