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Genetic diversity analysis of *Mucuna pruriens* (L.) germplasm on the basis of qualitative and reproductive traits

Isha Thakur and Jai Kumar

Abstract
Plants genetic diversity is the key component of agricultural system & genetic improvement of crops can be accelerated when broad genetic diversity is available. There is a need to undertake detailed characterization of Mucuna pruriens genetic resources in India to reinforce its crop improvement program. Therefore, to evaluate the diversity among germplasm of Mucuna pruriens on the basis of qualitative and reproductive traits became an essential researchable area. The research was held at AICRP research field of Birsa Agricultural University, Ranchi, Jharkhand. Maximum contribution towards genetic diversity in Mucuna pruriens germplasm was observed by L-dopa content (40.22%), followed by pod yield/plant (11.59%), seed length (11.23%) and number of flowers/inflorescence (9.06%). Minimum contribution was shown by weight of 10 dried pods (0.36%) followed by 100 seed weight and seed yield/plant (0.72%), pod length (1.09%) and number of seeds/pod and pod width both (1.45%). The clustering pattern of twenty four germplasm of Mucuna pruriens showed that cluster I comprise of 22 germplasm and cluster II comprises only 2 germplasm. Germplasm *viz*. IIHR MP_1, IIHR MP_2, IIHR MP_4, IIHR MP_5, IIHR MP_6, IIHR MP_7, IIHR MP_8, DMAPR MP_1, DMAPR MP_3, DMAPR MP_4, DMAPR MP_5, DMAPR MP_6, DMAPR MP_7, DMAPR MP_8, Ranchi MP_1, Ranchi MP_2, Ranchi MP_3, Ranchi MP_4, Ranchi MP_5, Ranchi MP_6, Ranchi MP_7, Ranchi MP_8 were grouped into cluster I and IIHR MP_3, DMAPR MP_2 were grouped in Cluster II by multivariate analysis of genetic divergence of Mahalonobis "D^2" statistics. Maximum intra-cluster divergence was recorded in cluster II (50.53) followed by cluster I (34.96). However, inter-cluster divergence was recorded very high between clusters I and cluster II (123.52). In mean performance of clusters for plant length, cluster I had the higher mean value (8.59m) than cluster II (8.38m) with reverse relation with L-Dopa content; thereby suggested that smaller plant length yield more L-dopa content. The principal component analysis of Mucuna pruriens germplasm indicated that the germplasm such as IIHR MP_3, Ranchi MP_6, DMAPR MP_2, Ranchi MP_2, DMAPR MP_4 and DMAPR MP_1 created maximum genetic diversity among studied germplasm.

Keywords: *Mucuna pruriens*, genetic diversity, cluster, L-dopa, crop improvement

Introduction
Genetic diversity is a broad term encompassing all the variability occurring among different genotypes with respect to total genetic make-up of genotypes related to single species or between species. Natural variation is essential for conservation and utilization of plant genetic resources, so it is important to collect, evaluate and conserve wide range of genetic variation within a species and genus [8]. Genetic diversity becomes more important in context of climatic change and associated unforeseen events as it may serve as the reservoir of many novel traits conferring tolerance to different biotic and abiotic stresses. Use of crop genetic resources in crop improvement programmes should be the ultimate objective of germplasm resource

management and improvement in both qualitative and quantitative characters of a crop should be the main aim of any breeding programmes [10]. Since *Mucuna pruriens* is a seed propagated crop, it is essential to assess the quantitative and reproductive traits for further crop improvement. No systematic information is available regarding the evaluation and screening of high yielding germplasm of *Mucuna pruriens*, hence screening of viable germplasm is essential for the recommendation of its commercial cultivation. Scientific name of species is *Mucuna pruriens* (L.) DC., which belongs to the family Fabaceae, sub family Papilionaceae. It is widespread in tropical and sub-tropical regions of the world. Its Hindi name is Kewach and English name is Velvet bean. Seeds of *Mucuna pruriens* are known to produce the unusual non-protein amino acid 3-(3, 4-dihydroxyphenyl)-1-alanine (L-Dopa) which is used as anti-Parkinson's drug. Besides its medicinal uses, *M. pruriens* is used as an important fallow and green manure crop. Since the plant is a legume, it fixes nitrogen and fertilizes soil by soil amelioration, conservation and fertility improvement [3]. Keeping in view the importance of genetic diversity and evaluation of quantitative parameters, a systematic research trial was undertaken on *Mucuna pruriens* (L.) DC to know the sources and contribution of different traits towards genetic divergence of *Mucuna pruriens* germplasm, clustering pattern of *Mucuna pruriens* germplasm based on quantitative traits, average intra and inter cluster distance and mean performance of clusters for quantitative traits

