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Research Note

Genetic grouping of selected RILs for yield and attributing traits in determinate type of Indian bean

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Abstract
The Indian bean is a well-known legume and green vegetable crop in India. An attempt was made to analyze the diversity pattern in selected recombinant inbred lines (RILs) of determinate type of Indian bean using principal component analysis (PCA) and D^2 statistics. Data from eleven yield component traits were used to carry-out the analysis. Clustering of lines was performed using modified (sequential) tocher method, and results were also compared with original tocher method. PCA analysis showed that first three principal components collectively accounted for 68.92% of total variation among the genotypes and had eigenvalues >1. The lines were grouped into five clusters using modified tocher method for cluster analysis, which has been shown to be superior to tocher method. Cluster-I and cluster-IV showed the highest inter-cluster distance (124.92) and crossing between genotypes from these two clusters was suggested to get desirable segregants. The study also suggested that PCA and D^2 analysis can be used alternatively to assess the genetic diversity pattern among genotypes.

Keywords: Principal component analysis, D^2 analysis, Modified tocher, RILs, Indian bean

The Indian bean, or *Lablab purpureus* (L.) Sweet, is one of the oldest cultivars of legumes and is used in a variety of products, including pulses, green vegetables, green manure, livestock feed, decorations, and medicines (Raghu *et al.,* 2018). It is cultivated mainly across dry, subtropical and semi-arid regions of Africa, Central and South America, West Indies and Southeast Asia (Naeem *et al.,* 2020). It is known by many other names across the globe, *viz*., Dolichos bean, Bonavist bean, Hyacinth bean, Lablab bean, Seim bean, Avare, Wal etc. (Al-snafi, 2017; Raghu *et al*., 2018). In Gujarat, it is popular with different names like '*Papdi*', '*Wal'* and '*Valor'* (Kyada *et al*., 2022; Patel *et al*., 2022, Patel *et al*., 2023). It is farmed in rainfed regions either as a pure stand or in combination with other crops including peanut and castor, as well as grains like finger millet, sorghum, bajra and maize (Raghu *et al*., 2018). It has the ability to fix nitrogen in the soil and can be cultivated alternating with cereals for this purpose. In addition to having a good number of carbs, lipids, fibre and minerals, its seeds and pods are rich source of protein (20-28%). (Naeem *et al*., 2020). Additionally, there are not many cultivable determinate and photo-insensitive varieties (Kyada *et al*., 2022). These qualities allow for the growing of crops during any season, and more than one season can be taken each year.

Moreover, the information about genetic diversity is important to select parents for getting segregating

progenies with greater variability after hybridization (Murthy and Arunachalam, 1966). Multivariate analysis techniques such as principal component analysis (PCA) (Pearson, 1901) and D^2 statistics (Mahalanobis, 1936; Rao, 1952) are used to get the diversity pattern of genotypes. The PCA is specifically used to reduce the dimension of the data without losing much information to study the dispersion pattern of genotypes more easily (Jolliffe and Cadima, 2016). The Modified or Sequential Tocher method of clustering has an advantage over the Tocher method illustrated by Rao (1952). The sequential or modified Tocher method prevents the effects of previously formed groups on subsequent grouping (Vasconcelos *et al.,* 2007). So, the total number of clusters can be reduced and the unnecessary greater number of clusters with singular genotypes get included in the nearest cluster. Thus, it was suggested that clustering of genotypes is more appropriate by the modified tocher method than that of the original tocher method (Vasconcelos *et al.,* 2007).

Therefore, this study was conducted to analyse genetic diversity among the selected determinate type F_5 RILs (recombinant inbred lines) developed from four crosses using PCA and Modified Tocher method of clustering.

