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# Variability in L-Dopa and other biochemical composition of *Mucuna pruriens* (L.) an underutilized tropical legume

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ABSTRACT

*Mucuna pruriens* (L.) is an important underutilized legume medicinal plant. It is an herbaceous annual twining climber. Its seeds are used to extract the L-Dopa (L-3, 4-dihydroxyphenylalanine) to the preparation of the drugs and are used in the treatment of Parkinson's disease. Mucuna seeds are rich source of L-Dopa in addition to protein and other minerals. In the present study estimation of L-Dopa and other biochemical traits in 58 genotypes of velvet bean has been analyzed to identify the genotypes having maximum seed yield coupled with high L-Dopa to the preparation of drugs. The L-Dopa is varied significantly from 2.94 to 6.91% among mucuna genotypes. The levels of total phenol content and tannins varied significantly among genotypes and ranged from 27.73 to 103.50 mg/100 g and 0.18 to 0.96 mg/100 g DW, respectively. The protein content in the estimated genotypes of Mucuna seed greatly varied from 19.08% to 38.18% with a mean value of 28.16%. Significantly maximum L-Dopa, total phenol content, total tannin, crude protein, fat and carbohydrate was recorded in IIHR MP 62-1 (6.91%), IIHR MP 62-2 (103.50 mg/100 g), IIHR MP 63-1 (0.96 mg/100 g), IIHR MP-74 (38.18%), IIHR MP 95 (7.54%) and IIHR MP 62-3 (64.36%), respectively. Significantly low L-Dopa, total phenol content, total tannin found in IC 332432 (2.94%), IIHR MP 89-1 (27.73 mg/100 g), IIHR MP 10 (0.183 mg/100 g), respectively. Biochemical traits viz., crude protein, fat and carbohydrate found less in EC 17827 (19.08%), IIHR MP 62-3 (3.10%) and IIHR MP 74 (47.81%), respectively. Cluster analysis based on biochemical data revealed that the cluster I showed highest mean value for L-Dopa and total phenol content, cluster III for total tannin and cluster VI for crude protein. The results showed that intra cluster distance was maximum in cluster VI (167.51) and maximum inter cluster distance was between cluster I and V (2014.94). Total phenol content was the main contributor to the total genetic divergence. The traits fat and L-Dopa were moderately contributing and carbohydrate was least contributing to the total divergence of the estimated biochemical traits. The genotypes belonging to the cluster with maximum inter cluster distance are genetically more divergent and these genotypes could be used in the hybridization programme of velvet bean to evolve high L-Dopa type to meet the demand of drug industry.

## 1. Introduction

Velvet bean (*Mucuna pruriens* L.) is an important underutilized medicinal plant belonging to the family Fabaceae. It is indigenous to India and other tropical areas including Central and South America. In India about 14 species are found in the foothills of Himalayas, the plains of West Bengal, Madhya Pradesh, Karnataka, Kerala, Andhra Pradesh and Uttar Pradesh, the Andaman and Nicobar Islands and Sri Lanka (Farooqi et al., 1999). It is an herbaceous annual twining climber and grows to 3–8 m in height. It has trifoliate leaves. Its flowers are creamy white, light purple to deep purple in colour and are self pollinated. The fruit is called as pod and slightly curved at both ends and appears as sigmoid shape. The pod is 6–12 cm in length and each pod consists of 4–6 seed. The seed colour is varied from white, black and mottle with mosaic. The seed size varies from small, medium to bold seed. The pods are covered with reddish orange coloured long stiff hairs that can cause itching and dermatitis when it comes in contact with skin. It gives a yield about 1.3 to 2.5 t/ha (Gurumoorthi et al., 2003)