Material and Methods

Genetic diversity in twenty four germplasm of *Mucuna pruriens* was estimated by multivariate analysis of genetic divergence of Mahalanobis "D^2" statistics [7]. Treating D^2 as the square of generalized distance, all germplasm were grouped into a number of clusters, according to the methods described by Tocher [9]. The replicated data of all the 24 germplasm were subjected to genetic divergence analysis. Variances and covariances for all the characters were calculated and a dispersion table was prepared. Wilk's criteria were used to test the significance differences in mean values of all the characters. Using the common error dispersion matrix, the D^2 value was calculated for each pair of germplasm among all possible combination. Average intra and inter cluster distances were calculated for all the clusters. For calculating the inter-cluster distance, two clusters were taken at a time and total distance between the populations falling in these two clusters was calculated.

Data recorded for genetic divergence studies were % contribution of factors towards genetic divergence, average intra & inter cluster D^2 value and mean performance of clusters. Statistical analysis of the data was carried out and the relative contribution of different twenty characters to the total D^2 between each pair of germplasm was calculated based on the magnitude of the D^2 value due to each character.

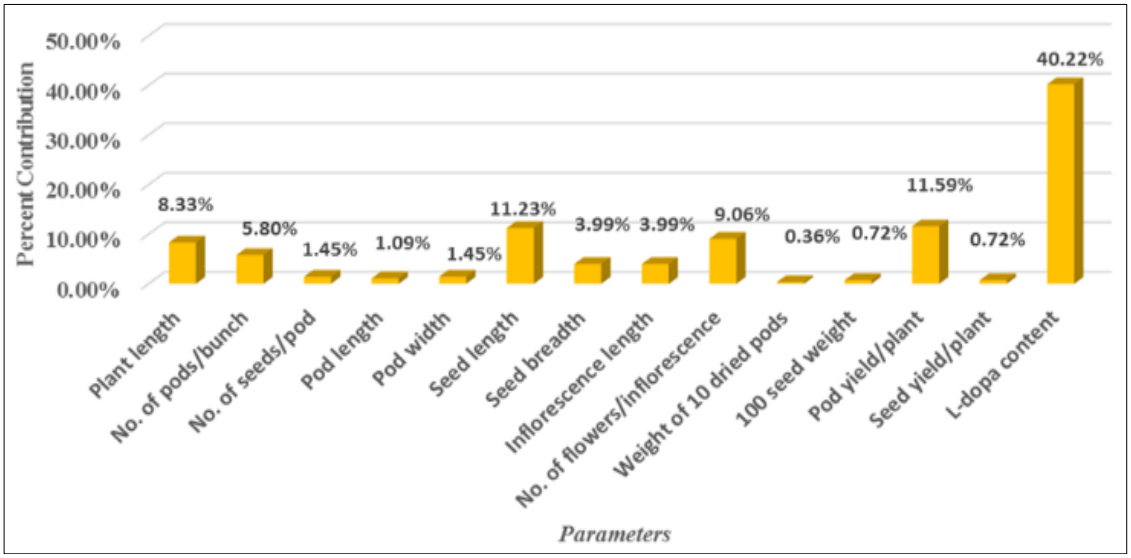
Results and Discussion

Among different sources of genetic diversity in *Mucuna pruriens* germplasm L-Dopa content in seeds (40.22%) of *Mucuna pruriens* germplasm contributed maximum towards its diversity followed by pod yield/plant (11.59%) and seed length (11.23%). Rest of the parameters shown minimum impact on genetic diversity of *Mucuna pruriens* germplasm namely number of flowers/inflorescence (9.06%) > plant length (8.33%) > number of pods/bunch (5.80%) > seed breadth = inflorescence length (3.99%) > number of seeds/pod = pod width (1.45%) > pod length (1.09%) > 100 seed weight = seed yield/plant (0.72%) > weight of 10 dried pods (0.36%). It is reported that dry root yield per plant, root length and plant height were very potent in contributing towards divergence in Ashwagandha [2]. Characters like number of fruits per plant, average fruit weight, plant height and fruit yield contributed maximum towards genetic divergence in Tomato [11]. Leaf number contributed most toward divergence (15.8%), followed leaf length (12.1%) in Kalmegh [5].

