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Diversity analysis of mungbean (*Vigna radiata* L.) germplasm under different seasons

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Abstract
The investigation was conducted with thirty-six mung bean genotypes under two seasons i.e., *Kharif* 2019 and *zaid* 2020 to identify the diverse genotypes for utilization in hybridization through genetic diversity analysis. Thirty-six genotypes were grouped into seven clusters. Cluster I and cluster II were polygenotypic and were the largest clusters each comprising of 18 and 25 genotypes in *Kharif* and summer respectively. During the *Kharif* season, Cluster II showed maximum intracluster D^2 value. Cluster I showed maximum intracluster D^2 value followed by cluster II for the summer season. The number of seeds per pod (43.33%) and biological yield per plant (g) (44.29%) contributed towards maximum genetic divergence. Entries from cluster II and cluster I were found to be superior and highly diverse, thus suitable as parents for hybridization. The potential genotypes based on the D^2 statistics under both seasons were found as SML 1831, PDM-139, TM-37 and Ganga-8.

Keywords: D^2, divergence, genetic diversity, mungbean

Introduction
Mungbean [*Vigna radiata* (L.) Wilczek] is a prominent pulse crop of Asian countries. Among the thirteen food legumes grown in India, it is the third leading pulse crop of India next to chickpea and pigeon pea. The seeds are a magnificent source of minerals (calcium, iron, zinc, potassium and phosphorus), vitamins (folate and vitamin K), dietary fibres (Keatinge *et al.*, 2011) [1] and are consumed as dal or sprouts. Mungbean is cultivated in all three seasons in India, with *Kharif* being the major season for cultivation. However, summer cultivation from February to June is the most suitable cultivation period when there is plenty of sunshine, high temperature and low humidity that keep insects and disease infestations under check. In India, the total area covered under mungbean is 4.1 m ha with a production of 1.9 m tonnes with a productivity of 467 kg/ha in the country during 2017-18. The area covered under mungbean in Madhya Pradesh is 2.97 lakh ha with a production of 2.20 lakh tons (DAC&FW, 2018) [2]. To increase production and productivity, there is a need of developing high yielding mega varieties of mungbean. Being an autogamous legume crop it faces the constraint of having a narrow genetic base. Recently, mungbean productivity has been unstable due to the impact of biotic and abiotic stresses. The large yield gap requires pre-breeding efforts to broaden the genetic base suitable for screening superior and efficient genotypes. In every crop improvement programme, genetic diversity among the population is required for the improvement of resistance and yield. Genetically diverse parents produce more desirable segregants. Genetic divergence analysis is of prime importance as it helps in formulating an effective breeding programme. Thus, D^2 statistics is a powerful tool to measure genetic divergence within a set of genotypes. It classifies the genotypes in a comprehensive manner into groups of distinct orders based on similarities in one or more traits, and thus points in the right direction in the choice of parents for hybridization. It was reported that the grouping pattern of the diverse genotypes suggested no parallelism between genetic divergence and the geographical distribution of the genotypes (Das *et al.*, 2001) [3]. The present study aims to analyse the genetic diversity of thirty-six recently evolved mungbean genotypes.

Materials and Methods
The investigation was carried out consisting of thirty-six new breeding genotypes of mungbean which includes released varieties and advanced breeding lines. The experiment was conducted during *Kharif* 2019 and Summer 2020 in RCBD having three replications at Seed Breeding Farm, JNKVV, Jabalpur, Madhya Pradesh. Observations were recorded for two phenological traits and eleven quantitative traits.

Five random plants were selected to record observations and the replicated data of traits was subjected to genetic divergence analysis using D^2 statistic (Mahalanobis, 1936) [4] as suggested by C.R. Rao (Rao, 1952) [5]. All the mungbean genotypes were grouped into respective clusters on the basis of values following Tocher's method.

The estimation of 'Ë' (Wilk's criterion) was done using the following relationship.

'Ë' =(E)/ (E+V)

Where

(E)= Determination of error sum of squares and sum of products matrix.