The experiment took place at N. M. College of Agriculture, NAU, Navsari (Gujarat), during the *Rabi* 2019-20. There were 46 determinate genotypes used in the experiment, of which 44 were RILs and two were standard varieties (GNIB-21 and GNIB-22) **(Table 1)**. Four crosses, including GNIB-21 × GP-1, GNIB-21 × GP-189, GNIB-21 × GP-167, and GNIB-21 × GPKH-120 had been done to derive F_5 progenies. Among the parents, GNIB-21 was kept as the common female parent for its determinate growth habit. All genotypes were analyzed in randomized block design (RBD) with three replications. There were fifteen plants each row, spaced 60 cm x 20 cm, and suggested agronomic activities were carried out in order to successfully raise the crop. Eleven quantitative characters, including days to maturity (DM), days to 50% flowering (DFF), plant height (PH) (cm), pods per raceme (PPR), pods per plant (PPP), racemes per plant (RPP), pod length (PL) (cm), pod width (PWD) (cm), pod weight (PW) (gm), seeds per pod (SPP) and seed yield per plant (SYPP) (gm) were examined. The data was collected on 10 randomly selected plants from each line and each replication for each trait, and their mean values were used in data analysis, with the exception of days to 50% flowering and days to maturity, which were recorded on a collective basis.

The data were subjected to principal component analysis (PCA) (Pearson 1901) and D^2 analysis (Rao 1952) and genotypes were clustered according to both tocher and modified tocher method (Vasconcelos *et al.,* 2007) using Rstudio (v4.1.2; R core team 2021) via the R packages biotools (v4.2; da Silva *et al.,* 2021) and FactoMineR (Le *et al.,* 2008). The comparison between original tocher method and sequential or modified tocher method of clustering was done, and comparison of PCA and D^2 analysis was also carried out by super imposing PCA scatter plot of genotypes with clusters.

Principal component analysis: Principal components having eigenvalue more than unity were considered as major principal components. A scree plot based on eigenvalues indicated that eigen values is decreasing gradually for each principal component **(Fig. 1)**. Based on that, the first three principal components (PC) were selected out of eleven PCs, each of three having more than 10 % of the explained variance (of total inertia) cumulatively contributing 68.92 % of the total variation among genotypes **(Table 2)**.

The correlations of the variables (coordinates) with PCs are given in **Table 3** and represented in correlation circle **(Fig. 2)**. The first principal component PC-1 contributed 37.72 % of total variation and had positive association with PPP, PPR, RPP, PL, SPP and SYPP whereas pod weight was negatively correlated with PC-1. The PC-2 presented 19.56 % of total variation among genotypes

Table 1. List of RILs and varieties of Indian bean under study

S. No.	Genotypes	S. No.	Genotypes	S. No.	Genotypes	S. No.	Genotypes	S. No.	Genotypes
1	R-1-32	11	R-1-39	21	R-1-44-1-A	31	R-3-28-1	41	R-3-30-2
2	R-1-34-2	12	R-1-40-1-A	22	R-1-44-1-B	32	R-3-33-1	42	R-3-30-3
3	R-1-34-4	13	R-1-40-1-B	23	R-3-45-1	33	R-3-33-2	43	R-3-30-4
4	R-1-35-2	14	R-1-40-1-C	24	R-3-45-2	34	R-3-33-3	44	R-3-30-5
5	R-1-35-3	15	R-3-41-1	25	R-3-46-1	35	R-3-13-1-A	45	GNIB-21
6	R-1-36-2	16	R-1-41-3	26	R-3-17-1	36	R-3-13-1-B	46	GNIB-22
7	R-1-37	17	R-3-42-1	27	R-3-19-1-A	37	R-3-14-1		
8	R-3-37-2	18	R-1-42-2	28	R-3-19-1-B	38	R-3-14-2-A		
9	R-1-39-1	19	R-3-42-2	29	R-3-19-2	39	R-3-14-2-B		
10	R-3-39-1	20	R-1-43-1	30	R-3-19-3	40	R-3-30-1		

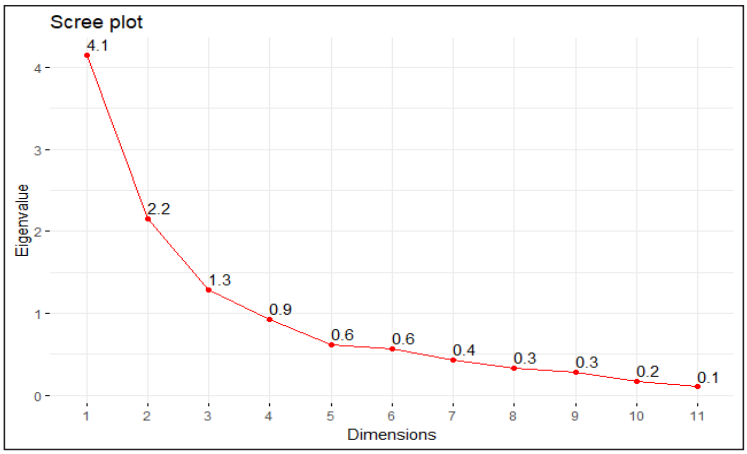

Fig. 1. Scree plot of principal components based on eigenvalues. Dimensions represents principal components