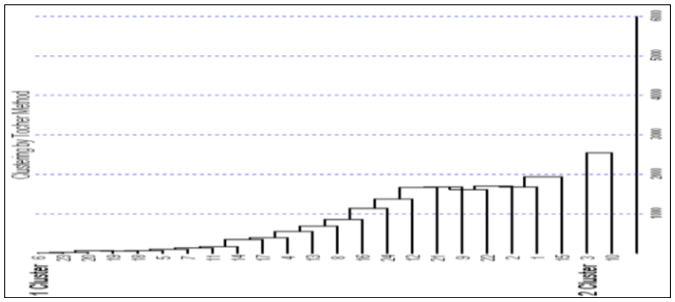
Table 1: Sources of genetic diversity in *Mucuna pruriens* germplasm with their percentage contribution

Sl. No.	Source	Percentage contribution	Rank w.r.t. % contribution
1.	Plant length	8.33%	5
2.	No. of pods/bunch	5.80%	6
3.	No. of seeds/pod	1.45%	8
4.	Pod length	1.09%	9
5.	Pod width	1.45%	8
6.	Seed length	11.23%	3
7.	Seed breadth	3.99%	7
8.	Inflorescence length	3.99%	7
9.	No. of flowers/inflorescence	9.06%	4
10.	Weight of 10 dried pods	0.36%	11
11.	100 seed weight	0.72%	10
12.	Pod yield/plant	11.59%	2
13.	Seed yield/plant	0.72%	10
14.	L-dopa content	40.22%	1

Relative contribution of different characters towards divergence in Kalmegh accessions was shown maximum by dry herbage yield/plant (48.40%) followed by number of leaves/plant (24.53%), leaf stem/ratio (8.56%), plant height (7.04%), plant spread (5.01%), leaf width (4.86%) and least by leaf length (1.60%) [6]. Analysis of variance and D^2 statistics revealed significant differences in all the metric traits and sufficient inter-cluster distances indicating considerable diversity among the accessions. In a study of genetic diversity with thirteen genotypes in string bean, it was observed that phenotypic variation was greater than that of genotypic and environment variations for all the characters [1]. In tomato, based on D [2] values of eight related characters, genotypes were grouped into eight clusters [11]. Clustering pattern indicated that there was no association between geographical distribution of genotypes and genetic divergence. The characters like number of fruits per plant, average fruit weight, plant height and fruit yield contributed maximum towards genetic divergence.



Graph 1: Sources of genetic diversity in *Mucuna pruriens* germplasm with their percentage contribution



Graph 2: Clustering pattern of *Mucuna pruriens* germplasm based on quantitative traits

Graph 2 represents cluster diagram of 24 germplasm of *Mucuna pruriens*, cluster I comprises maximum number of germplasm (twenty-two) and cluster II comprises 2 germplasm only. Study of clustering pattern indicated that germplasm IIHR MP_3 and DMAPR MP_2 were quite different to other germplasm, distributed in divergent cluster. Cluster I comprised of 22 genotypes namely IIHR MP_6, Ranchi MP_7, Ranchi MP_4, Ranchi MP_3, Ranchi MP_2, IIHR MP_5, IIHR MP_7, DMAPR MP_3, DMAPR MP_6, Ranchi MP_1, IIHR MP_4, DMAPR MP_5, IIHR MP_8, DMAPR MP_8, Ranchi MP_8, DMAPR MP_4, Ranchi MP_5, DMAPR MP_1, Ranchi MP_6, IIHR MP_2, IIHR MP_1, DMAPR MP_7. Cluster II comprises only 2 genotypes namely IIHR MP_3, DMAPR MP_2. Nature and magnitude of genetic divergence in thirty five chilli genotypes of different geographical origin using Mahalanobis D^2 statistics was studied by [12] and grouped them into six clusters. The cluster-II was the largest with 16 genotypes followed by cluster – III with six and cluster-V with five genotypes. Higher inter-cluster distances were main cause of heterogeneity in composition of clusters. In evaluation of genetic diversity of Kalmegh from 53 accessions, D^2 values for all the pairs of accessions ranged from 0.01 to 76.98 thereby indicating considerable diversity in the Kalmegh material [6]. Based on D^2 values, all the 53 accessions were grouped into five clusters. There were 31 accessions in cluster I, 9 in cluster II, 6 in cluster III, only 2 in cluster IV and 5 accessions in cluster V.

