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Genetic Diversity Assessment in Dolichos Bean (*Lablab purpureus* L.) Based on Principal Component Analysis and Single Linkage Cluster Analysis

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ABSTRACT

Background: Dolichos bean occupies a unique position among the legume vegetables of Indian origin for its high nutritive value and wider climatic adaptability. Despite its wide genetic diversity, no much effort has been undertaken towards genetic improvement of this vegetable crop. Knowledge on genetic variability is an essential pre-requisite as hybrid between two diverse parental lines generates broad spectrum of variability in segregating population. The current study aims to assess the genetic diversity in dolichos genotypes to make an effective selection for yield improvement.

Methods: Twenty genotypes collected from different regions were evaluated during year 2016-17 and 2017-18. Data on twelve quantitative traits was analysed using principal component analysis and single linkage cluster analysis for estimation of genetic diversity.

Result: Principal component analysis revealed that first five principal components possessed Eigen value > 1, cumulatively contributed > 82.53% of total variability. The characters positively contributing towards PC-I to PC-V may be considered for dolichos improvement programme as they are major traits involved in genetic variation of pod yield. All genotypes were grouped into three clusters showing non parallelism between geographic and genetic diversity. Cluster-I was best for earliness and number of cluster/plant. Cluster-II for vine length, per cent fruit set, pod length, pod width, pod weight and number of seed /pod, cluster III for number of pods/cluster and pod yield /plant. Selection of parent genotypes from divergent cluster and component having more than one positive trait of interest for hybridization is likely to give better progenies for development of high yielding varieties in Dolichos bean.

Key words: Dolichos bean, Genetic diversity, Principal component analysis, Single linkage cluster analysis.

INTRODUCTION

Dolichos bean (*Lablab purpureus* L.) is an important leguminous crop grown by nutri-gardeners and commercial vegetables grower for green pods, tender leaves, immature green seeds as vegetable and dry seeds as pulse throughout country. It has wide adaptability in different diverse climate conditions such as arid, semi-arid, subtropical and humid region. Crop is rich source of protein, minerals and vitamins besides a significant contributor to agriculture sustainability because of its ability to fix the atmospheric nitrogen as a leguminous crop (Basu *et al.* 1999) and high drought tolerance capacity. Despite its importance, this crop remained underutilized due to low productivity of the existing varieties, long gestation period, photosensitivity, indeterminate growing nature (Magalingam *et al.* 2013). India is one of the primary centres of origin and a large variability exists for plant and pod characters. Green, fleshy, string less pods coupled with high yield are preference of consumers and growers. There is a need of genetic restructuring of dolichos bean considering the consumer preference for attractive fleshy green pods with high yield to bridge the gap between potential yield and actual yield realised at framers field. Genetic variability is pre requisite for effective improvement programme of any crop (Azmat *et al.* 2011). Diverse genetic back ground of parental lines provides a desirable allelic variation to attempt novel combinations (Taran *et al.* 2005). A good understanding of genetic variability in different characters is a useful tool for identification of suitable genes to improve the genetic behaviour of crop (Nwangburuka *et al.* 2011). Classification of genetic variability, among the genotype is valuable for maintenance and further utilization as parental line for development of improved varieties (Amurrio *et al.* 2007). It is necessary to utilize existing natural genetic resources with minimum genetic erosion (Nissar *et al.* 2011). Moreover principal component analysis (PCA) is a useful tool for measuring variability in plant genetic resources for agronomic and physiological traits (Julier *et al.* 1995, Veasey *et al.* 2001, Bhargava *et al.* 2007). PCA is a descriptive method, which shows the pattern of co-variation of characters among the individual (Rhodes and Martin, 1972).

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However, PCA is not adequate for representation of characters in terms of relative importance, when numerous characters are considered simultaneously (Nwangburuka *et al.* 2011). To compliment the result of such multivariate analysis, single linkage cluster analysis is an essential analytical technique to classify the variation existing in germlasm. Single linkage cluster analysis (SLCA) is an agglomerative technique which shows the patterns of relationship between the individuals of population (Ario and Odulaza, 1991). Thus the study was conducted with an objective to identify the major characters responsible for variation among the accessions and to classify potential parental stock into groups employing the combined multivariate analysis.