(E+V) =Determination of error + Varieties sum of squares a sum of products matrix

Results

Genetic diversity generalized distance (D2)

Under *Kharif* season, Wilk's Ë criterion value (1.80082) for genetic divergence among 36 genotypes of mungbean was found highly significant (Table 1). The V statistics value (2516.012) was also highly significant at 455 degrees of freedom. Under the summer season, Wilk's Ë criterion value (1.76628) and the V statistics value (1602.274) was highly significant at 455 degrees of freedom for genetic divergence among 36 genotypes of mungbean was found highly significant (Table 2). Genotypes differed significantly when all the traits were considered simultaneously. Highly significant differences within the population for most of the studied traits were noted through the analysis of variance. The D^2 values corresponding to possible comparison among 36 new breeding genotypes were computed separately in the analysis.

Contribution of individual traits towards genetic divergence: Under *Kharif* season, the maximum contribution towards genetic divergence was shown by the number of seeds per pod (43.33%) followed by the number of pods per plant (20.63%), the number of seeds per plant (11.75%), pod length (10.63%), biological yield per plant (3.65%), the trait days to flowering initiation has no contribution towards divergence. (Table 3) Under the summer season, the traits *viz*. biological yield per plant (g) (44.29%) contributed most towards genetic divergence followed by remaining traits in descending order *viz*. 100 seed weight (17.94%), harvest index (9.84%), seed yield per plant (7.94%), days to maturity (5.56%), number of pods per plant (3.97%), number of nodes per plant (3.65%) (Table 3) The characters having meaningful contribution toward total genetic divergence belonged to cluster II. The above results were confirmed when the number of times each character appeared first in rank is considered. The traits that appeared a higher number of times first in ranks as depicted in Fig.1 and Fig. 2 also contributed substantially to a higher percentage of divergence toward total divergence in D^2 statistics.

Grouping of genotypes into different clusters

On the basis of D^2 values, the thirty-six genotypes were grouped into five clusters in *Kharif* whereas in seven clusters under summer season, following Tocher's method (Table 4). Cluster I and cluster II were polygenotypic {largest cluster with 18 genotypes (cluster II) in *Kharif* and 25 genotypes in summer (cluster I), respectively} while the remaining 2 clusters in *Kharif* and 5 clusters in summer were monogenotypic. Cluster IV in *Kharif* consisted of 3 genotypes. Critical examination of clustering patterns reveals that the genotypes originating from different eco-geographical regions were grouped together into different clusters showing similarities in one or more traits. Similar cluster findings have been reported by Tiwari *et al.* (2012) [6], Narasimhulu *et al.* (2013) [7], Thippani *et al.* (2013) [8], Sen *et al.* (2016) [9], Rasal *et al.* (2017) [10], Sofia *et al.* (2017) [11] and Wesly *et al.* (2020) [12].

Intra and inter cluster divergence D^2 values

The average intra-cluster and inter-cluster D^2 values were estimated as per the procedure given by Singh and Choudhary (1979) [13] are presented in Table 5 and the cluster mean values are summarized in Table 6. Genotypes grouped into the same cluster were slightly divergent from one another when the cumulative averages of characters were considered. Statistical distances indicated the genetic diversity among clusters. The average cluster mean values under both seasons are represented in Table 5. During the *Kharif* season, Cluster II showed maximum intra-cluster D^2 value (141.28) followed by cluster I (116.97).

