Genetic variability and divergence studies on seed traits and L-dopa content of Mucuna pruriens (L.) DC. accessions

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Abstract

Mucuna pruriens (L.) DC. belongs to the Fabaceae family and is widely distributed across the tropical and subtropical regions of the world. Seeds of Mucuna are the industrially important source of natural L-dopa. The present study is intended to screen out the superior Mucuna germplasm which can be utilized for commercial cultivation. A total of 59 accessions of *M. pruriens* (L.) DC. were collected from various geographical locations across India and evaluated for seed-associated agronomic traits and L- dopa content in the seed. Morphological observations of seed samples revealed a wide variation in seed color pattern including complete white, black and striped/mottled. Quantitative traits such as 100 seed weight (90.30-150.59gm), seed length (5-19.3 mm), width (2.66-13.00 mm), thickness (0.90–8.67 cm), length width ratio (1.17–2.26), length thickness ratio (1.50–7.40), width thickness ratio (0.89-6.00) seed geometric diameter (2.34–12.60 mm), arithmetic mean diameter (2.88–13.33 mm), seed sphericity (41.89–78.75), seed surface area (17.19-498.51 mm^2) and L-dopa content (1.79–6.05%) were evaluated in order to compare the features of the accessions collected from different places. The inter-association between morphological observations and L-dopa content was measured using principal component analysis and Ward's hierarchical clustering, as these are of vital importance in selecting the best accession of velvet bean for breeding programs.

Introduction

Mucuna pruriens (L.) DC. is a legume of high economic importance and a notable underutilized tribal pulse with a diploid chromosome number (2n = 2x = 22). It is a member of the family fabaceae, sub family Papilionaceae with its natural habitats in tropical areas such as the region of Asia (Eastern India, Southern China and Malaysia), South America and Africa (Wilmot-Dear 1984a; 1987b; Pathania et al. 2020). According to Moura et al. (2016), the plant originated and first diversified in the paleotropics before the pantropical spread of its seeds through multiple oceanic long-distance into the horn of Africa. In India, the genus Mucuna up till date is represented by 12 species of which *M. pruriens* consists of three varieties (Misra et al. 2021). *M. pruriens* otherwise known as Cowitch, cowhage, Kawaanch, velvet bean and atmagupta (Anonymous, 1985) is a self-pollinated plant. Infante et al. (1990) reported that Mucuna type referred to as "Cowitch" and "Cowhage" produce abundant, long stinging hairs on the pod, which when in contact with human skin causes intense itchy dermatitis as a result of mucunain. However, the other type which is non stinging with appressed silky hairs is known as velvet bean.

Szabo and Tebbett (2002) described the plant as a yearly climbing vegetable that have been widely explored and utilized as nutraceutical, pharmaceutical and food. Traditionally, velvet bean is used and consumed by certain ethnic groups in different countries of the world (Janardhanan et al. 2003). For instance, in Asia, America, Africa and Pacific Island, it is cultivated for its pods used as vegetable and young leaves as animal fodder (Lampariello et al. 2012). Moreover, the plant is also cultivated as cover crop (or green fertilizer) for weed control and to add fertility to the soil in addition to reducing nematode population (Queneherve et al. 1998). The beans of the plant aside being processed into flour for consumption are very rich in starch, protein, and fiber (Taylor 2005), thus enhancing their functional application in various food products. In India, different parts of the plant have been incorporated into more than 200 locally formulated medications (Sastry and Kavathekar 1990) and have been found to exhibit various biological activities such as anticarcinogenic, antiviral, antimicrobial, anti-inflammatory, antihypertensive, anti-Parkinson, antioxidant with action against nervous disorder (Adebowale and Lawal 2003; Sadh et al. 2018). It was further reported that the Mucuna seeds are of therapeutic value with different preparations from the seeds explored in the management of several free-radical mediated diseases including ageing, rheumatoid, arthritis, diabetes, male fertility and nervous disorders (Rakesh and Praveen 2020).

The seeds of Mucuna are of economic importance because of its valuable phytochemicls and nutritional substances. Of these important phytochemical is L-3,4- dihydroxyphenyl alanine (L-dopa), a non-protein amino acid (2–7%) that is active against Parkinson disease (Soares et al. 2014). Other chemical compounds found aside L-dopa in the seeds of Velvet beans are glutathione, lecithin, gallic acid, and beta-sitosterol (Kavitha and Thangamani 2014) with seed oil containing four principal fatty acids i.e., stearic, oleic, linoleic and palmitic acids. There is high demand for the seeds of *M. pruriens* and this has increased many folds in India as well as in the international drug market (Farooqi 1999) due to its resourcefulness. Hadapad et al. (2018), opined that greater emphasis should be given to seed yield per plant, and seed test weight to enhance L-DOPA yield. Mucuna seeds have been found to be larger and bulkier than the seeds of most common legumes, with variability in kernel size and shape that may be important for domestic processing (Ezeagu et al. 2003) and affect quality attributes.