MATERIALS AND METHODS

Present investigation was carried out during year 2016-17 and 2017-18 at experimental farm of ICAR-Central Institute for subtropical Horticulture Rehmankhera Kakori, Lucknow in block I. The experimental site situated at 26° 45′ to 27° 10' N latitude, 80° 30′ to 80° 5′ E longitude and 123 m above the sea level. Twenty diverse genotypes of dolichos bean collected from different regions of India were used in the study (Table 1).

Seeds were sown in first week of July in both the year at 120 × 60 cm inter and intra row spacing at depth 5cm. The experiment was conducted in a randomised complete block design with three replications. The soil of experimental field was loamy in nature with 6.8 pH and 0.36 dsm^{-1} EC. Plants were staked and supported. Uniform agronomic cultural practices were adopted to obtain the good phenotypic expression of characters. Five plants from each replication were randomly selected to record the 12 quantitative traits representing vegetative characteristics of plants related to yield and yield attributes. Data collected on the quantitative characters were analysed using the SAS Microsoft Windows 9.2 (SAS Institute, 2011). PCA and SLCA were used to assess the genetic variation and percentage similarity within the genotype. The PCA produced Eigen – vectors and principal component score were used to assess the relative discriminatory power of its axis and their associated characters. The cluster procedure was used to identify distinct groups of 20 genotypes on the basis of genetic relationship using the character variation. SLCA analyse the position of accessions into a dendogram at an interval of 5% level of dissimilarity starting from 100% level of dissimilarity.

RESULTS AND DISCUSSION

Variability in phenotypic characters expressed by range, standard deviation and coefficient of variation reflected significant variation among the delineated genotypes (Table 2). The vine length ranged from 1.70 to 5.90 meter. The highest vine length of 5.90 meter was recorded by genotype CISH-DC-16 whereas minimum vine length of 1.70 meter was recorded with CISH-DC-18. Number of primary braches

Table 1: Details on the Dolichos bean genotypes used for genetic diversity analysis.

Genotype	Collection site	Latitude	Longitude	Altitude(m.asl)	Genotype	Collection site	Latitude	Longitude	Altitude(Meter)
CISH-DC-1	Malihabad Lucknow U.P.	26.9 2°N	80.72° E	128.00	CISH-DC-11	Malihabad Lucknow U.P.	26.9 2°N	80.72° E	128.00
CISH-DC-2	Malihabad Lucknow U.P.	26.9 2°N	80.72° E	128.00	CISH-DC-12	Malihabad Lucknow U.P.	26.9 2°N	80.72° E	128.00
CISH-DC-3	Malihabad Lucknow U.P.	26.9 2°N	80.72° E	128.00	CISH-DC-13	Mursidabad West Bengal	24.18°N	88.27° E	10.00
CISH-DC-4	Malihabad Luc know U.P.	26.9 2°N	80.72° E	128.00	CISH-DC-14	Mursidabad West Bengal	24.18°N	88.27° E	10.00
CISH-DC-5	Malihabad Lucknow U.P.	26.9 2°N	80.72° E	128.00	CISH-DC-15	Malda , West Bengal	24.40°N	87.45° E	17.00
CISH-DC-6	Bakshi ka Talab Lucknow U.P.	26.59°N	80.53° E	128.00	CISH-DC-16	Malda , West Bengal	24.40°N	87.45° E	17.00
CISH-DC-7	Bakshi ka Talab Lucknow U.P.	26.59°N	80.53° E	128.00	CISH-DC-17	Mursidabad West Bengal	24.18°N	88.27° E	10.00
CISH-DC-8	Bakshi ka Talab Lucknow U.P.	26.59°N	80.53° E	128.00	CISH-DC-18	Mursidabad West Bengal	24.18°N	88.27° E	10.00
CISH-DC-9	Bakshi ka Talab Lucknow U.P.	26.59°N	80.53° E	128.00	CISH-DC-19	Malda , West Bengal	24.40°N	87.45° E	17.00
CISH-DC-10	Bakshi ka Talab Lucknow U.P.	26.59°N	80.53° E	128.00	CISH-DC-20	Malda , West Bengal	24.40°N	87.45° E	17.00

Table 2: Variability for different traits of dolichos bean genotypes.