The highest inter-cluster divergence was observed between genotypes of clusters II and V (1317.65), followed by clusters II and III (862.25), II and IV (706.28), IV and V (666.26) and cluster II and I (453.77). During the summer season, Cluster I showed a maximum intra-cluster D^2 value (31.04) followed by cluster II (23.12). The highest inter-cluster divergence was observed between genotypes of cluster V and VII (291.33), followed by cluster VI and VII (237.79) III and VII (196.81), IV and VII (191.20), IV and VII (166.14). The genotypes belonging to the clusters separated by high statistical distance could be used in the hybridization programme for obtaining a wide spectrum of variation among the segregates. It is true that more the divergence between genotypes, grand would be the heterotic outcome obtained when the hybrid programme is planned to develop promising varieties (Bekele *et al*., 2012) [14]. Under *Kharif* season, Cluster I (116.97) was a polygenotypic cluster with 13 genotypes and was nearest to cluster III (221.78), followed in ascending order by cluster IV (260.09), V (399.46) while cluster II (453.77) was farthest or more distantly related to cluster I. Cluster II (141.28) was the largest poly-genotypic cluster with 18 genotypes and was nearest to cluster IV (706.28) followed by cluster III (862.25) however it was placed at a maximum distance to cluster V (1317.65). Cluster III, Cluster VI and Cluster V were mono-genotypic.

Whereas under summer season, Cluster I (31.04) was the largest poly-genotypic cluster with 25 genotypes and was nearest to cluster IV (53.21), following in ascending order were cluster II (59.57), III (61.1), VI (69.18), V (70.32) while cluster VII (130.22) was farthest or more distantly related to cluster I. Cluster II (23.12) was a poly-genotypic cluster with 6 genotypes, but nearest to cluster VII (82.55) followed by cluster IV (132.42), cluster VI (155.41) however it was placed at a maximum distance to cluster V (166.14). Cluster III, Cluster VI, Cluster V, Cluster VI and Cluster VII were mono-genotypic.

Clusters mean showing importance of grouped genotypes: Cluster means for different characters revealed the substantial

differences among the clusters for all the characters (Table 6). Early flowering was observed in the genotypes of cluster V (33.67 days), while delayed flowering was noticed in the genotypes of cluster III (36.67 days). Days to maturity ranged from 65.33 days in cluster V to 67.89 days in cluster II. The cluster means for the number of pod clusters per plant ranged from 2.33 in cluster V to 9.27 in cluster II. The number of pods per plant ranged from 5.55 in cluster III to 19.48 in cluster II. Pod length varied from 5.43 cm in cluster V to 7.79 in cluster II. The seeds per pod were maximum in cluster IV (14.35) and minimum in cluster V (8.06). Seeds per plant varied from 60.00 in cluster V to 223.91 in cluster II. Biological yield and harvest index has shown maximum values in cluster II (14.41 g and 36.81 respectively) while minimum values were recorded by cluster V (8.54 g and 13.89 respectively). Hundred seed weight ranged from 2.08 in cluster V to 2.83 in cluster II. The genotypes in cluster II recorded high seed yield per plant (4.92 g), while genotypes in cluster V recorded low seed yield per plant (1.17 g).

During the summer season, Cluster II recorded the highest value for 100 seed weight (4.33) and seed yield per plant (6.51). Cluster III recorded the highest value for the number of seeds per pod (10.00), the number of seeds per plant (222.10), pod length (8.77) and harvest index (33.92) and while it has low values for the number of nodes per plant (5.66). Cluster IV recorded the highest values for the number of pod clusters per plant (11.11), while it has low values for days to flowering initiation (39.33), the number of seeds per pod (6.77) and seed yield per plant (3.43). Cluster V recorded the least values for most of the traits *viz*. days to maturity (71.00), the number of primary branches per plant (1.66), pod length (7.66). Cluster VI has shown the highest value for most of the traits *viz*. days to flowering initiation (44.00), number of primary branches per plant (3.44), number of nodes per plant (12.55), number of pods per plant (24.44) while it obtained the least value for biological yield per plant (14.94). Cluster VII recorded the highest values for days to maturity (76.67) and biological yield per plant (46.65), while it has low values for the number of pod clusters per plant (4.55), the number of pods per plant (16.11) and the number of seeds per plant (141.18) and harvest index (11.75). Inter-crossing of the genotypes from these clusters could be recommended to create a broad spectrum of variability followed by efficient selection for these characters. These findings were in conformity with the results of Sofia *et al*. (2017) [11].