Table 2. Principal component analysis of eleven quantitative traits in Indian bean

Principal components	Eigenvalue	Percentage of variance	Cumulative percentage of variance
PC1	4.15	37.72	37.72
PC2	2.15	19.56	57.28
PC3	1.28	11.64	68.92
PC4	0.93	8.44	77.37
PC5	0.62	5.63	83.00
PC6	0.56	5.11	88.11
PC7	0.43	3.90	92.00
PC8	0.33	2.97	94.98
PC9	0.29	2.59	97.57
PC10	0.17	1.50	99.07
PC11	0.10	0.93	100

Table 3. Correlations of the variables with the first three principal components

Variables	PC1	PC2	PC3
DFF	0.168	-0.776	0.463
DM	0.204	-0.682	0.588
PH	-0.041	0.557	0.384
RPP	0.791	0.109	-0.053
PPP	0.907	-0.123	-0.014
PW	-0.460	0.387	0.297
PPR	0.837	-0.154	-0.060
PL	0.754	0.252	0.005
PWD	-0.040	0.568	0.686
SPP	0.757	0.351	0.078
SYPP	0.758	0.253	0.032

DFF=Days to 50% flowering DM=Days to maturity PH=Plant height (cm)
PPP=Pods per plant PW=Pod weight (g) PPR=Pods per raceme
PWD=Pod width (cm) SPP=Seeds per pod SYPP=Seed yield per plant (g)
RPP=Racemes per plant PL=Pod length (cm)

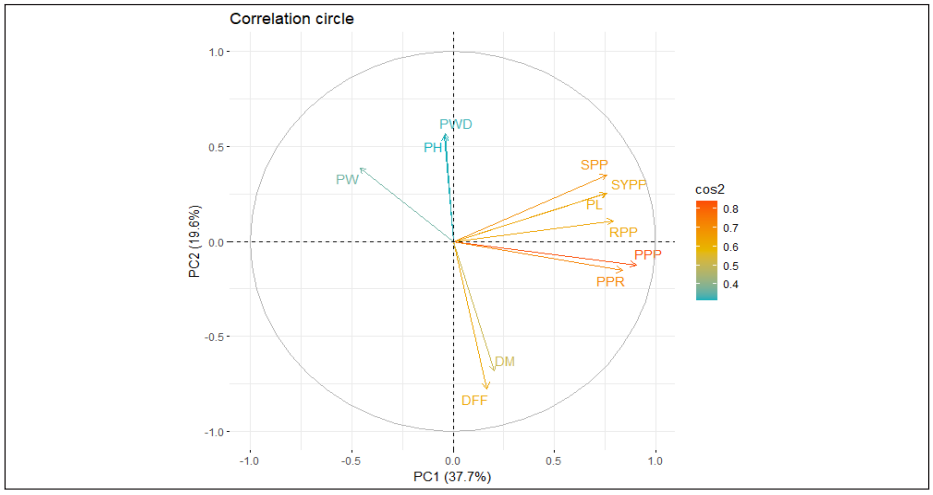

Fig. 2. Correlation circle. Vector (arrow) length and colour represents quality of representation of variables to the principal components. The angle between vectors indicates correlation among variables

and had positive correlation with PH, PWD, PW and SPP. The PC-3 accounted for 11.64 % of total variation with positive correlations with DFF, DM and PH.