Table 2: Clustering pattern of twenty four genotypes of *Mucuna pruriens* based on genetic divergence among growth, yield and reproductive parameters

Clusters	No. of Genotypes	Genotypes
I	22	IIHR MP_1, IIHR MP_2, IIHR MP_4, IIHR MP_5, IIHR MP_6, IIHR MP_7, IIHR MP_8,DMAPR MP_1, DMAPR MP_3, DMAPR MP_4, DMAPR MP_5, DMAPR MP_6, DMAPR MP_7,DMAPR MP_8, Ranchi MP_1, Ranchi MP_2, Ranchi MP_3, Ranchi MP_4, Ranchi MP_5, Ranchi MP_6, Ranchi MP_7, Ranchi MP_8
II	2	IIHR MP_3, DMAPR MP_2

Table 3: Average intra and inter-cluster divergence among *Mucuna pruriens* germplasm

Clusters	I	II
I	34.96	123.52
II		50.53

Maximum intra-cluster divergence was recorded in cluster II (50.53) followed by cluster I (34.96). However, inter-cluster divergence was recorded very high between clusters I and II (123.52). Significant differences in all the metric traits and sufficient inter-cluster distances were recorded in Kalmegh [6]. D^2 values for all the pairs of accessions ranged from 0.01 to 76.98 thereby indicating considerable diversity in the Kalmegh. Average intra-cluster distance ranged from 0.34 to 4.06, while inter-cluster distance ranged between 3.95 and 6.85. Cluster III and V showed maximum inter-cluster divergence (6.85), thus indicating wide diversity between the accessions of these two clusters. The minimum inter-cluster distance occurred between clusters I and V, suggestive of close relationship. On the basis of average divergence (D^2) cluster V was the most divergent from the rest. High inter-cluster D^2 values recorded between cluster II and III and cluster III and VI in Kalmegh [5] indicated the possibility of raising transgressed hybrids from cross hybridization programs using divergent parents of these four clusters.

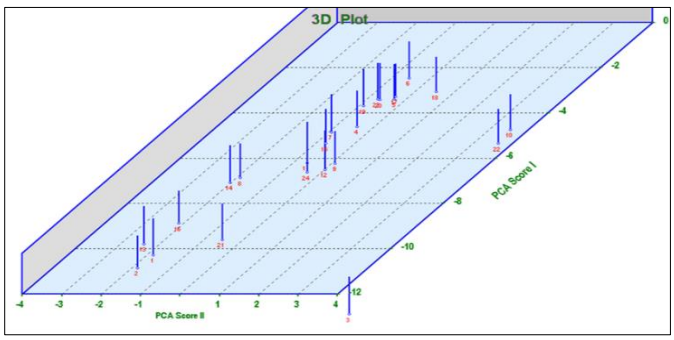
Table 4: Mean performance of clusters

Clusters	Cluster Means													
	Plant length (m)	No. of pods/ Bunch	No. Of seeds/pod	Pod length (mm)	Pod width (mm)	Seed length (mm)	Seed breadth (mm)	Inflorescence length (cm)	No. of flowers /inflorescence	Weight of 10 dried pods (g)	100 seed weight (g)	Pod yield/plant (Kg)	Seed yield/plant (Kg)	L-Dopa content (Kg)
I	8.59	6.14	4.36	89.25	17.05	15.70	12.26	10.86	10.42	64.21	84.83	0.24	0.14	3.35
II	8.38	11.36	3.21	66.10	15.03	13.83	11.50	21.86	36.59	27.36	41.08	0.02	0.01	3.79

Table 4 represents mean performance of clusters for growth, reproductive and yield characters of different *Mucuna pruriens* germplasm. For plant length, cluster I had the higher mean value (8.59 m) than cluster II (8.38 m) thereby suggested that smaller plant length yield more L-Dopa content. However, seed yield/ plant (0.14 Kg) and pod yield/plant (0.24 Kg) was higher in cluster I. For number of pods/bunch, cluster II (11.36) had higher mean value followed by cluster I (6.14) thereby suggested that more number of pods/bunch yield more L-Dopa content. For number of seeds/pod, cluster I (4.36) had higher mean value than cluster II (3.21).