Conclusion

From the above diversity analysis, significant and high values for different traits reveals that under the *Kharif* season, selection of genotypes for the medium duration from Cluster II and Cluster IV can be rewarding. Genotypes from Cluster II were found superior for yield and yield contributing traits like the number of seeds per plant, pod length, 100 seed weight and seed yield per plant. On the basis of genetic divergence, it can be concluded that genotypes from cluster II under *Kharif* season recorded high cluster mean values for most of the economic traits, and genotypes from Cluster I and Cluster II are found to be highly diverse with large inter-cluster distances and thus can be utilized in hybridization programme. Divergence studies under the summer season revealed that for yield contributing traits such as the number of seeds per plant, 100 seed weight, biological yield and seed yield per plant, genotypes from Cluster II have recorded higher cluster mean values and thus could be selected as superior parents in hybridization programme. Also, genotypes from cluster I and cluster II have shown an ample amount of diversity and can be utilized for the development of a heterotic pool.

Therefore, the selection of divergent parents based on these characters might be useful for heterosis breeding as well as to obtain a large number of segregants in the subsequent generations. Thus, on the basis of inter-cluster distance, cluster means, characters with high contribution to D^2 values, dendrogram and by comparing the mean values of all the genotypes; the following four genotypes *viz*., TM-37, Ganga-8, PDM-139 and SML-831 would be the most potent genotypes in the future breeding programme.

Future scope

- Diverse genotypes of Cluster I and Cluster II may be utilized for hybridization and isolation of superior lines segregants.
- Diverse lines having desirable agronomic and quality traits may be conserved to prevent genetic erosion.
- Further molecular studied for assessment of diversity using markers may be performed to establish the phylogeny at genomic level.

Table 1: Wilks Test under *Kharif* season

Determinant of Error Matrix			**1.15944E-1**
Determinant of Error + Variety Matrix			6.43843E+12
Wilk's Criterion			1.80082E-14
M	79.5	V statistics	2516.01200
Degree of Freedom	455	Probability	0.00000

Table 2: Wilks Test under summer season

Determinant of Error Matrix			**2.38397E+4**
Determinant of Error + Variety Matrix			1.34972E+13
Wilk's Criterion			1.76628E-09
M	79.5	V statistics	1602.274
Degree of Freedom	455	Probability	0.000

Table 3: Percentage contribution towards diversity by different traits

Traits	**Times ranked**		**Contribution%**	
	K	**S**	**K**	**S**
SPP	273	8	43.33%	1.27%
PPP	130	25	20.63%	3.97%
SPPI	74	0	11.75%	0.00%
PL	67	6	10.63%	0.95%
BY	23	279	3.65%	44.29%
PBPP	20	1	3.17%	0.16%
PCPP	19	8	3.02%	1.27%
NPP	9	23	1.43%	3.65%
DM	6	35	0.95%	5.56%
HI	6	62	0.95%	9.84%
100SW	2	113	0.32%	17.94%
SYPP	1	50	0.16%	7.94%
DFI	0	20	0%	3.17%

Note: Where, DM- days to maturity, DFI- days to flowering initiation, PBPP-Primary branches per plant, NPP-nodes per plant, PCPP-pod cluster per plant, PPP-pods per plant, SPP- seeds per pod, SPPI - seeds per plant, PL - pod length, HI - harvest index, BY-biological yield per plant, 100SW- 100 Seed weight, SYPP- seed yield per plant