Selection of suitable parents for quality breeding and improvement is premised on the existence of genetic diversity in a crop plant, which involves evaluation and categorization of genetic differences. The degree of genetic diversity coupled with estimates of genetic parameters (i.e., genetic and phenotypic coefficient of variations) and heritability for traits under selection are central to designing of effective breeding program and its successful execution. Collection, analyses, and conservation of wide range of genetic diversity within and across various species of crop plants are crucial to their usage both for current and future benefits (Quedraogo 2001; Malviya et al. 2021).Some reports have been documented on genetic diversity assessment in Mucuna plants using molecular approach (Patil et al. 2016; Chinapolaiah et al. 2018; Lepcha and Sathyanarayana 2021). Misra et al. (2021) assessed genetic diversity and extent of variability in Mucuna Adans. (Fabaceae) in

Odisha, Eastern India using some botanical characters and chemical evaluation of L-dopa, while some qualitative and reproductive traits were employed for same purpose by Mahesh and Sathyanarayana (2011) and reproductive traits by Hemkumar et al. (2021). Seed physical dimensions have been evaluated in hemp (Sacilik et al. 2003), lentil (Amin et al. 2004), fenugreek (Altunlas et al. 2005), sunflower (Baumler et al. 2006), linseed (Selvi et al. 2006) and sesame (Azeez and Morakinyo 2011). There is dearth of information on seed physical dimensions of Mucuna species and its usefulness in the assessment of genetic diversity. As such, the present study was conducted to assess the variability in physical dimensions and L-DOPA content of the seeds of *M. pruriens* germplasm and investigate the degree of association among the traits and their utility in selection.

Materials and methods

Seeds of fifty-nine (59) *M. pruriens* accessions (both itching and non-itching types) collected from different parts of India's terrain were evaluated for L-dopa content and seed physical traits. Ten seeds were randomly selected from collections of each accession and three linear dimensions such as length (L), width (W) and thickness (T) of the seeds were measured using Vernier caliper. Hundred seed weight was determined using weighing balance. Seed geometric mean diameter was determined according to Tarighi et al. (2011)

$$Dg = (LWT)^{1/3}$$

Where; *Dg* is the geometric mean diameter, *L* is the length, *W* is the width, *T* is the thickness.

Arithmetic mean diameter was calculated following the method of Isık and Unal (2007).

$$Da = (L + W + T)/3$$

The sphericity (Φ) for the Mucuna seeds was calculated as a function of the three principal dimensions (Mohsenin 1970) and reported as average of ten determinations.

$$\Phi = [(LWT)^{1/3}/L] * 100$$

The surface area (As) was measured using equation that shows the analogy with a sphere of same geometric mean diameter (Sathe 1984).

$$As = \pi Dg^2$$

Extraction and Quantification of L-DOPA

Seeds of *M. pruriens* collected were grinded into fine powder and L-dopa content was determined and quantified by reversed phase high-performance liquid chromatography (RP-HPLC) method described by Hasegawa et al. (2011). Dried sample of powdered seeds (100mg) was weighed and ultrasonically extracted for 15 minutes using 5ml of acetonitrile/water/ formic acid (50:50:1). The supernatant was transferred to a 20ml volumetric flask after being centrifuged at 1,300 g for 10 min. The precipitate was further centrifuged after being extracted ones again with 5mL of acetonitrile/water/formic acid (50:50:1). The volume of the combined supernatants from the extractions was adjusted to 20 ml using acetonitrile/water/formic acid (50:50:1). This solution was filtered using a polytetrafluoroethylene membrane filter with a 0.45μm pore size and the filtrate was diluted ten times with acetonitrile, water, and formic acid (50:50:1). HPLC was performed using a PU-2089 apparatus equipped with ultraviolet (UV) detector (Model LC-2030 PLUS (IND), SHIMADZU and C18 reverse phase column (250 x 4.6 mm, 5 μm). Ammonium formate buffer of 10 mmol/l (pH 3.5)/acetonitrile (3:7) served as the mobile phase. The injection volume was 10 μl, and the mobile phase's flow rate was set at 1.0 ml/min. The column temperature was maintained at 40°C with UV detector set at 280 nm.

Data analysis

Data collected on seed physical dimensions were subjected to analysis of variance using SPSS software version 22 while statistically significant means were separated using Duncan Multiple Range Test ($P < 0.05$).

Result and discussion

Seed traits, among other characteristics, are key elements of crop improvement and a promising path for further study into the genetic control of seed development ((Maiti et al. 1994; Gonzalez-Andres and Ortiz 1995). Significant differences were observed among the genotypes for each trait indicating that all of the characters genotypes are variable. According to the descriptive statistics for the L-dopa content in dried seed powder *M. pruriens* seeds have an L-dopa content that ranges from 1.79–6.05% respectively. Furthermore it has been noticed that 55.93% accessions have L-dopa content lower than 4% and 44.03% accessions have higher L-dopa content up to 6% which can be considered as a good germplasm for plant breeding programs. Results from a comparable study were published by Tewari (1999); Singh et al. (1995a, 2008b); Raina et al. (2018) and Chinapolaiah et al. (2019). Significant variation of L-dopa content in Mucuna genotypes could be due to difference between the genetic makeup of genotypes, geographic location and environment effect. In the current study, it was found that wild varieties with itching trichomes have higher level of L-dopa in their seeds than non-itchy types do.

