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Association and diversity analysis for yield attributing traits in advance generation of green gram (*Vigna radiata* (L.) Wilczek)

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Abstract
The present investigation reveals the association between yield and its contributing traits in a set of advanced generation of green gram genotypes. Seed yield per plant showed significant and positive correlation with plant height followed by pod length, pods per cluster and pods per plant whereas, 100 seed weight and harvest index showed positive but non-significant correlation. Path analysis indicated that the pod length has the highest positive direct effect on seed yield per plant, followed by harvest index, primary branches per plant, 100 seed weight, pods per cluster, protein content and pods per plant. Cluster analysis revealed divergence in 25 accessions by making four clusters. Cluster II and cluster IV showed highest inter cluster distance signifying that hybridisation between genotypes from these two clusters will exploit heterosis at a greater level. Selection criteria based on pod length, pods per cluster, protein content, 100 seed weight and seed yield per plant can be devised for further improvement of these genotypes.

Keywords: Green gram, cluster analysis, divergence, path analysis

Introduction
Mung bean [*Vigna radiata* (L.) Wilczek] is the third most important legume crop grown in India after chickpea and pigeon pea (Ahmad *et al*. 2019) [2]. It is also known as moong, green bean, moong bean and golden gram. It is a self-pollinated crop with diploid nature having 2n=2x=22 chromosomes and a genome size of 579 Mb (Kang *et al*. 2014) [13]. Nowadays, domestic consumption of green gram has increased, because of its rising popularity in Indian ethnic foods and perceived health benefits (Datta *et al*. 2012) [5]. It is a good source of minerals (calcium, iron, zinc, potassium and phosphorus), vitamins (folate and vitamin K) and dietary fibres (Keatinge *et al*. 2011) [14]. Along with this, it is a staple source of plant-based protein in India completing a balanced human diet. Its average protein content is nearly 24 per cent which is rich in lysine, an essential amino acid for humans (Ramakrishnan *et al*. 2018) [30]. Moreover, green gram has a rich nutritional profile, its 100 g of mature grain contains, 1.2 g of fat, 62.6 g of carbohydrate, 16.3 g dietary fibre and 347 kilocalories of energy (Majhi *et al*. 2020) [21]. Apart from their nutritional benefit they also play an important role in cropping systems due to the short life cycle and the ability to fix nitrogen through rhizobacteria resulting in increased soil fertility and improved soil health (Shiv *et al*. 2017) [33].

According to fourth advanced estimates (2019-20), the annual production of green gram in India is 2460 thousand tonnes whereas total production in 2018-19 was 2455.37 thousand tonnes with productivity of 516 kg/hectare (Anonymous, 2020) [3] which makes India largest producer of green gram. Despite this, India still lacks self-sufficiency to feed its growing population in case of green gram. The main reasons behind this problem includes smaller seed size, susceptibility to different viral diseases (Latha *et al*. 2019) [19] and poor genetic makeup (Narasimhulu *et al*. 2016) [24] of green gram. To make India self-sufficient in green gram production, breaking the present bottleneck in its productivity is the need of time. To achieve these goals, our research unleashes different traits which affects the green gram yield in direct or indirect manner and acquaint us with the knowledge to employ them for increasing yield gains.

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Green gram has moderate genetic variability but low yield potential due to multiplicative interactions of various yield attributing traits affected by environmental factors. For overcoming these problems, an association study is an important strategy for devising selection criteria for yield and its component traits. Correlation coefficient analysis measures the mutual relationships between various plant characters pairs and determines the component characters on which selection can be based for improvement in yield (Singh, 2001) [35]. Yield depends on many other component characters which are further related with each other, making correlation studies important. Determination of correlation and path coefficients between yield and its component traits is important for the selection of desirable plant types in green gram breeding programmes. Hence, path analysis was done to determine the direct and indirect effect of the independent components on the dependent component. Success in recombination breeding rely on the adequate selection of genotypes as parents for yielding high heterotic crosses and transgressive segregants (Shiv *et al*. 2017) [33]. Also, diversity has a key role in plant breeding experiments as it has capacity to buffer against different types of environments. Hence, Genetic divergence can be used as alternative parameter with reasonable efficacy in the selection of parental lines (Shweta, 2013) [34]. Still, in these recent years very few studies have been reported regarding diversity analysis using this method in green gram. To fill this gap in green gram research, this population was also analysed using D square test to know if any diversity present in these genotypes.