Based on the correlation circle of first two principal components (PC-1 and PC-2), the traits were divided in three groups. The first group comprised SPP, SYPP, PL and RPP, which were positively correlated with both PCs. The second group was negatively correlated with PC-1 and positively correlated with PC-2, including the traits, PWD, PH and PW. The third group, composed of PPP, PPR, DM and DFF was negatively correlated with PC-2 and positively correlated with PC-1 **(Fig. 2)**. The correlation circle can also give an idea about correlation between variables as the cosine of the angle between vectors representing variables (Jolliffe and Cadima, 2016). The variable vectors grouped together were positively correlated. If the angle between two vectors is < 90°, it indicates strong positive correlations between two variables, whereas wide angle >90° indicates negative correlations between variables. However, the perpendicular vectors (= 90°) indicates the independence of the variables (Sabaghnia *et al*., 2011). Thus, it can be concluded that SYPP, SPP, PL, RPP, PPP and PPR were positively correlated with each other **(Fig. 2)**. Whereas, negatively correlated traits positioned on opposite quadrants, indicating that PPR was negatively correlated with PW, PH, and PWD. Shibli *et al*. (2021) had also observed positive correlation between pod yield per plant, racemes per plant and pods per plant.

The cos^2 (squared coordinates or squared cosine) value indicates quality of representation of variables (proportion of explained variability of each trait) on factor maps (Kassambara, 2017). The variables which are far from

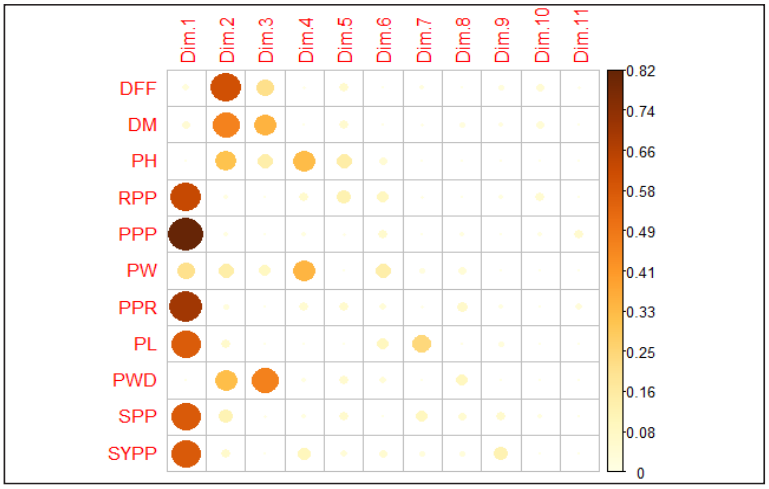

Fig. 3. Quality of representation of variables to the principal components. Dim (dimension) denotes principal components. The colour and size of filled circle indicates Cos2 values (quality of representation)

origin, close to circumference of the circle and with red arrows had high cos^2 and high quality of representation of variables **(Fig. 2)**. The cos^2 values indicated that all variables were well represented combinedly by PC-1 and PC-2 **(Fig. 3)**.

Pods per plant had the highest contribution (19.831 %) to PC-1 while days to 50% flowering had the highest contribution (27.956 %) to PC-2 **(Table 4)**. The traits which did not contribute to PC-1, contributed to PC-2 and vice versa. The variables that are more parallel to the X-axis are more contributing to the PC-1 and same is true for Y-axis and PC-2 **(Fig. 2)**. The average contribution of the particular trait to both principal components was given by (C1×Eigen1) + (C2×Eigen2) / Eigen1 + Eigen2 **(Table 4; Fig.4)**. Where, C1 and C2 are contributions of variables to PC-1 and PC-2, respectively, and Eigen 1 and Eigen 2 are the eigenvalues of PC-1 and PC-2, respectively (Kassambara, 2017). The red dashed line indicates the average expected contribution of variables to both principal components assuming a similar contribution to each PC (1/11×100 = 9.09%). The average contribution to PC-1 and PC-2 was obtained by (9.09×Eigen1) + (9.09×Eigen2) / Eigen1 + Eigen2 (Kassambara, 2017). The PPP, PPR, SPP, SYPP, RPP, PL and DFF contributed more than expected values to the PC-1 and PC-2.