Descriptive statistics for the seed morphological traits showed that the seed length among the accessions varied from 5-19.3mm, seed width ranges from 2.66-13.00mm and seed thickness was calculated between 0.90-8.67mm. For all of the accessions examined in the study, it was observed that the seed length was greater than their width and thickness. Length width ratio (1.17–2.26), length thickness ratio (1.50–7.40), width thickness ratio (0.89-6.00) seed geometric diameter(2.34–12.60 mm), arithmetic mean diameter (2.88–13.33 mm), seed sphericity (41.89–78.75), seed surface area (17.19-498.51 mm^2) and the hundred seed weight (19.30-150.59gm) among different accessions varied significantly and also comparable to the previous studies by Sathyanarayana and Mahesh (2012); Lepcha and Sathyanarayana (2021); Raina and Misra (2018). An important agronomic trait that can be used in breeding efforts to create desirable cultivars is seed weight since the heavier the seeds, the better the price they command (Alfieri et al. 2017; Elliott et al. 2007; Gondwe et al. 2019). The present study revealed that 44.06% accessions are having less then 50gm of 100 seed weight and considered as small type, 27.11% having more than 50gm considered as medium type and 21.81% with 100 seed weight more than 100gm which are desirable for breeding programs to increase yield component (Krishnamurthy et al. 2002). Merely 1.6% of accessions with L-dopa concentration higher than 4% have 100 seeds weighing more than 100gm. Dessalegn et al. (2008) reported a similar finding, that coffee beans with a low 100-bean weight contained much more caffeine than heavy beans.

Principle component analysis

A two-dimensional principal component analysis was performed using the seed morphological traits and the central variability of all traits was displayed. Similar studies on seed trait were reported on bambara ground nut by Esan et al. (2023); Olanrewaju et al. (2021); Stoilova et al. (2013) in cowpea and Khatun et al. (2022) in country bean revealed that first principle component explained the greater proportion of the total variability explained (76.119) followed by second (10.077) and third (6.888). As suggested by Arshad et al. (2010) that the characters positively affected to first three principal components might be taken into account while choosing the best hybrids without losing yield potential. Kaiser (1960) used the eigenvalue criterion to select statistically significant principal component. The first component contributes the maximum eigenvalue (9.134) followed by Two (1.209) and three (0.827) respectively. The first component had high positive loadings from Seed Length (0.929), Seed Width (0.965), Seed Thickness (0.984), Seed Geometric Diameter (0.987), Arithmetic Mean Diameter (0.977), Seed Sphericity (0.902), Seed Surface Area (0.971) and 100 seed weigh (0.857). The second compound had high positive loading from Seed Length (0.075), Seed Width (0.225), Length-Thickness Ratio (0.514), Width-Thickness Ratio (0.703), Seed Geometric Diameter (0.050), Arithmetic Mean Diameter (0.088) and Seed (0.514), Surface Area (0.114) and 100 seed weigh (0.302), however the third compound had high positive loading from all attributes except seed sphericity and L-Dopa content.

Correlation coefficient analysis

For systematic breeding to determine seed yield and shape, the metrics measure on seeds, their correlation, and relationship are crucial (Mahalingam et al. 2020; Adebisi 2004; Adewale et al. 2013). Correlation coefficient analysis revealed that LDP content is positively correlated with LWR (0.320**), WTR (0.409**) and negatively correlated with all the other seed characters indicating that seed size may not be a selection criterion for identifying a genotype with high L-dopa content (Raina et al. 2012). Seed length (SLT) has significant correlation with SWT (0.940**) which is crucial for farmers as they prefer the seeds with big size. Thus, these two factors are crucial for size improvement (Valombola et al. 2019). STH (0.926**), SGD (0.970**), AMD (0.982**), SSC (0.705**), SSA(0.954**) and HSW(0.789**). Seed width (SWT) has positive correlation with STH (0.936**), SGD (0.978**), AMD (0.980**), SSC (0.821**), SSR (0.972**) and HSW (0.868**). Highly positive correlation of thickness (SHT) was observed with SGD (0.982**), AMD (0.971**), SSC (0.971**), SSA (0.966**) and HSW (0.808**). Length width ratio (LWR) has positive correlation with WTR (0.092). Length thickness ratio (LTR) and width thickness ratio (WTR) (0.943**) has significant correlation. Seed geometric diameter (SGD) has positive correlation with AMD (0.997**), SSC (0.845**), SSA (0.988**) and HSW (0.840**). Arithmetic mean diameter (AMD) positively correlated with SSC (0.812**), SSA (0.985**), HSW (0.839**). Seed sphericity (SSC) has positively correlated with SSA (0.808**) and HSW (0.691**). Seed surface area (SSA) and HSW (0.876**) was also correlated significantly (Table no 3). Similar attributes were reported by Enyew et al. (2021) in 324 accessions of Sorghum for agronomic traits, 44 Bambara groundnut accessions by Khan et al. (2022) for yield attributes, 40 accessions of Mustard for yield and oil content by Yadav et al. (2021), 24 accessions of Jatropha by Kaushik et al. (2007) for seed trait and oil content and 79 accessions of African yam bean by Adewale et al. (2012).