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and the yield of total biomass 20–30 t/ha and dry matter ranged from 7 to 9 t/ha. Velvet bean is a potential crop for cultivation and it can tolerate to a number of environmental stresses such as drought, low soil fertility and high soil acidity. It is widely used in traditional Ayurvedic Indian system of medicine for the management of male fertility, nervous disorder and as an aphrodisiac. It is a constituent for more than 200 indigenous drug formulations. Mucuna seeds are used for extraction of L-Dopa which is used to relief the symptoms of Parkinson disease. Mucuna seeds are rich source of L-Dopa a non protein phenolic amino acid and precursor of the brain neurotransmitter dopamine (Vadivel and Pugalenthi, 2008). The preparations made from the seeds of mucuna are used for the management of ageing, rheumatoid arthritis, diabetes, male infertility and nervous disorders (Raina and Khatri, 2011). Besides L-Dopa, nicotine, physostigmine, serotonin, bufotenine, choline, *N-N* dimethyl tryptamine and some indole compounds are the other phytochemicals present in the other parts like roots, stems, leaves of velvet bean (Tripathi and Upadhyay, 2002). In addition to L-Dopa, pharmacologically active compounds methylated and non-methylated tetra hydroisoquinoline are also present in its seeds (Siddhuraju and Becker, 2001). Although all plant parts of mucuna such as leaf, stem, seed and root have been reported to possess medicinal values but great importance has been given to the seed for extraction of high L-Dopa. In addition to medicinal values, Mucuna fixes nitrogen and it is used as a green manure and cover crop by the farmers (Farooqi et al., 1999). Mucuna seed contains high concentration of bioactive compounds such as free phenolics, tannins, L-Dopa, phytic acid in addition to protein (26–29%) and other nutrients (Pugalenthi et al., 2005). In India, the tonic prepared from Mucuna seeds are used for male vitality in traditional medicine (Misra and Wagner, 2007). Pharma industry needs high seed yield in association with high L-Dopa content to extract high L-Dopa yield by the demand of drug manufacturing industries to the treatment of the Parkinson's disease. Raina et al. (2012) screened 34 accessions of *Mucuna pruriens* for L-Dopa and found that the range of L-Dopa was 3.29 to 5.44% in the seeds. The variability of biochemical composition of mucuna was reported by earlier workers with few genotypes only and available information is meagre. Keeping this in view, biochemical composition of 58 genotypes collected from different parts of the India are studied in the present work to identify high L-Dopa genotypes those could be used in hybridization programme of velvet bean.

## 2. Material and methods

A total of fifty eight genotypes of velvet bean were used for the estimation of biochemical analysis. The genotypes which were collected from the different parts of India are being maintained at the field gene bank of ICAR-Indian Institute of Horticultural Research, Bengaluru. The genotypes consist of both itching and non itching types, where non itching types in which some of the advance breeding lines and two released varieties i.e. *Arka Aswini* and *Arka Dhanwantri* are grown during the year 2014 at the experimental plots of medicinal plants at ICAR-IIHR, Bengaluru. The matured pods were harvested and seeds are separated from the pods. The seed material which are made into fine powder and used for analysis of biochemical traits. The L-Dopa estimated according to the protocol developed by Shivananda et al. (2003) by using analytical instrument ultra high performance liquid chromatography (UHPLC).

### 2.1. Extraction and estimation of L-Dopa

About 50 mg of seed powder was taken in a conical flask and added 25 ml of 0.1 M $H_3PO_4$ and sonicated for 15 min. It was filtered and sufficient amount was added through filter paper to make up to 50 ml with double distilled water. The pure L-Dopa was obtained from Sigma. A stock solution of L-Dopa was prepared by dissolving an accurately weighed 100 mg L-Dopa standard in 100 ml of 0.1 M $H_3PO_4$ in a volumetric flask. From the stock solution prepared different concentration of 10, 20, 30, 50,100 and 200 ppm solution and standard curve was prepared and depicted in Fig. 1. Quantitative estimation of L-Dopa was done by Shimadzu Nexera $X_2$ ultra high-performance liquid chromatography (UHPLC) with the following conditions: Shimadzu Shim-Pack XR-ODS III, (2 mm X 150 mm size); detector-PDA detector set at 280 nm; time: 4 min; mobile phase of 2.8 pH Sodium dihydrogen phosphate solution ($NaH_2PO_4$ $2H_2O$), flow rate: 0.4 mL/min, injection volume: 5 µL, Retention time of L-Dopa:1.94 to 1.96 min.