Table 4: Distribution of genotypes among various clusters

Cluster	No. of genotypes		Genotypes	
	K	S		
I	13	25	Pusa M 19-42, MH 1344, OBGG 102, IPM 02-3, VGG 17-038, IPM701-4, IPM 604-1, IPM 205-7, TRCRM-147, PDM-11, SL-668, Pusa Vishal, TMB-136	IPM 701-4, VGG 16-045, Pusa BM-8, OBGG 102, IPM 604-1, Shikha, Virat, OBGG 101, SL-668, SKNM-1608, HUM-1, JBM 136, TMB-136, Pusa 9531, WBSM 48-5, VGG 17-040, MH 1451, IPM 410-3, IPM 610-2, LGG 460, VGG 17-038, IPM 205-7, Pusa M 19-41, VGG 17-015, PDM-11
II	18	6	VGG16-045, SML1825, SML 1831, IPM 610-2, OBGG101, VGG17-015, VGG17-040, MH1451, Pusa M 19-41, JBM 136, TJM-3, PDM-139, PDM11, LGG460, TM-37, Ganga-8, HUM-1, Shikha, Virat	TM-37, Ganga-8, MH1344, PDM-139, Pusa M 19-42, SML 1831
III	1	1	WBSM 48-5	Pusa Vishal
IV	3	1	SKNM -1608, Pusa 9531, IPM 410-3	TRCRM-147
V	1	1	Pusa BM-8	IPM 02-3
VI		1		SML 1825
VII		1		TJM-3

Note: K: *Kharif* S: Summer

Table 5: The average intra and inter-cluster D^2 values under both seasons

Clusters		I	II	III	IV	V	VI	VII
I	K	116.97	453.77	221.78	260.09	399.46	-	-
	S	31.04	59.57	61.10	53.21	70.32	69.18	130.22
II	K		141.28	862.25	706.28	1317.65	-	-
	S		23.12	132.42	89.60	166.14	155.41	82.55
III	K			0.00	123.77	363.06	-	-
	S			0.00	115.94	94.02	41.32	196.81
IV	K				113.76	666.26	-	-
	S				0.00	49.47	58.07	191.20
V	K					0.00	-	-
	S					0.00	53.49	291.33
VI	S						0.00	237.79
VII	S							0.00

Note: K: *Kharif* S: Summer

Table 6: Cluster means showing importance of grouped traits under both seasons

Clusters		DFI	DM	PBPP	NPP	PCPP	PPP	SPP	SPPI	PL	BY	100SW	HI	SYPP
I	K	34.28	65.92	2.47	6.51	6.52	11.82	10.71	125.56	7.40	12.51	2.83	27.56	3.36
	S	40.99	73.52	2.05	6.55	6.01	19.50	7.87	154.58	8.07	23.60	3.20	20.25	4.55
II	K	35.19	67.89	2.34	8.44	9.27	19.48	11.63	223.91	7.79	14.41	2.70	36.18	4.92
	S	40.89	73.67	1.89	6.20	6.68	22.00	7.28	160.31	7.73	34.74	4.33	19.13	6.51
III	K	36.67	65.67	1.89	3.55	4.55	5.55	13.34	74.00	5.89	6.66	1.97	25.55	1.65
	S	41.33	75.33	2.11	5.66	6.22	22.11	10.00	222.10	8.77	16.42	2.55	33.92	5.54
IV	K	35.56	67.33	1.89	5.81	3.37	6.07	14.35	86.56	7.53	13.65	2.59	20.86	2.40
	S	39.33	73.67	2.55	11.89	11.11	23.89	6.77	164.19	7.64	25.00	2.18	13.69	3.43
V	K	33.67	65.33	1.44	3.77	2.33	7.44	8.06	60.00	5.43	8.54	2.08	13.89	1.17
	S	42.33	71.00	1.66	7.44	7.78	24.22	9.14	220.55	7.33	16.04	1.15	15.85	2.55
VI	S	44.00	76.00	3.44	12.55	8.22	24.44	8.41	204.81	8.47	14.94	1.87	25.52	3.81
VII	S	42.33	76.67	2.00	6.22	4.55	16.11	8.75	141.18	8.70	46.65	3.96	11.75	5.47

Note: Where, DM- days to maturity, DFI- days to flowering initiation, PBPP-Primary branches per plant, NPP-nodes per plant, PCPP-pod cluster per plant, PPP-pods per plant, SPP- seeds per pod, SPPI - seeds per plant, PL - pod length, HI - harvest index, BY-biological yield per plant, 100SW- 100 Seed weight, SYPP- seed yield per plant