Cluster analysis

The degree of genetic variation is revealed through cluster analysis, which is helpful for plant breeding (Sultana et al. 2006). A distribution of 59 *M. pruriens* accessions classified in to IV clusters. Cluster-I consisted of maximum number of genotypes and have 14 genotypes that are 50.87% of the total genotypes evaluated. The genotypes in this cluster indicate had narrow genetic diversity among them. The higher number genotypes next to these cluster was cluster-II consist of twelve accessions (21.05%), followed by clusters III and IV, each of which had nine accessions (15.78%) (Table 4). It has been suggested by (Abdulkareem et al. 2015) that even though accessions within a cluster have similar genetic traits, environmental influences over time may have caused them to diverge morphologically. The cluster mean values recorded in the present study presented in Table 5 indicated appreciable variation among the clusters. According to the values cluster IV represents highest number of accessions with superior characteristics for seed associated characters followed by cluster III, cluster I and cluster II respectively while cluster II included the accessions with higher L-dopa content followed by cluster III, I and IV. The average intra and inter cluster distance (Table 6) revealed that the genetic diversity among the genotypes were generally superior for one or few characters which made them divergent from other clusters. The genotypes from distantly related clusters are likely to produce more transgressive segregants on hybridization and selection (Geetha et al. 2011). Maximum distance (417.537) was observed between clusters III and cluster II giving scope for hybridization between the genotypes of these clusters for improvement of yield and yield contributing traits followed by cluster II and IV (286.622) and cluster I and III (283.458) were the minimum indicating close relationship between those clusters. The highest inter cluster distance was observed in cluster IV (57.933) while the minimum distance was found in cluster III (30.583).

Understanding the relationships between different variables at the beginning of any breeding programme enhances the likelihood of choosing superior genotypes with a wide range of desirable traits. According to Gizachew and Gebeyaw (2021), the genotypes in the cluster with the greatest intercluster distance are genetically more diverse, and these genotypes might be used in the velvet bean hybridization programme to evolve high L-Dopa types in order fulfill the needs of the pharmaceutical business.

Conclusion

The enormous genetic variability is a prerequisite for establishing a breeding program. The success of breeding relies on the amount of genetic variety present in the crop gene pool. The present investigation found significant difference between the L-dopa content and seed-associated traits. The principle, component analysis and correlation analysis of these attributes in combination with different sets of characters revealed an undesirable negative correlation between 100 seed weight and L-dopa content, a promising antiparkinson drug. As a result, these qualities can be enhanced through hybridization and selection to develop a cultivar with a high yield.

Declarations

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Author contributions

Methodology: [SP, RK], Investigation: [SP], Writing: [SP, MA], Supervision: [RK], Validation: [SP, MA, RK].

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Confict of interest

The author declares no confict of interest.

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Tables

Table 1
Variability for L-Dopa content and seed associated characters among *M. pruriens* accessions

Character	Mean	Range	SD	Std. error	CV%	LSD5%
L-Dopa (%)	3.67	1.79–6.05	1.06	0.14	28.83	0.28
Seed length (mm)	11.20	5.00-19.33	3.94	0.52	35.11	1.03
Seed Width (mm)	7.88	2.66-13.00	3.30	0.43	41.89	0.87
Seed Thickness (mm)	4.69	0.90–8.67	2.67	0.35	56.95	0.70
Length width ratio	1.49	1.17–2.26	0.23	0.03	15.56	0.06
Length thickness ratio	3.09	1.50–7.40	1.53	0.20	49.45	0.40
Width thickness ratio	2.07	0.89-6.00	0.95	0.12	45.94	0.25
Seed geometric diameter (mm)	7.36	2.34–12.60	3.32	0.44	45.13	0.87
Arithmetic mean diameter (mm)	7.92	2.88–13.33	3.23	0.42	40.75	0.85
Seed sphericity (Φ)	63.20	41.89–78.75	10.23	1.34	16.18	2.69
Seed surface area (mm^2)	204.33	17.19-498.51	155.54	20.42	76.12	40.90
100 Seed weight(gm)	68.19	19.30-150.59	40.86	5.37	59.92	10.74

Table 2
Principle component analysis

Attributes	Principal Components		
	1	2	3
L-Dopa %	-0.671	-0.107	-0.355
Seed Length (mm)	0.929	0.075	0.279
Seed Width (mm)	0.965	0.225	0.003
Seed Thickness (mm)	0.984	-0.071	0.034
Length-Width Ratio	-0.584	-0.482	**0.629**
Length-Thickness Ratio	-0.817	0.514	0.224
Width-Thickness Ratio	-0.701	**0.703**	0.025
Seed Geometric Diameter (mm)	**0.987**	0.050	0.084
Arithmetic Mean Diameter (mm)	0.977	0.088	0.124
Seed Sphericity (%)	0.902	-0.179	-0.370
Seed Surface Area (mm^2)	0.971	0.114	0.111
100-Seed Weight (gm)	0.857	0.302	0.065
Eigen Value	9.134	1.209	0.827
% Variance Explained	76.119	10.077	6.888
Cumulative Variance Explained	76.119	86.196	93.085
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Table 3
Correlation coefficients between L-DOPA% and seed physical dimensions