Finally, the dispersion pattern of genotypes is represented in PC-1-2 biplot, representing a considerable amount of variability among genotypes **(Fig. 5)**. The vector colour gradient is showing the contribution of the particular trait to both principal components. PC-1 and PC-2 are depicted on X-axis and Y-axis, respectively. The most diverse genotypes for various traits are those that are far from the origin, such as genotypes 35 (R-3-13-1-A), 9 (R-1-39-1), 23 (R-3-45-1) and 27 (R-3-19-1-A). These genotypes may be used in future breeding programmes for hybridization and for the improvement of Indian bean. Genotypes near the PC-1 axis and far right from origin had high values for seed yield, pods per plant, racemes

Table 4. Contribution of different traits to the principal components

Variables	PC1	PC2	PC3	Average contribution to PC-1 and PC-2
DFF	0.680	27.956	16.756	9.995
DM	1.000	21.638	27.031	8.048
PH	0.041	14.420	11.520	4.952
RPP	15.082	0.549	0.224	10.119
PPP	19.831	0.707	0.015	13.300
PW	5.104	6.972	6.906	5.742
PPR	16.888	1.097	0.284	11.495
PL	13.703	2.956	0.002	10.033
PWD	0.038	15.002	36.704	5.148
SPP	13.801	5.728	0.476	11.044
SYPP	13.831	2.976	0.081	10.124
Total	**100**	**100**	**100**	**100**

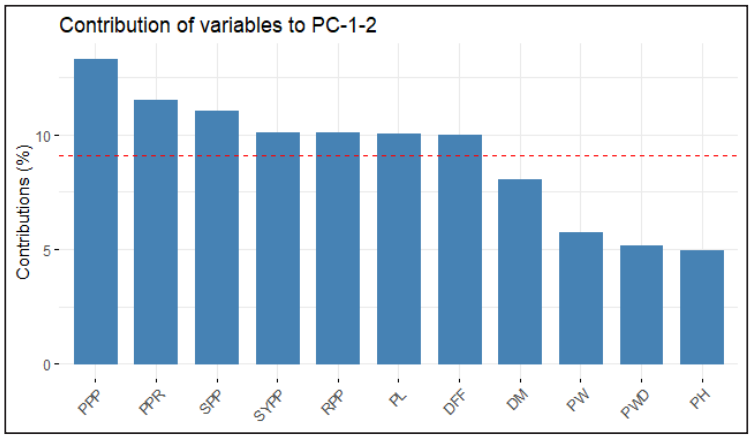

Fig. 4. An average percentage contribution of traits to the first two principal components. Red line indicates average expected contribution of traits assuming similar contribution from all the traits

per plant and pods per raceme, whereas far left from the origin would be having less values for these traits. Thus, PCA can be useful in identification of diverse genotypes and importance of traits to the groups of genotypes. Cluster analysis: In this study, the original tocher method produced nine clusters including four clusters of single genotypes **(Table 6)**, whereas it was reduced to five clusters using the sequential or modified tocher method **(Table 5)**. Genotypes from cluster IV of the tocher method was included in cluster III by using the modified tocher

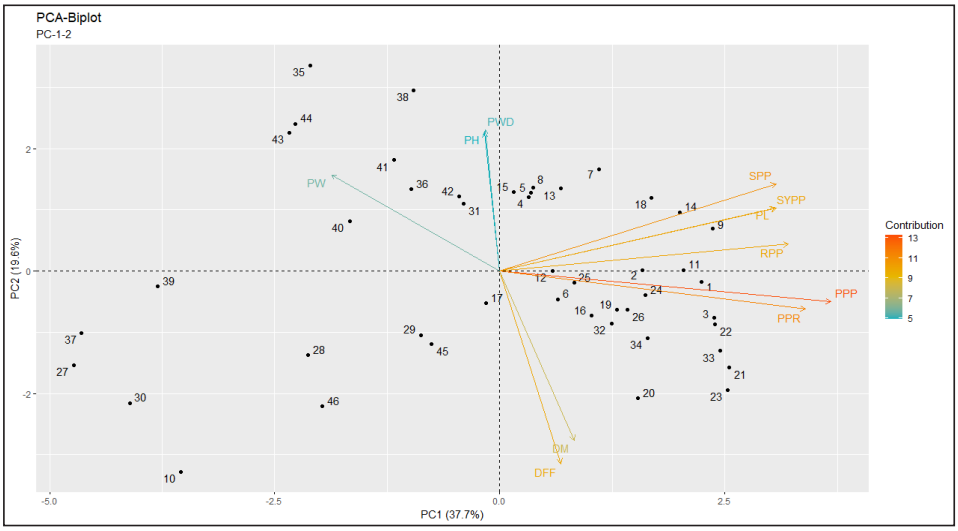

Fig. 5. Principal component analysis biplot of PC-1 and PC-2. Scattered numbers across the plot indicates serial number of genotypes enlisted in Table 1. The colour of vectors indicates average contribution of variable to the PC-1 and PC-2. The length of vectors indicates quality of representation of variables to the PCs.