It may be concluded that less number of seeds/pod had less seed yield/plant and pod yield/plant but more L-Dopa content. Cluster I had higher mean value for pod length (89.25 mm) and pod width (17.05 mm) than mean values of pod length (66.10 mm) and pod width (15.03 mm) for cluster II. Pod yield/plant and seed yield/plant had shown significantly direct relation with pod length and pod width. Cluster I had higher mean value for seed length (15.70 mm) and seed breadth (12.26 mm) than mean values of seed length (13.83 mm) and seed breadth (11.50 mm) for cluster II. However, a different trend was followed in reproductive parameters. Cluster II had higher mean values of inflorescence length (21.86 cm) and number of flowers/ inflorescence (36.59) than mean values of inflorescence length (10.86 cm) and number of flowers/ inflorescence (10.42). Therefore, plants containing more inflorescence length and number of flowers/ inflorescence yield more L-Dopa content. Cluster I was recorded with higher mean values of weight of 10 dried pods (64.21 Kg) and 100 seed weight (84.83 Kg) than mean values of weight of 10 dried pods (27.36 Kg) and 100 seed weight (41.08 Kg) for cluster II.

From the perusal of data it may be inferred that for higher the means values of plant length, number of pods/bunch, number of seeds/pod, L-Dopa content was higher. However, higher mean values of pod length, pod width, seed length, seed breadth yield lesser L-Dopa content. Higher mean values of reproductive parameters namely inflorescence length and number of flowers/inflorescence yield higher L-Dopa content. Higher mean values of weight of 10 dried pods, 100 seed weight yield higher L-Dopa content whereas lower mean values of pod yield/plant and seed yield/plant yield more L-Dopa content.



Graph 3: Principal component analysis of *Mucuna pruriens* germplasm

Principal component analysis of different *Mucuna pruriens* germplasm indicated that components with PCA score with positive values of PCA Score II namely IIHR MP_3, Ranchi MP_6, DMAPR MP_2, Ranchi MP_2, DMAPR MP_4, DMAPR MP_1 are responsible for higher magnitude of variance in the germplasm. Principal component analysis of different *Mucuna pruriens* germplasm indicated that components with PCA score with positive values of PCA Score II namely IIHR MP_3, Ranchi MP_6, DMAPR MP_2, Ranchi MP_2, DMAPR MP_4, DMAPR MP_1 are responsible for higher magnitude of variance in the germplasm. Therefore, parents from these germplasm may be selected in further genetic divergence studies.

Less polymorphism by the RAPD markers compared to the morphological markers in Kalmegh was observed by [13]. The principal component analysis (PCA) based on the RAPD markers revealed that the studied Malaysian Kalmegh accessions were distributed to three distinct groups. They found accessions P_2, P_3 and P_6 as the most distant parents, and considered them indicator for the weak crossability responses of accessions P_2 and P_3 to accession P_6. Principal component analysis of yield characters of Kalmegh showed maximum genetic advance by stem weight (82.47%), followed by dry weight (75.73%), leaf weight (74.10%), and fruiting index (73.18%), thus maximum improvement in these parameters is possible through appropriate breeding strategies for obtaining maximum dry biomass and seed yield from Kalmegh germplasm [4].

Conclusion

The results from given analysis indicated that 24 accessions of *Mucuna pruriens* are diverse for several morpho-agronomical characters with potential for exploitation in breeding programs. Thus, the study concluded that contribution of L-dopa content (40.22%), followed by pod yield/plant (11.59%), followed by seed length (11.23%) and number of flowers/inflorescence (9.06) was more towards the genetic diversity of *Mucuna pruriens* germplasm. Parents from cluster I (contained 22 germplasm) and cluster II (2 germplasm) may be selected for crossing under hybridisation program. The principal component analysis of *Mucuna pruriens* germplasm indicated that the germplasm such as IIHR MP_3, Ranchi MP_6, DMAPR MP_2, Ranchi MP_2, DMAPR MP_4 and DMAPR MP_1 created maximum genetic diversity among studied germplasm. Through germplasm evaluation, estimation of yield based on pattern of response of genotypes, agronomic treatments across environments and reliable guidance for selecting the best genotypes may be done.

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