Variable	Range		Genotypes		Mean	SD	CV (%)
	Min	Max	Min	Max			
Vine length (m)	1.70	5.90	CISH-DC-18	CISH-DC-16	4.45	1.15	25.74
Number of primary branch/plant	5.67	11.00	CISH-DC-5	CISH-DC-13	7.85	1.69	21.58
Days to first flowering	65.00	225.00	CISH-DC-1	CISH-DC-20	128.83	57.18	44.38
Number of flower /cluster	11.33	28.00	CISH-DC-6	CISH-DC-3	18.46	4.38	23.70
Percent fruit set	39.24	94.74	CISH-DC-20	CISH-DC-15	73.78	12.68	17.18
Number of cluster /plant	20.00	70.33	CISH-DC-2	CISH-DC-11	50.73	12.70	25.04
Number of pods /cluster	6.67	19.67	CISH-DC-3	CISH-DC-13	13.47	3.38	25.09
Pod length(cm)	5.55	16.80	CISH-DC-3	CISH-DC-6	11.22	2.85	25.36
Pod width(cm)	1.03	2.25	CISH-DC-1	CISH-DC-6	1.40	0.33	23.37
pod weight (g)	3.60	11.22	CISH-DC-20	CISH-DC-7	7.01	2.32	33.01
Number of seed /pod	2.97	5.97	CISH-DC-18	CISH-DC-16	4.91	0.77	15.76
Pod yield/plant (g)	1.90	10.17	CISH-DC-20	CISH-DC-7	4.78	2.41	50.51

Table 3: Principal component analysis of 12 traits in 20 genotypes of dolichos bean.

Variables	PC-I	PC-II	PC-III	PC_IV	PC-V
Vine length (m)	0.32	0.38	0.25	-0.20	-0.03
Primary branch / plant	-0.05	0.23	0.25	0.17	0.67
Days to first flowering	0.16	-0.35	0.11	0.16	-0.14
Number of flower /cluster	0.13	0.52	-0.37	-0.13	-0.08
Percent fruit set	0.27	-0.20	0.05	0.63	0.19
Number of cluster /plant	0.08	0.30	0.51	0.08	-0.43
Number of pods /cluster	0.35	0.31	-0.29	0.35	0.13
Pod length(cm)	0.35	-0.17	-0.10	-0.49	0.24
Pod width(cm)	0.33	-0.23	0.06	0.08	-0.38
pod weight (g)	0.41	-0.30	-0.08	-0.27	0.18
Number of seed /pod	0.10	-0.01	0.60	-0.18	0.23
Pod yield/plant (g)	0.50	0.12	0.00	0.06	-0.08
Eigen value	3.53	2.12	1.81	1.29	1.15
Proportion (%)	29.45	17.67	15.07	10.74	9.61
Cumulative (%)	29.45	47.12	62.19	72.93	82.53

ranged 5.67 (CISH-DC-5) to 11.00 (CISH-DC-13) plant. Days taken for first flowering determine the harvest time which ranged from 65.00 (CISH-DC-1) to 225.00 days (CISH-DC-20) Number of flower/cluster ranged from 11.33 (CISH-DC-5) 28.00(CISH-DC-3). Fruit set percentage ranged from 39.24 to 94.74 %. Genotype CISH-DC-15 recorded maximum fruit set 94.74% whereas minimum fruit set (39.24%) was recorded by CISH-DC-20. Number of cluster/ plant which directly associated with yield ranged from 20 (CISH -DC-2) to 70.3 (CISH-DC -11). Number of pods/cluster ranged from 6.67 (CISH-DC-3) to 19.67 (CISH-DC-13). Pod length ranged from 5.55 cm (CISH-DC-3) to 16.30 cm (CISH-DC-6). Pod weight, which is one of the most important trait for determining yield of crop ranged from 3.60 g (CISH-DC-20) to 11.22 g (CISH-DC-7). Number of seeds/pod varied from 2.97 (CISH-DC-18) to 5.97 (CISH-DC-16). Pod yield / plant ranged from 1.89 kg (CISH-DC-20) to 10.17 kg (CISH-DC-7). The genotypes having highest values for particular desirable traits may be selected as parents for future breeding aiming for genetic improvement programme of yield in dolichos. Similar observations have been reported by Parmar *et al.* 2013 and Peer *et al. 2018* in the germplasm evaluation of Dolichos bean.