Table 5. Distribution of genotypes in different clusters using modified tocher method

Clusters	Size	Membership
I	27	R-1-32, R-1-34-2, R-1-34-4, R-1-35-2, R-1-35-3, R-1-36-2, R-1-37, R-3-37-2, R-1-39-1, R-1-39, R-1-40-1-A, R-1-40-1-B, R-1-40-1-C, R-3-41-1, R-1-41-3, R-3-42-1, R-1-42-2, R-3-42-2, R-1-44-1-A, R-1-44-1-B, R-3-45-1, R-3-45-2, R-3-46-1, R-3-17-1, R-3-33-1, R-3-33-2, R-3-33-3
II	11	R-3-19-2, R-3-28-1, R-3-13-1-A, R-3-13-1-B, R-3-14-2-A, R-3-14-2-B, R-3-30-1, R-3-30-2, R-3-30-3, R-3-30-4, R-3-30-5
III	5	R-3-39-1, R-3-19-1-B, R-3-19-3, GNIB-21, GNIB-22
IV	2	R-3-19-1-A, R-3-14-1
V	1	R-1-43-1

Table 6. Distribution of genotypes in different clusters using the original tocher method

Clusters	Size	Membership
I	27	R-1-32, R-1-34-2, R-1-34-4, R-1-35-2, R-1-35-3, R-1-36-2, R-1-37, R-3-37-2, R-1-39-1, R-1-39, R-1-40-1-A, R-1-40-1-B, R-1-40-1-C, R-3-41-1, R-1-41-3, R-3-42-1, R-1-42-2, R-3-42-2, R-1-44-1-A, R-1-44-1-B, R-3-45-1, R-3-45-2, R-3-46-1, R-3-17-1, R-3-33-1, R-3-33-2, R-3-33-3
II	8	R-3-19-2, R-3-28-1, R-3-13-1-B, R-3-14-2-A, R-3-30-1, R-3-30-2, R-3-30-3, R-3-30-4
III	2	GNIB-21, GNIB-22
IV	2	R-3-19-1-B, R-3-19-3
V	3	R-3-19-1-A, R-3-14-1, R-3-14-2-B
VI	1	R-3-39-1
VII	1	R-1-43-1
VIII	1	R-3-13-1-A
IX	1	R-3-30-5

method, whereas genotypes in cluster VI, VIII and IX of the tocher method was included in cluster II and III of the modified tocher method leaving only one cluster with single genotype.

Of these five clusters, cluster-I had the highest number of genotypes (27), followed by cluster-II (11), cluster-III (5), cluster-IV (2) and cluster-V having single genotypes. All the traits contributed to total divergence having maximum contribution by SPP (11.67%) and lowest contribution by PL (6.38%). SPP, PPP, SYPP and PPR each contributed more than 10 % to the total divergence **(Table 7)**. Cluster-I recorded the highest mean for SYPP (13.92 g) and cluster-IV had the lowest mean for SYPP (6.54 g) **(Table 7)**. Cluster-III recorded the lowest mean for PH (49.31 cm) while cluster-V had the highest mean for PH (60.54 cm) **(Table 7)**.

The intra cluster distance was lower than inter cluster distance **(Table 8)**. Similar results were also observed by Hadavani *et al*. (2018). The maximum inter cluster distance was observed between cluster-I and cluster-IV (124.92) indicating genotypes is diverse between these two clusters and can be used in a breeding programme for hybridization for exploiting heterosis and getting desirable recombinants in segregating progenies. Whereas, the lowest inter cluster distance was recorded between cluster-I and cluster-V (63.54) suggesting that genotypes between these two clusters are less diverse. Additionally, superimposing a clusters ellipse on a PCA biplot (PC-1-2) demonstrated a distinct and consistent pattern of clustering and dispersion **(Fig. 6)**. This indicates that both multivariate techniques *i.e*., Principal component analysis (PCA) and D^2 analysis can be used alternatively to determine the diversity pattern of genotypes effectively. This is also in accordance with Islam (2008). It is shown here that the RILs are developed from four crosses having a common female parent GNIB-21 for its determinate nature, so the differences among RILs are said to be ultimately based on genetic differences between different male parents used in the crosses.