Attributes	SLT	SWT	STH	LWR	LTR	WTR	SGD	AMD	SSC	SSA	HSW
LDP	-0.630**	-0.617**	-0.613**	0.320**	0.468**	0.409**	-0.631**	-0.635**	-0.497**	-0.617**	-0.614**
SLT		0.940**	0.926**	-0.391**	-0.651**	-0.577**	0.970**	0.982**	0.705**	0.954**	0.789**
SWT			0.936**	-0.662**	-0.665**	-0.513**	0.978**	0.980**	0.821**	0.972**	0.868**
STH				-0.502**	-0.817**	-0.727**	0.982**	0.971**	0.971**	0.966**	0.808**
LWR					0.386**	0.092	-0.535**	-0.523**	-0.676**	-0.530**	-0.581**
LTR						0.943**	-0.752**	-0.716**	-0.910**	-0.695**	-0.537**
WTR							-0.646**	-0.609**	-0.763**	-0.594**	-0.418**
SGD								0.997**	0.845**	0.988**	0.840**
AMD									0.812**	0.985**	0.839**
SSC										0.808**	0.691**
SSA											0.876**

*Represents significant correlation at $P < 0.05$. L-dopa content (LDP), seed length (SLT), Seed width (SWT), thickness (STH), length width ratio (LWR), length thickness ratio (LTR), width thickness ratio (WTR) seed geometric diameter(SGD), arithmetic mean diameter (AMD), seed sphericity (SSC), seed surface area (SSA) and hundred seed weight (HSW).

Table 4
Composition of Euclidean clusters for L-Dopa and seed traits in Accessions of plants

Cluster Studied	No of Accessions	Accession No
I	29	Mp027, Mp037, Mp025, Mp031, Mp029, Mp034, Mp033, Mp038, Mp021, Mp030, Mp022, Mp041, Mp020, Mp024, Mp026, Mp023, Mp028, Mp019, Mp035, Mp036, Mp039, Mp032, Mp048, Mp042, Mp043, Mp053, Mp058, Mp040, Mp049
II	12	Mp006, Mp046, Mp044, Mp050,Mp 045, Mp047, Mp015, Mp057, Mp052, Mp007, Mp056, Mp017
III	09	Mp001, Mp059, Mp014, Mp011, Mp018, Mp004, Mp013, Mp003, Mp008
IV	09	Mp002, Mp009, Mp010, Mp016, Mp051, Mp055, Mp005, Mp054, Mp012

Table 5
Cluster mean values for L-Dopa and seed traits in 59 accessions studied

Attributes	Cluster I	Cluster II	Cluster III	Cluster IV
L-Dopa %	3.23	4.43	3.60	2.73
Seed Length (mm)	14.09	6.94	11.76	16.44
Seed Width (mm)	10.69	4.38	7.59	12.44
Seed Thickness (mm)	6.86	1.76	4.77	8.29
Length-Width Ratio	1.32	1.61	1.57	1.32
Length-Thickness Ratio	2.06	4.52	2.56	1.99
Width-Thickness Ratio	1.56	2.85	1.67	1.50
Seed Geometric Diameter (mm)	10.09	3.70	7.43	11.97
Arithmetic Mean Diameter (mm)	10.54	4.36	8.04	12.39
Seed Sphericity	71.80	53.17	63.74	73.07
Seed Surface Area (mm^2)	320.88	44.77	177.35	450.32
100-Seed Weight (gm)	96.76	37.08	46.32	132.63

Table 6
Estimates of between and within cluster Euclidean distances for L-Dopa and seed traits in 59 accessions studied

Clusters	I	II	III	IV
I	51.013	134.393	283.458	152.462
II		51.325	**417.537**	286.622
III			30.583	133.597
IV				57.933