Coefficient of variation (%) reflected the high magnitude of variability among the evaluated phenotypic traits. Highest coefficient of variation was observed for pod yield /plant (g) (50.51%) followed by days taken to first flowering (44.39%), pod weight (33.01%) indicated an appreciable variability in delineated genotypes which is a prerequisite for crop improvement programme. The results are in accordance with findings of the Ali *et al*. 2005.

Principal component analysis transforms a number of possible correlated variables into smaller number of uncorrelated variables by reducing the outliers. Eigen values with more than one has high explanatory power of original variables. Based on degree of divergence, 20 genotypes were grouped into five principal components having Eigen value > 1 accounted for 82.53% of total variability. The first principal component (PC-I) explained 29.44% of total variation and the traits which positively contributed towards the first component were pod yield /plant (0.497), pod weight (0.407) pod length (0.345) pod with (0.332) whereas primary branch /plant contributed negatively to this component. Numbers of flowers/cluster (0.518) and vine length (0.383) were found to be the main traits contributing towards PC-II which were responsible for 17.67 % of total variability. The traits positively contributing towards PC- III were number of seeds /plant (0.600) number of clusters/plant (0.514) which was responsible for 15.07 % of total variability. PC-IV accounted for 10.74 % of total variability positively contributed by percent fruit set (0.631), number of pods / cluster (0.354) whereas 9.61% of the total variability was positively contributed by PC V from primary branches/plant (0.666). The traits with largest absolute closer to unit within the first component influence the clustering more than those with lower absolute value closer to zero (Chahal and Gosal, 2002). Thus the differentiation within the delineated genotypes in different principal component was because of high contribution of few characters rather than small contribution of each character. The bi-plot of PC-I and PC-II showed a considerable variability presenting a dispersion pattern of delineated genotypes (Fig 1). The genotypes having negative values for PC-I and PC-II had smaller pods with less number of seeds /pods, low pod weight and yield. The genotypes with maximum positive loadings for PC-I and negative for PC-II were characterised with high number of flowers/cluster, number of flower clusters/plant and number of pods/cluster. The characters which are positively contributing towards PC-I to PC-V are important because they explain more than 82.53% of total variability. The dataset revealed higher contribution from the traits *viz.*, pod yield /plant, pod weight, pod length, pod width, number of flower/cluster, vine length and primary branches/plant. The genotypes grouped in the separate clusters (Fig 2) as shown by dendrogram are to be considered important during the selection of parents for hybridization or direct selection. Similar findings were earlier reported by Hadwani *et al*. 2018 in dolichos bean. The use of PCA to identify the most divergent genotypes in germplasm collection has also been reported (Singh *et al* 2017, Singh *et al*, 2020). The scatter plot generated through PCA illustrated the diverse genotypes located farther from the point of origin. Genotypes namely CISH-DC-12, CISH-DC-20, CISH-DC-3 and CISH-DC-11 were found most divergent for different traits (Fig 1). These diverse genotypes may be employed as parents in hybridisation in future breeding program in hybridization. Generally, it is customary to select one of the important variables from these identified groups for targeted improvement programme. Hence, PC I for pod yield/plant PC II for number of flower /clusters, PC III for number of seeds/pod, PC IV for maximum fruit set percentage and PC-V for number of primary branches /plant. The results demonstrated the usefulness of PCA in the identification of traits that contributed most variation within a group of genotypes. This technique has more practical utility in the selection of parental lines for breeding purposes.