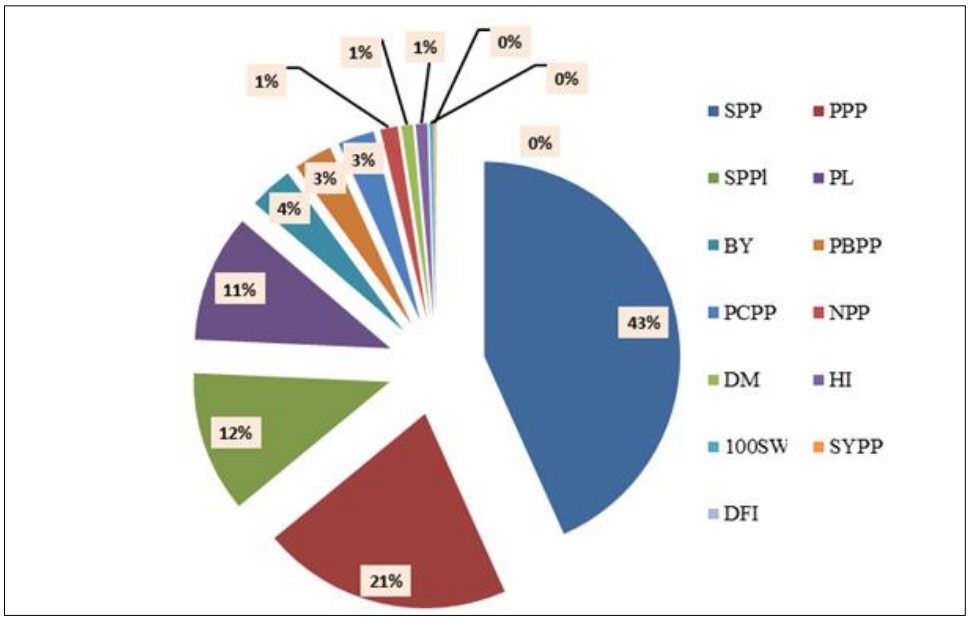

Fig 1: Percentage contribution towards diversity by different traits under kharif season

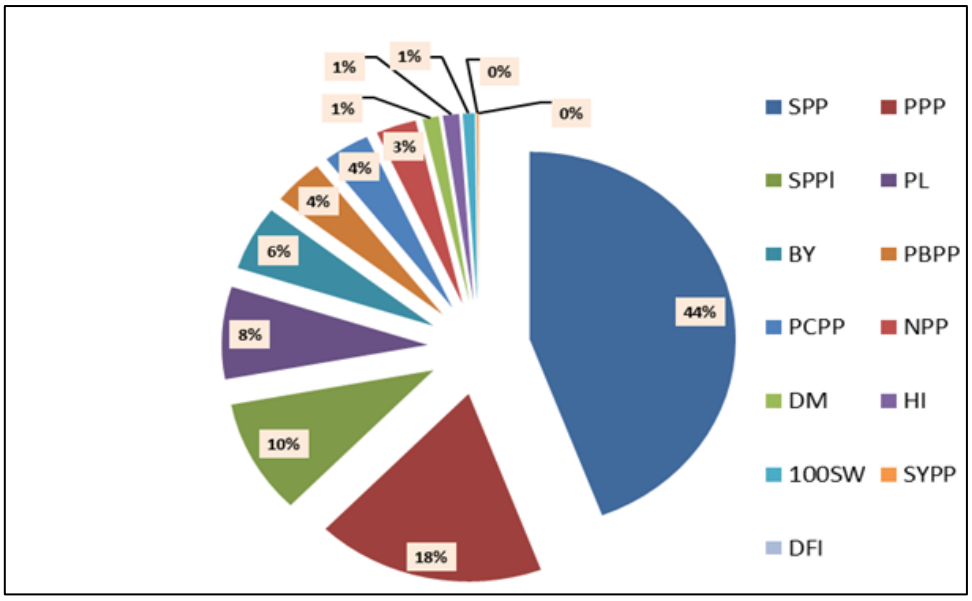

Fig 1: Percentage contribution towards diversity by different traits under summer season

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