Figures

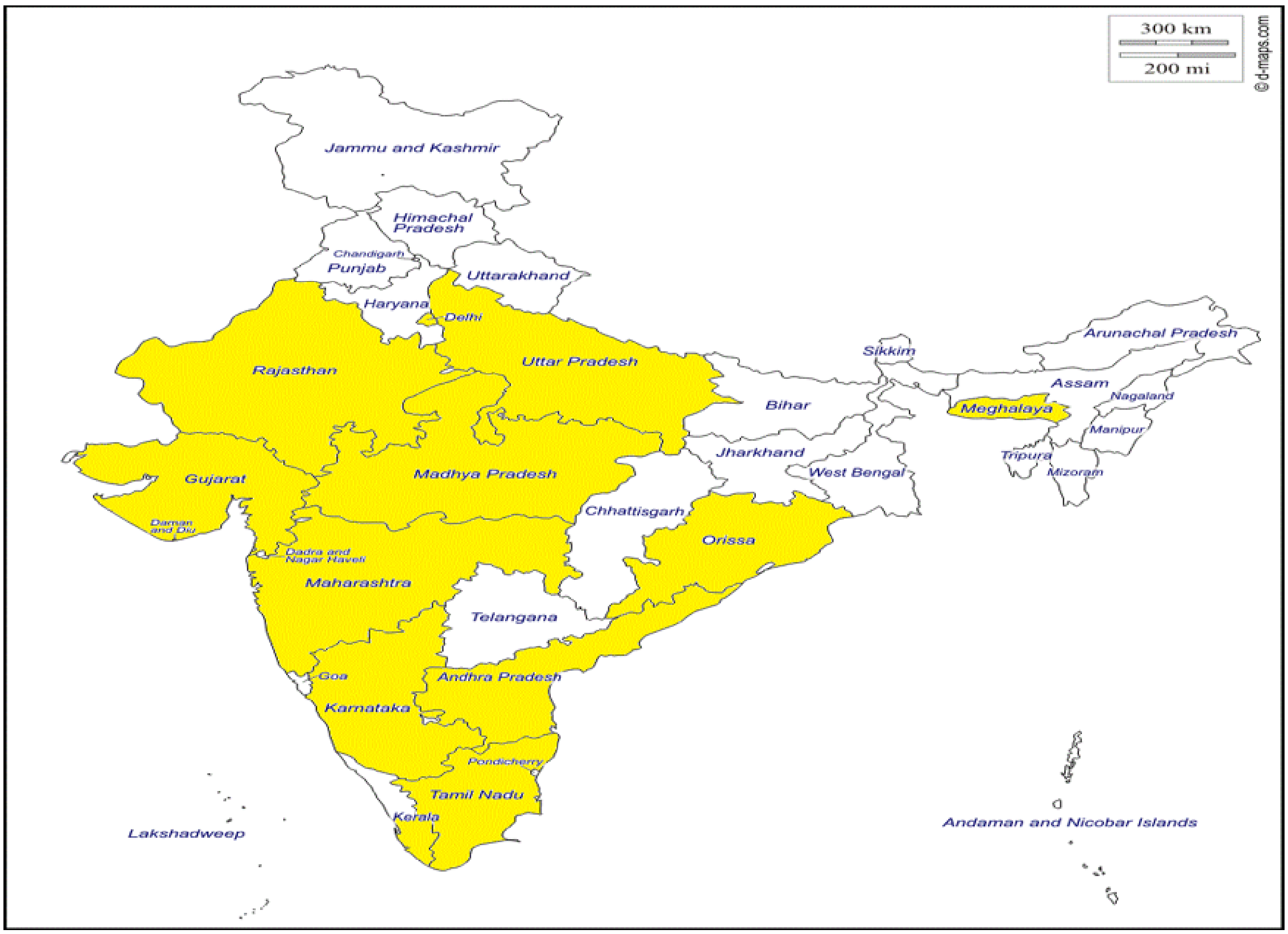

Figure 1

Map showing the locations of study area

Figure 2

Variability in *M. pruriens* seeds

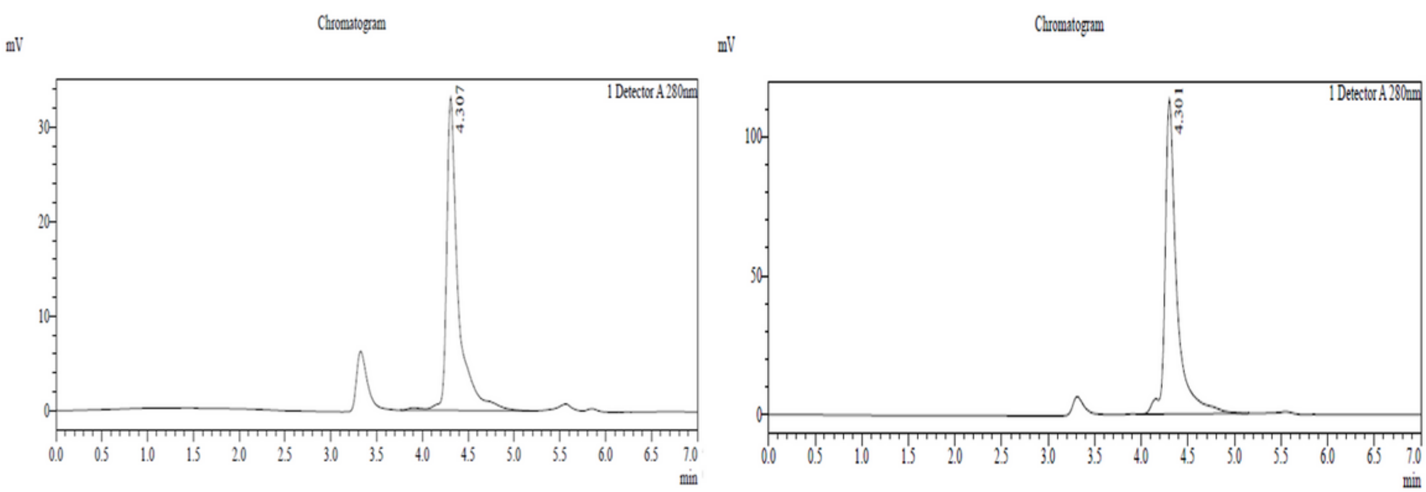

Figure 3

HPLC chromatogram of L-dopa content (minimum (1.79), Maximum (6.05)