Materials and Methods
A set of twenty genotypes of advance generation along with five check varieties raised in randomized block design (Table 1). The research material was grown in three replications during summer, 2018 at the experimental area of College farm, N. M. College of Agriculture, Navsari Agricultural University, Navsari, Gujarat, India. Each genotype planted in a single row comprising 20 plants with 60 cm x 15 cm inter and intra row spacing respectively. All recommended cultural practices were followed to raise a healthy crop. Ten desirable plants were selected based on phenotypic superiority from each genotype to record the data on ten morphological traits *viz*, Days to 50 per cent flowering, plant height, primary branches per plant, pods per cluster, pods per plant, pod length, seed per pod, 100 seed weight, harvest index, seed yield per plant and one quality parameter i.e. protein content. Regarding protein content, seed harvested from ten individual plants were bulked with in a genotype in each replication. These pooled samples were then used for estimation of protein content using micro Kjeldahl method which was done by chromic acid digestion followed by distillation and was quantified by titrating with sulphuric acid (Trivedi *et al*. 1999) [39]. To determine the association between different characters phenotypic and genotypic correlations were computed as per the method of Panse and Sukhatme (1957) [27]. The cause and effect relationship between two variables cannot be known from a simple correlation coefficient. Therefore, path analysis suggested by Wright (1921) [40] and Dewey and Lu (1959) [6] was applied. The estimation of genetic divergence among these genotypes was done through Mahalanobis D square analysis (1936) [20]. Following this the classification of genotypes into separate clusters was carried out using the tocher's process.

Table 1: List of genotypes subjected to association studies and diversity analysis

Sr. No.	Genotype	Pedigree	Sr. no	Genotype	Pedigree
1.	13A	Meha × Pusa vishal	14.	56C	Meha × GM-4
2.	34A	Meha × Pusa vishal	15.	59C (10)	Meha × GM-4
3.	34A (7)	Meha × Pusa vishal	16.	59C (5)	Meha × GM-4
4.	42A	Meha × Pusa vishal	17.	65C	Meha × GM-4
5.	62A	Meha × Pusa vishal	18.	79C	Meha × GM-4
6.	64A	Meha × Pusa vishal	19.	37D	Meha × GJM-1008
7.	48B	Meha × GJM-1006	20.	47D	Meha × GJM-1008
8.	65B	Meha × GJM-1006	21.	Meha	Check variety
9.	94B	Meha × GJM-1006	22.	GAM-5	Check variety
10.	95B	Meha × GJM-1006	23.	GM-6	Check variety
11.	18C	Meha × GM-4	24.	GM-7	Check variety
12.	19C	Meha × GM-4	25.	GM-4	Check variety
13.	40C	Meha × GM-4			

Result and Discussion
Yield is a complex trait affected by many other contributing component traits. Being a quantitative trait in nature it is affected by genotype (G) × environment (E) interaction. Hence to bring a change in yield, a deep understanding of the extent of interrelationships among yield and yield attributing traits is necessary.

Correlation studies
Correlation studies analyses the relationship of dependent variable yield with its independent component traits; thus, the association of various traits would determine their relative significance to improve yield. In the present research, the majority of the cases have high genotypic correlation as compared to their phenotypic correlations for all the characters (Table 2) indicating little influence of environment and the presence of an inherent association between various characters. Seed yield per plant was found highly significant and positively correlated with plant height ($r_g = 0.52$) followed by pod length ($r_g = 0.49$), pods per cluster ($r_g = 0.48$) and pods per plant ($r_g = 0.44$). The positive association of plant height, pod length, pods per cluster and pods per plant with seed yield per plant were also reported by Ramakrishnan *et al*. (2018) [30], Ahmad *et al*. (2019) [2], Mohammed *et al*. (2020) [22] and Majhi *et al*. (2020) [21]. The days to 50 per cent flowering has a non-significant but negative correlation with seed yield per plant which supports the report of Ahmad *et al*. (2019) [2]. On the contrary, Majhi *et al*. (2020) [21] found significant and negative correlation of days to 50 per cent flowering with seed yield per plant. Harvest index ($r_g=0.15$) and 100 seed weight ($r_g=0.13$) showed positive but non-significant association with seed yield per plant. Supporting