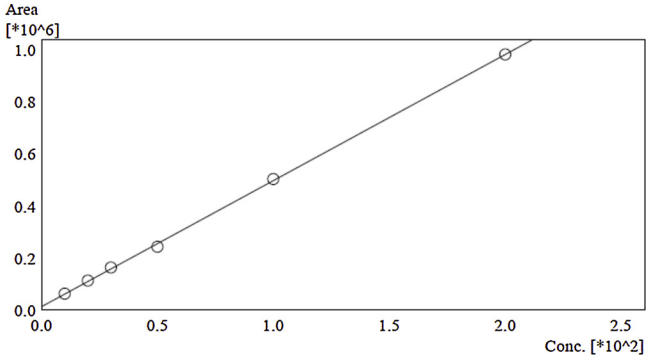


**Fig. 1.** Calibration curve using different concentration of standard L-Dopa.

$$\frac{\text{Area of the sample} \times \text{Standard weight(mg)} \times \text{Sample dilution} \times \text{Purity of standard}}{\text{Area of the standard} \times \text{Standard dilution} \times \text{Sample weight(mg)} \times 100} \times 100$$

The crude protein content was estimated by multiplying the percentage of Kjeldhal nitrogen by a factor 6.25 (AOAC, 1990). Crude lipid content was estimated using soxhlet apparatus (AOAC, 2005). Carbohydrate was estimated by calculation of difference method (Muller and Tobin, 1980). Total phenol content estimated using Folin Ciocalteu Reagent method (Bray and Thorpe, 1954). Weighed about 50 mg of the Mucuna seed powder was added in 15 ml of 80% ethanol in volumetric tube. It was mixed thoroughly and kept overnight. The supernatant extract of 0.2 ml taken into fresh test tube and added 8.3 ml of distilled water. The addition of FCR (Folin Ciocalteu Reagent) 0.5 ml to the sample and as well as to the blank (without sample) and mixed properly. After 10–15 min 1 ml of 20% $Na_2CO_3$ added and mixed thoroughly and incubated for 1 h. Standard curve was prepared using different concentration of gallic acid. From the standard curve concentration of total phenol content was calculated in the test sample and expressed as mg phenols /100 g material.

$$\text{Formula} = \frac{\text{total volume of extract(ml)}}{\text{Volume taken for estimation(ml)}} \times \text{Concentration} \times \frac{1}{\text{weight of the sample taken for extraction(g)}}$$

The total tannins were estimated according to Makkar et al. (1993) with minor modifications. Biochemical compounds were estimated using triplicate of the seed samples of *Mucuna* genotypes. Biochemical variables were correlated, they were transformed into uncorrelated linear combination through pivotal condensation method. The genetic divergence among 58 genotypes of Mucuna for six biochemical characters was assessed through Mahalanobis $D^2$ statistics following the procedure given by Rao (1952). The average $D^2$ values have been used for constellation of genotypes into clusters in such a way that the genotypes in the cluster had smaller average $D^2$ values than those belonging to different clusters. The simultaneous significance of mean differences was tested by analysis of dispersion. The F value is highly significant indicating large differences between the means of the genotypes based on the pooled effect of six biochemical characters further