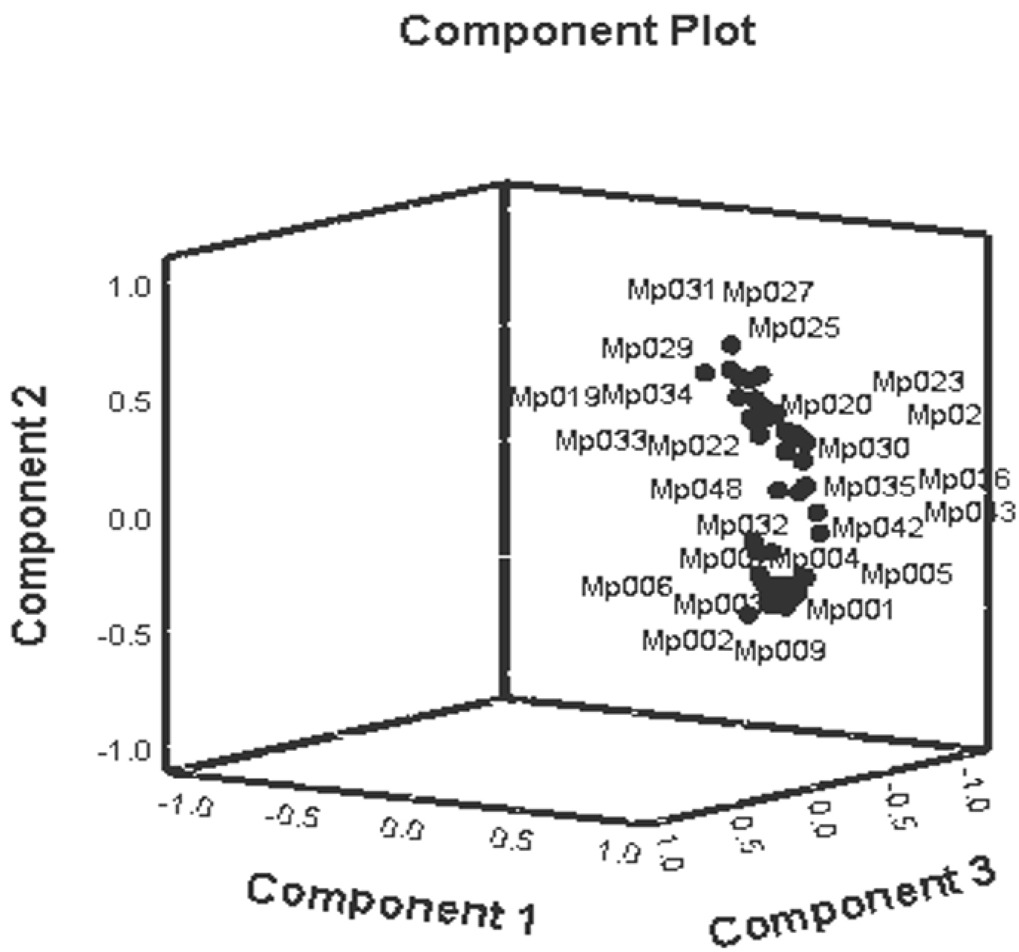

Figure 4

Ordination of the 59 accessions of plant studied on principal components (axes) 1, 2 and 3 from cluster analysis of L-Dopa and seed trait