these results, Majhi *et al.* (2020) [21] in his two crosses and Mohan *et al.* (2019) [23] found positive non-significant association of 100 seed weight with seed yield per plant. Protein content showed non-significant associations with seed yield per plant and at phenotypic level, it was negatively correlated indicating that increase in protein content may reduce yield. Kumar *et al.* (2013) [16] also reported negative association of protein content with seed yield. In all the cases, however, more focus should be placed on the genotypic correlations as more genetic gain due to correlated response to selection can be achieved from these heritable correlations. Similar results in green gram were also reported by Tabasum *et al.* (2010) [37], Javed *et al.* (2014) [12], Ahmad *et al.* (2014) [1], Pathak *et al.* (2014) [29], Hemavathy *et al.* (2015) [11] and Keerthinandan *et al.* (2016) [15].

Table 2: Genotypic and phenotypic correlations of seed yield per plant with other characters in 25 genotypes of green gram

Characters		PH (cm)	PB	PPC	PPP	PL (cm)	SPP	100 SW (g)	HI (%)	PC (%)	SYP (g)
DF	r_g	0.39**	0.82**	0.12	0.58**	-0.44**	-0.20	-0.29*	-0.22	0.46**	-0.12
	r_p	0.23*	0.37**	-0.02	0.22	-0.17	0.06	-0.21	-0.17	0.37**	-0.10
PH (cm)	r_g		0.93**	-0.038	0.98**	0.13	-0.054	-0.16	0.02	0.19	0.52**
	r_p		0.22	0.14	0.43**	0.22	0.100	-0.11	0.05	0.13	0.37**
PB	r_g			0.64**	1.84**	-0.14	0.08	-0.71**	-0.27*	0.51**	-0.01
	r_p			0.027	0.28*	-0.15	-0.02	-0.25*	0.09	0.19	-0.03
PPC	r_g				0.094	-0.15	0.05	-0.09	-0.27*	-0.032	0.48**
	r_p				0.29*	0.20	0.30**	-0.07	-0.03	-0.018	0.36**
PPP	r_g					-0.32**	-0.37**	-0.47**	-0.46**	0.063	0.44**
	r_p					0.02	0.09	-0.10	0.03	0.048	0.16
PL (cm)	r_g						0.79**	0.73**	0.09	-0.003	0.49**
	r_p						0.58**	0.38**	0.08	-0.013	0.26*
SPP	r_g							0.34**	0.12	-0.14	-0.01
	r_p							0.17	0.22	-0.077	0.10
100 SW (g)	r_g								-0.32**	0.007	0.13
	r_p								-0.17	0.005	0.17
HI (%)	r_g									-0.63**	0.15
	r_p									-0.44**	0.22
PC (%)	r_g										0.11
	r_p										-0.08

*, ** Significant at 5.0 and 1.0 per cent level of significance, respectively

r_g = Genotypic correlation coefficient, r_p = Phenotypic correlation coefficient

DF: Days to 50% flowering, PH: Plant height (cm), PB: Primary branches per plant, PPC: Pods per cluster, PPP: Pods per plant, PL: Pod length (cm), SPP: Seed per pod, 100 SW: 100 Seed weight (g), HI: Harvest index (%), PC: Protein content (%), SYP: Seed yield per plant (g)

Path coefficient analysis

Among the component traits studied, Path analysis revealed that the traits like pod length (1.15), harvest index (1.13), primary branches per plant (0.89), 100 seed weight (0.70), pods per cluster (0.54), protein content (0.45) and pods per plant (0.24) exhibited high and positive direct effects on seed yield per plant as depicted in Table 3. Similar results were also obtained by Gadakh *et al.* (2013) [8], Kumar *et al.* (2013) [17], Patel *et al.* (2014) [28], Titumeer *et al.* (2014) [38], Raturi *et al.* (2015) [32], Garg *et al.* (2017) [9], Ramakrishnan *et al.* (2018) [30], Ahmad *et al.* (2019) [2], Mohammed *et al.* (2020) [22]. The above-mentioned traits were identified as superior seed yield components. Therefore, the genotypes having good performance for these characters can be used for further improvement of green gram. Conclusively, there is a true relationship between these characters and the seed yield per plant and direct selection for these characters can be carried out for the green gram improvement programs. A negative direct effect was observed on seed yield per plant with days to flowering (-0.35), plant height (-0.60) and seeds per pod (-1.35) indicating the selection for these traits may have an undesirable impact on yield. A negative direct effect of similar traits including days to flowering, plant height and seeds per pod on seed yield per plant was also observe by Rathor *et al.* (2015) [31], Ramakrishnan *et al.* (2018) [30], Azam *et al.* (2018) [4], Mohan *et al.* (2019) [23] and Ahmad *et al.* (2019) [2]. Moreover, the residual effect at genotypic level was 0.15 which suggest that the traits under study could capture around 85 per cent of the accountable variation which contributes towards yield improvement in green gram. The overall view of path analysis pointed out that for improving yield in green gram, selection advantage should be given to more pods per cluster, pods per plant, pod length and harvest index. As Pod length has the high positive direct effect on seed yield per plant along with positive and highly significant genotypic correlation it can be used as principle character during selection for improving yield.