Single linkage cluster analysis grouped all genotypes into three clusters by quantifying cluster means of all traits and their individual contribution (Table 4). The maximum number of genotypes were accommodated in cluster III (8) followed by cluster I (6) and cluster II (6) comprising 40, 30 and 30 % share of the total population, respectively. On the cluster mean basis, cluster I was important for days to first flowering (47.83) earliness and number of clusters /plant (54.44). Cluster II is important for the traits *viz.*, vine length (4.58), per cent fruit set (79.30), pod length (11.97), pod width (1.53) pod weight (7.58) and number of seeds /pod (5.14) whereas cluster III three was important for primary branches /plant (8.88), number of flowers/cluster (19.79), number of pods/cluster (14.96) and pod yield /plant (5.13). The genotypes of highest cluster mean of specific traits can be utilized as parents for realising targeted genetic

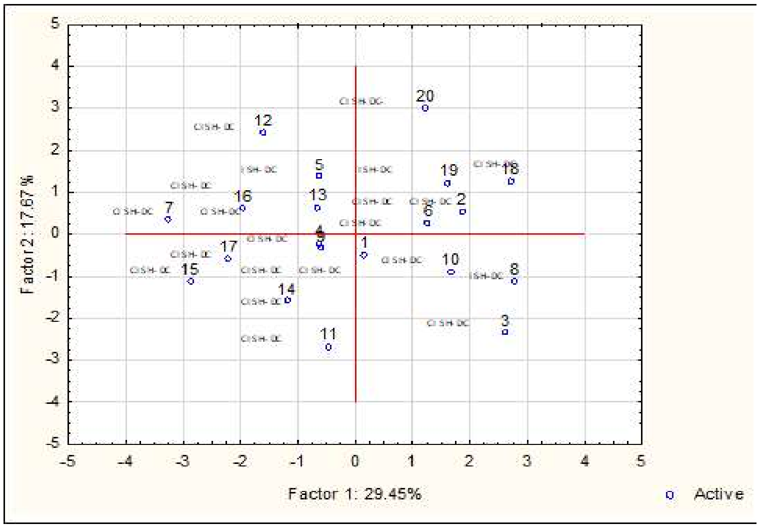

Fig 1: Scatter diagram of Bi-plot presenting dolichos bean genotypes.

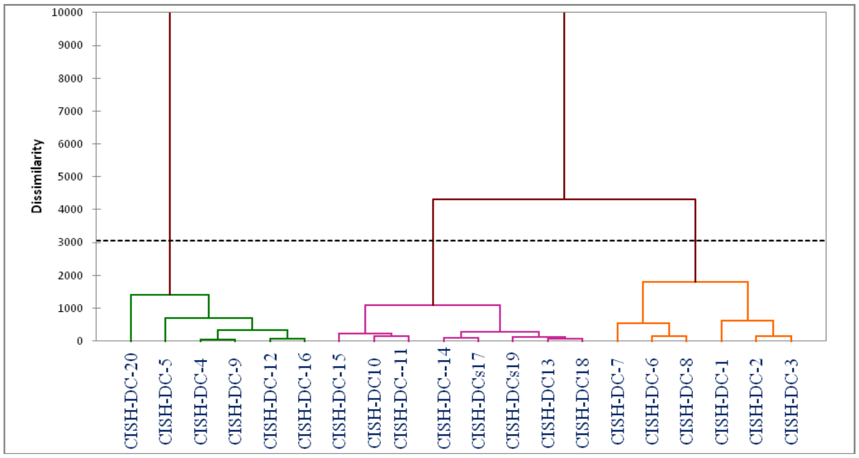

Fig 2: Dendogram depicting the genetic relationship among the dolichos bean genotypes based horticultural traits produced by ASH analysis (scale- Euclidean distance at .05).

Table 4: Cluster mean values for twelve important horticultural traits along with number and percent contribution of genotypes in each cluster.