The principal component analysis made it easy to determine the divergence of genotypes by plotting genotypes in a scatter plot of only the first two principal components, which covered seven traits with more than expected contributions. Similarly, by cluster analysis, crosses were suggested between genotypes belonging to different clusters. Among which, crosses between genotypes of cluster-I and cluster-IV will be more heterotic than others and important to get desirable segregants. These determinate RILs can be a valuable resource for improvement in the determinate type of cultivar that can be utilized by breeders to develop and improve the varieties and increase the scope of cultivation of Indian bean. The modified tocher method was validated to be good as compared to the original tocher method of clustering by omitting unnecessary single genotype clusters and reducing the effect of one group in the grouping of other genotypes.

Table 7. Cluster means for different traits and contribution of traits to the total diversity

Variables	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	% Contribution
DFF	45.12	43.58	45.60	46.50	48.33	8.50
DM	87.51	86.64	87.80	88.83	92.00	9.27
PH	53.46	59.67	49.31	49.42	60.54	8.35
RPP	5.79	5.04	4.66	4.20	5.24	7.52
PPP	38.73	25.85	24.65	19.80	47.56	11.64
PW	0.52	0.56	0.48	0.67	0.49	6.62
PPR	6.64	5.00	4.88	4.71	6.93	10.83
PL	5.66	5.32	5.00	4.60	5.70	6.39
PWD	1.34	1.41	1.24	1.41	1.36	7.85
SPP	3.89	3.83	3.68	3.62	3.84	11.67
SYPP	13.92	11.55	8.41	6.54	10.27	11.36

Table 8. Inter and Intra cluster distances between different clusters

Clusters	cluster 1	cluster 2	cluster 3	cluster 4	cluster 5
cluster 1	**25.79**	64.42	85.76	124.92	63.54
cluster 2		**37.14**	72.69	79.63	105.46
cluster 3			**47.83**	65.75	89.27
cluster 4				**35.62**	113.73
cluster 5					**0**

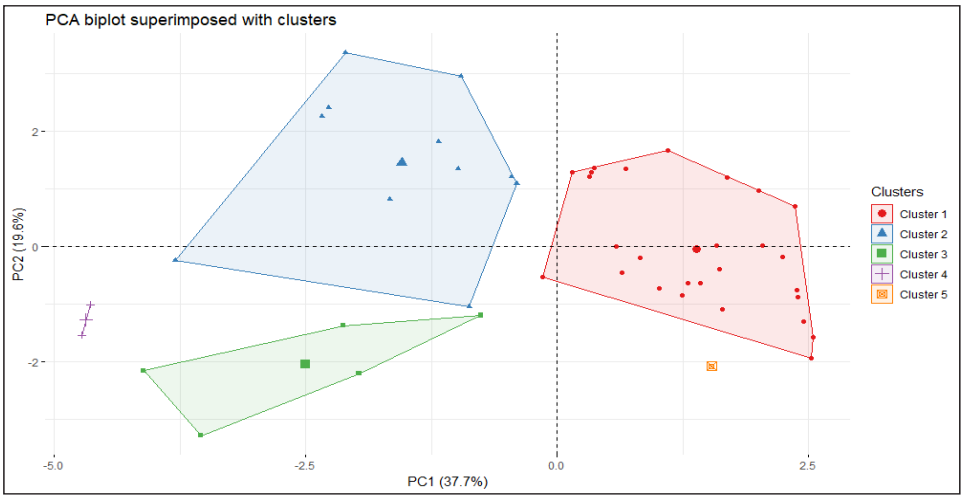

Fig. 6. PCA biplot of individuals superimposed with cluster ellipse indicating similarity of pattern of PCA and D2 cluster distribution of genotypes

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