**Table 1**
Mean performance of 58 *Mucuna* genotypes for biochemical parameters.

| Sl. No. | Germplasm | L-Dopa(%) | Total phenols (mg/100 g) | Total tannin (mg/100 g) | Protein (%) | Fat (%) | Carbohydrate (%) |
|---|---|---|---|---|---|---|---|
| 1. | IC 2199 | 3.17 | 74.71 | 0.25 | 25.39 | 6.60 | 57.51 |
| 2. | IC 21998 | 3.61 | 64.89 | 0.34 | 31.09 | 6.26 | 52.03 |
| 3. | IC2534 | 3.30 | 57.78 | 0.65 | 35.96 | 5.43 | 48.00 |
| 4. | IC202969 | 3.61 | 58.30 | 0.33 | 23.19 | 5.85 | 60.36 |
| 5. | IC33243 | 2.94 | 54.12 | 0.26 | 27.91 | 6.51 | 54.97 |
| 6. | IC 332432 | 3.62 | 65.49 | 0.27 | 27.75 | 5.42 | 54.99 |
| 7. | IC 83195 | 4.44 | 73.17 | 0.74 | 32.27 | 4.28 | 52.84 |
| 8. | EC17827 | 3.37 | 59.77 | 0.43 | 19.08 | 7.17 | 63.13 |
| 9. | EC2533A | 3.10 | 36.61 | 0.34 | 32.25 | 5.46 | 52.68 |
| 10. | EC 25334 | 3.92 | 33.75 | 0.41 | 32.71 | 5.22 | 51.46 |
| 11. | IIHR MP 5 | 4.09 | 68.11 | 0.26 | 30.52 | 5.89 | 52.98 |
| 12. | IIHR MP 7-1 | 4.90 | 76.91 | 0.52 | 25.83 | 4.17 | 58.90 |
| 13. | IIHR MP 9 | 3.65 | 72.82 | 0.34 | 25.04 | 5.38 | 58.97 |
| 14. | IIHR MP10 | 4.03 | 65.63 | 0.18 | 26.33 | 4.82 | 58.24 |
| 15. | IIHR MP 11 | 3.76 | 60.62 | 0.25 | 26.16 | 5.83 | 57.39 |
| 16. | IIHR MP 17 | 3.81 | 52.59 | 0.54 | 37.04 | 4.16 | 48.30 |
| 17. | IIHR MP 21 | 6.77 | 98.33 | 0.95 | 33.50 | 3.25 | 52.64 |
| 18. | IIHR MP 22 | 5.63 | 35.51 | 0.24 | 24.48 | 6.21 | 58.70 |
| 19. | IIHR MP 44 | 6.46 | 72.29 | 0.74 | 27.52 | 3.32 | 58.56 |
| 20. | IIHR MP 45 | 5.24 | 83.79 | 0.87 | 26.87 | 3.88 | 58.63 |
| 21. | IIHR MP 47 | 3.66 | 85.34 | 0.68 | 31.62 | 3.33 | 54.43 |
| 22. | IIHR MP 62-1 | 6.91 | 92.27 | 0.83 | 25.84 | 3.31 | 60.24 |
| 23. | IIHR MP 62-2 | 6.47 | 103.50 | 0.74 | 34.23 | 3.29 | 52.88 |
| 24. | IIHR MP 62-3 | 5.99 | 83.04 | 0.96 | 21.92 | 3.10 | 64.36 |
| 25. | IIHR MP 63 | 6.36 | 85.45 | 0.96 | 31.93 | 3.65 | 53.83 |
| 26. | IIHR MP 63-1 | 6.76 | 93.01 | 0.96 | 30.79 | 3.69 | 54.91 |
| 27. | IIHR MP 74 | 6.54 | 94.30 | 0.86 | 38.18 | 3.38 | 47.81 |
| 28. | IIHR MP 74-3 | 5.49 | 82.41 | 0.83 | 28.25 | 3.34 | 57.80 |
| 29. | IIHR MP 82 | 4.65 | 85.10 | 0.49 | 21.96 | 5.88 | 61.54 |
| 30. | IIHR MP 84 | 3.27 | 52.34 | 0.24 | 25.00 | 7.31 | 57.07 |
| 31. | IIHR MP 85 | 4.44 | 61.83 | 0.36 | 22.62 | 6.35 | 60.41 |
| 32. | IIHR MP 87 | 3.63 | 65.42 | 0.34 | 30.78 | 6.85 | 51.75 |
| 33. | IIHR MP 88 | 3.08 | 65.42 | 0.38 | 32.02 | 5.32 | 52.05 |
| 34. | IIHR MP 89 | 3.16 | 67.42 | 0.28 | 35.21 | 6.22 | 47.95 |
| 35. | IIHR MP 89-1 | 3.19 | 27.73 | 0.27 | 29.37 | 6.36 | 53.69 |
| 36. | IIHR MP 90 | 3.13 | 53.17 | 0.60 | 21.66 | 6.09 | 61.64 |