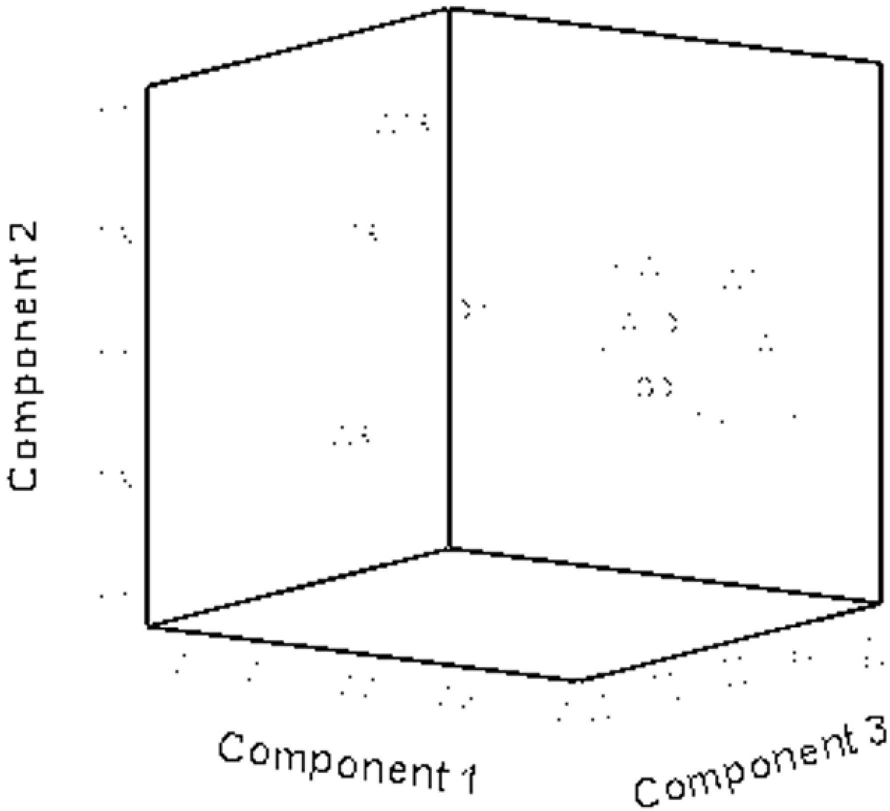

Figure 5

Ordination of the L-Dopa and seed traits of the 59 accessions studied on principal components (axes) 1, 2 and 3 from cluster analysis.

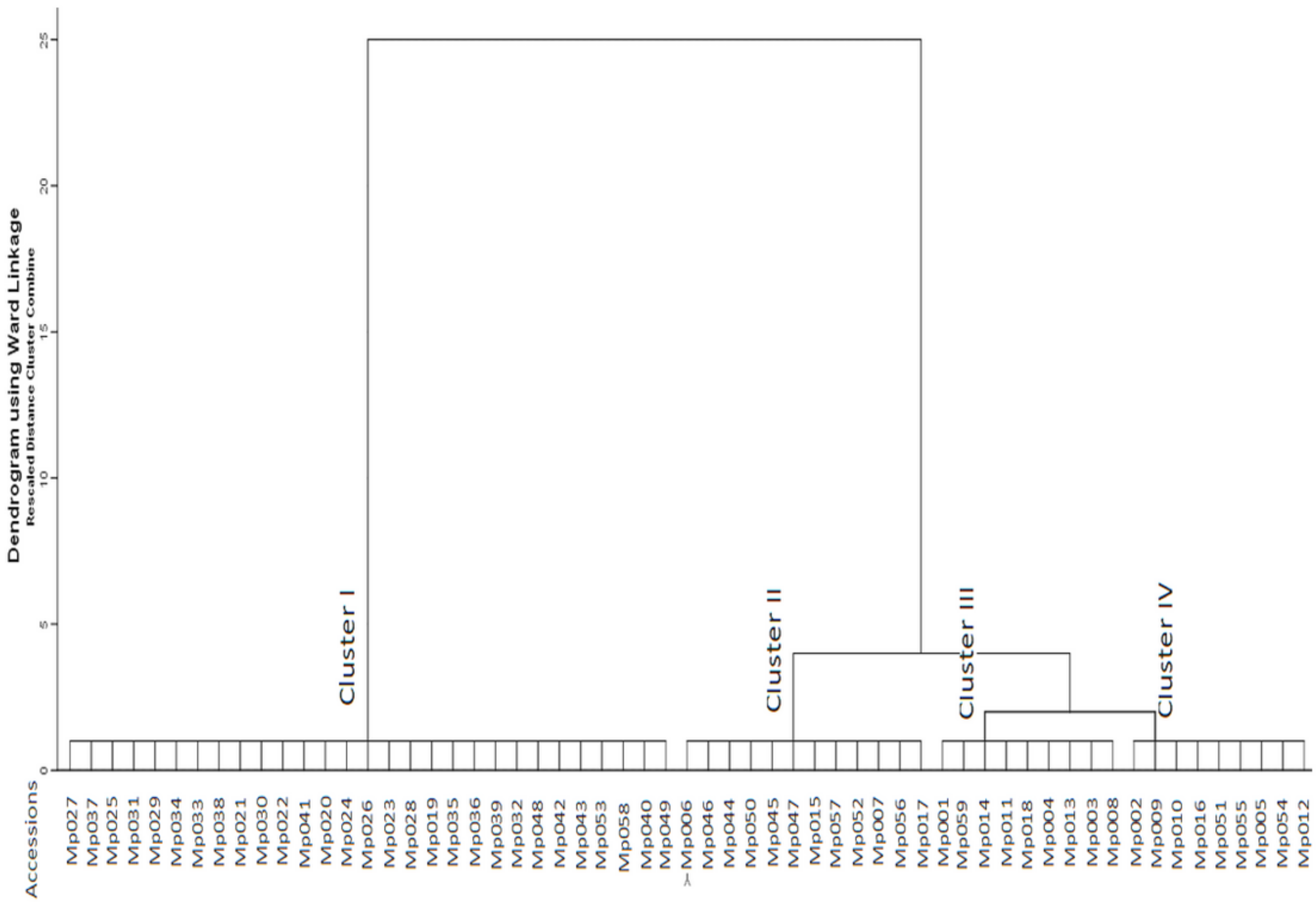

Figure 6

Dendrogram of 59 accessions of plant seeds studied using Ward linkage