Table 3: Genotypic path coefficient analysis depicting direct and indirect effects of ten different traits on seed yield per plant of green gram

Characters	DF	PH (cm)	PB	PPC	PPP	PL (cm)	SPP	100 SW (g)	HI (%)	PC (%)	Correlation with SYP (g)
DF	-0.35	-0.14	-0.28	-0.04	-0.20	0.15	0.07	0.10	0.08	-0.16	-0.12
PH (cm)	-0.23	-0.60	-0.55	0.02	-0.59	-0.08	0.03	0.10	-0.01	-0.11	0.52**
PB	0.73	0.82	0.89	0.57	1.64	-0.12	0.08	-0.63	-0.24	0.46	-0.01
PPC	0.07	-0.02	0.35	0.54	0.05	-0.08	0.03	-0.05	-0.15	-0.02	0.48**
PPP	0.14	0.24	0.44	0.02	0.24	-0.08	-0.09	-0.11	-0.11	0.02	0.44**
PL (cm)	-0.50	0.15	-0.16	-0.18	-0.37	1.15	0.92	0.84	0.11	0.00	0.49**
SPP	0.27	0.07	-0.12	-0.07	0.49	-1.07	-1.35	-0.46	-0.16	0.19	-0.01
100 SW (g)	-0.20	-0.11	-0.50	-0.07	-0.33	0.51	0.24	0.70	-0.22	0.01	0.13

| HI (%) | -0.25 | 0.02 | -0.31 | -0.31 | -0.52 | 0.11 | 0.14 | -0.36 | 1.13 | -0.71 | 0.15 |
| PC (%) | 0.21 | 0.09 | 0.23 | -0.01 | 0.03 | 0.00 | -0.06 | 0.00 | -0.28 | 0.45 | 0.11 |

*, ** Significant at 5.0 and 1.0 per cent level of significance, respectively. Residual effect = 0.15 (Bold figures show direct effect)
DF**:** Days to 50% flowering**,** PH: Plant height (cm), PB: Primary branches per plant, PPC: Pods per cluster, PPP: Pods per plant, PL: Pod length (cm), SPP: Seed per pod, 100 SW: 100 Seed weight (g), HI: Harvest index (%), PC: Protein content (%), SYP: Seed yield per plant (g)

D Square Analysis

D^2 analysis is a statistical method which is used for measuring genetic divergence among different accessions. D^2 analysis assists in identifying the diverse genotypes and grouping of the same based on genetic similarity for target traits (Gauhar *et al*. 2018) [10]. All the 25 accessions were grouped under four clusters based on Mahalanobis (1936) [20] D^2 methods of clustering (Figure 1). Among all the clusters, cluster II had the highest accessions followed by cluster III, Cluster I and cluster IV (Table 4). Cluster I to cluster IV was characterized by the highest mean value for days to 50 per cent flowering followed by plant height (Table 5). Moreover, cluster II also has high mean value for harvest index, cluster III for pods per plant, cluster I and cluster IV for protein content. Intra cluster distance reveals variability present in accessions occurring within the same cluster, higher intra cluster distance indicates that accessions have the potential to evolve more divergent breeding material and can be improved to attain maximum genetic advance (Singh *et al*. 2010). Furthermore, in our study intra cluster distance for cluster IV (D=29.9) was highest followed by Cluster III (D=23.46), Cluster I (18.52) and Cluster II (D=14.4) showing that cluster IV has comparatively more ability to develop divergent material. The inter cluster distance was highest (Figure 1) between the two clusters I and II (456.02) suggesting that hybridisation between genotypes from these two clusters may give many desirable segregants possessing different sets of genes which will also aid in generating some useful material after practicing selection. Following this, inter cluster distance between cluster II and cluster IV (263.87) was highest tailed by cluster II and cluster III (134.22), cluster I and cluster III (123.69), cluster I and cluster IV (114.84), cluster III and cluster IV (89.22). It is also advised that for capturing maximum variability, inter-cluster distance can be taken as a parameter for the selection of parents for the hybridization program (Natarajan *et al.*, 1988) [25]. The Relative contribution of each character to the total divergence was maximum for protein content (76.67%) similar results were also reported by Gadakh (2011) [7] and Panigrahi *et al.* (2014) [26]. The other major contributing traits were 100 seed weight (17%) and seed yield per plant (2.67%) as these are yield affecting traits so practicing selection on these traits may give desirable results. The contribution of remaining traits includes, Days to 50 per cent flowering (1%), Plant height (1%), harvest index (0.67%), pods per cluster (0.33%), pod length (0.33%) and seed per pod (0.33%). Considering the contribution of related traits to total divergence, focus can be driven towards such trait for further improvement.