Component	Cluster-I	Cluster-II	Cluster-III
Number of genotypes	6	6	8
Percent share of total genotypes	30 %	30%	40%
Position of genotypes in cluster	CISH-DC-1	CISH-DC-4	CISH-DC-10
	CISH-DC-2	CISH-DC-5	CISH-DC-11
	CISH-DC-3	CISH-DC-9	CISH-DC-13
	CISH-DC-6	CISH-DC-12	CISH-DC-14
	CISH-DC-7	CISH-DC-16	CISH-DC-15
	CISH-DC-8	CISH-DC-20	CISH-DC-17
			CISH-DC-18
			CISH-DC-19
Vine length (cm)	4.38	4.58	4.41
Primary branches / plant	7.44	6.89	8.88
Days to first flowering	74.83	210.33	108.21
Number of flowers /cluster	18.50	16.67	19.79
Percent fruit set	66.10	79.30	75.40
Number of clusters /plant	54.44	50.44	48.17
Number of pods /cluster	11.78	13.17	14.96
Pod length(cm)	10.34	11.97	11.32
Pod width(cm)	1.29	1.53	1.39
pod weight (g)	6.48	7.58	6.99
Number of seeds /pod	4.84	5.14	4.79
Pod yield/plant (g)	4.22	4.87	5.13

improvement for specific traits restructuring of dolichos bean for a particular character. Arya *et al*. (2017) also suggested that cluster having high mean values of specific traits may be used for hybridization programme to get superior recombinants.

Proximity matrix obtained suggests that the resolution for 20 genotypes of dolichos bean distributed in three clusters with a wide range of diversity for traits (Table 5). The highest inter cluster distance between clusters I and cluster II (136.237) followed by cluster-II and cluster-III (102.313) elucidated a high degree of divergence between the genotypes of these clusters. The hybrids developed from genotypes having maximum cluster distance resulted in high heterosis in yield and yield attributing traits (Kujur *et al.* 2017). Thus, crosses between the genotypes of cluster-I and Cluster-II and Cluster-II and cluster-III may be used in dolichos breeding for achieving maximum heterosis and isolating useful recombinants in segregating generation as well as introgressing useful traits in commercial dolichos cultivar. These findings are in conformity with the observations of Hadawani *et al.* 2018, Singh *et al.* 2017 and Birari and Ghanekar, 1992 that genotypes having placed farther in clusters resulted in a wide spectrum of variation when used in hybridization distance used in hybridization

Table 5: Average Inter cluster distance.

	Cluster-I	Cluster-II	Cluster-III
Cluster-I	0.00	136.24	35.43
Cluster -II		0.00	102.31
Cluster-III			0.00

resulted in a wide spectrum of variation in segregants. The genotypes grouped in same cluster presumably diverge very little from each other and does not produce desired segregates when used as parents for hybridization (Roy *et al.* 2013). Hence hybridization programme should be initiated with putative parents belonging to different characters. Therefore, parents with higher cluster distance are likely to be beneficial for further improvement in dolichos.

Dendogram obtained from single linkage cluster analysis by using the Euclidean distance depicted the clear relationship and exact position of genotype in clusters (Fig 2). ‘All the genotypes were distinct at 100 percent of dissimilarity and and formed three clusters at 65% of dissimilarity. Dissimilarity range 65 to 100% among the evaluated genotypes is enough to suggest the variability available in the dolichos bean genotypes. Distance among the genotype on the Euclidean scale reflected the position of genotype. CISH-DC-20, CISH-DC-3, CISH-DC-15, CISH-DC-5 and CISH-DC-2 were most divergent and potential for future breeding programmes in Dolichos bean and to isolate desirable recombinants for high pod yield. This study on genetic diversity assessment identified diverse genotypes in respect of pod yield that could be used as parental clones in hybridization and breeding programme in dolichos bean.

CONCLUSION

The principal component analysis and single linkage cluster analysis proved to be a better tool to assess the genetic diversity by providing the clear relationship among dolichos bean genotypes. The information on genotypes and distinct traits contributing for more variation facilitates breeders to develop newer high yielding cultivars. Selection of genotypes from divergent clusters for use in hybridisation may lead to genetic improvement in yield and quality of Dolichos bean.

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