Table 4: Cluster formation pattern of the green gram accessions

Cluster No.	No. of genotypes	Name of accessions
Cluster I	6	34A, 42A, 40C, 65, 56C, 64A
Cluster II	8	34A (7), 48B, 65B, 94B, 95B, 59C (5), 37D, 47D
Cluster III	7	13A, 62A, Meha, 18C, 19C, 59C (10), 79C
Cluster IV	4	GAM-5, GM-6, GM-7, GM-4

Table 5: Character means of various clusters for yield and its components

	DF	PH (cm)	PB	PPC	PPP	PL (cm)	SPP	SYP(g)	100 SW (g)	HI (%)	PC (%)
Cluster 1	50.61	47.14	2.92	3.28	18.84	6.71	9.44	3.69	3.98	15.07	29.27
Cluster 2	48.29	45.29	2.61	3.31	17.59	6.84	9.69	3.67	4.05	21.14	8.95
Cluster 3	48.81	44.94	2.80	3.36	20.77	6.73	9.38	4.25	3.90	19.13	19.40
Cluster 4	47.42	44.25	2.40	3.39	16.76	7.57	9.94	4.75	5.05	16.76	20.78

DF**:** Days to 50% flowering**,** PH: Plant height (cm), PB: Primary branches per plant, PPC: Pods per cluster, PPP: Pods per plant, PL: Pod length (cm), SPP: Seed per pod, 100 SW: 100 Seed weight (g), HI: Harvest index (%), PC: Protein content (%), SYP: Seed yield per plant (g)
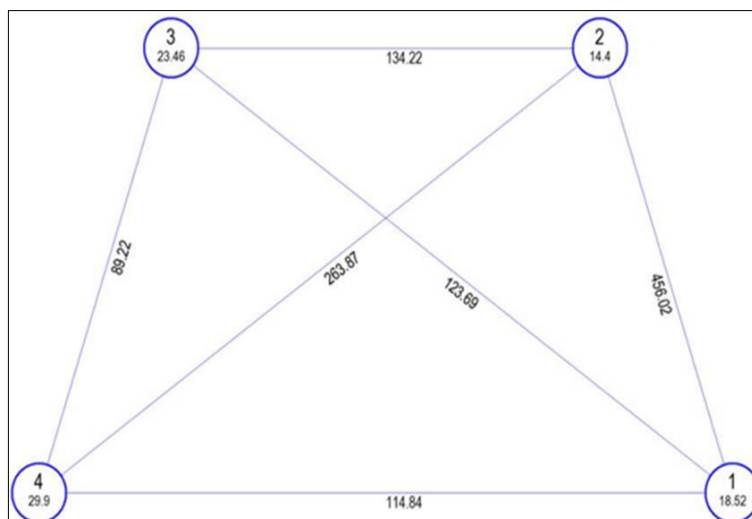
Fig 1: Cluster diagram for 25 genotypes of green gram

Conclusion

Based on association studies pod length and pods per cluster are the key components traits which contributes towards yield improvement in green gram. Following cluster analysis, selection of genotypes from cluster II and cluster IV for heterosis breeding will be effective as they are the most diverse groups. Protein content, 100 seed weight and seed yield per plant have high contribution towards total genetic divergence showing scope for selection criteria. Hence, selection for pod length, pods per cluster, protein content, 100 seed weight and seed yield per plant will lead to generate an improved population through a breeding programme enhancing the yield of green gram.

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