| 37. | IIHR MP 90-1 | 3.54 | 31.74 | 0.26 | 22.33 | 5.38 | 61.67 |
| 38. | IIHR MP 91 | 3.46 | 63.04 | 0.23 | 25.31 | 6.89 | 57.19 |
| 39. | IIHR MP 92 | 3.68 | 56.79 | 0.35 | 27.71 | 6.67 | 55.02 |
| 40. | IIHR MP 93 | 3.62 | 62.77 | 0.32 | 25.02 | 6.67 | 57.70 |
| 41. | IIHR MP 95 | 2.99 | 65.50 | 0.49 | 22.50 | 7.54 | 59.34 |
| 42. | IIHR MP 96 | 3.59 | 48.22 | 0.32 | 24.71 | 7.17 | 57.51 |
| 43. | IIHR MP 98 | 3.65 | 59.87 | 0.42 | 23.52 | 4.34 | 61.53 |
| 44. | IIHR MP 99 | 4.73 | 35.64 | 0.27 | 35.50 | 5.91 | 48.47 |
| 45. | IIHR MP 101 | 3.50 | 75.42 | 0.24 | 28.75 | 7.21 | 53.43 |
| 46. | IIHR MP 102 | 6.57 | 103.23 | 0.74 | 33.25 | 3.54 | 52.60 |
| 47. | IIHR MP 104 | 3.43 | 72.62 | 0.30 | 26.33 | 5.12 | 57.93 |
| 48. | IIHR MP 105 | 6.27 | 97.22 | 0.84 | 30.79 | 3.35 | 55.25 |
| 49. | IIHR Selection LP | 3.94 | 36.75 | 0.34 | 25.81 | 5.21 | 58.37 |
| 50. | IIHR Selection 1 | 4.22 | 48.35 | 0.32 | 31.31 | 5.17 | 52.79 |
| 51. | IIHR Selection 2 | 4.03 | 63.42 | 0.49 | 28.89 | 5.18 | 55.31 |
| 52. | IIHR Selection 3 | 3.11 | 57.45 | 0.27 | 27.73 | 6.82 | 54.84 |
| 53. | IIHR Selection 4 | 4.62 | 41.85 | 0.33 | 23.97 | 5.37 | 60.14 |
| 54. | IIHR Selection 8 | 3.10 | 68.23 | 0.35 | 26.98 | 6.61 | 55.85 |
| 55. | IIHR Selection 9 | 3.09 | 59.58 | 0.34 | 26.83 | 5.49 | 57.05 |
| 56. | IIHR Selection 10 | 4.06 | 39.78 | 0.29 | 22.06 | 5.70 | 61.63 |
| 57. | Arka Aswini | 3.97 | 66.42 | 0.43 | 25.87 | 6.18 | 57.33 |
| 58. | Arka Dhanwantari | 4.31 | 75.67 | 0.46 | 35.87 | 3.91 | 49.59 |
| | Mean | 4.27 | 65.39 | 0.47 | 28.16 | 5.31 | 55.93 |
| | Minimum | 2.94 | 27.73 | 0.183 | 19.08 | 3.10 | 47.81 |
| | Maximum | 6.91 | 103.50 | 0.96 | 38.18 | 7.54 | 64.36 |
| | S.E. | 0.06 | 0.51 | 0.02 | 0.05 | 0.10 | 0.55 |
| | C.D. 5% | 0.18 | 1.43 | 0.04 | 1.42 | 0.29 | 1.54 |
| | C.V (%) | 2.56 | 1.36 | 5.94 | 3.11 | 3.34 | 1.70 |

analyzed for computing $D^2$ estimates. Contribution of individual trait towards genetic divergence was quantified on the basis of coefficient of variation at genotypic and inter cluster levels (Vavilov, 1951).

## 3. Results and discussion

The mean performance of 58 genotypes of velvet bean for different biochemical parameters are presented in Table 1. Analysis of variance revealed that highly significant differences for biochemical characters studied indicating that great genetic variability among genotypes. The variability for bioactive compounds like L-Dopa content (2.94–6.91%), total phenol content, (27.73–103.50 mg/100 g), total tannin (0.18 to 0.96 mg/100 g) and other biochemical factors like protein (19.08–38.18%), fat (3.1–7.54%) and carbohydrate (47.81–64.36%)

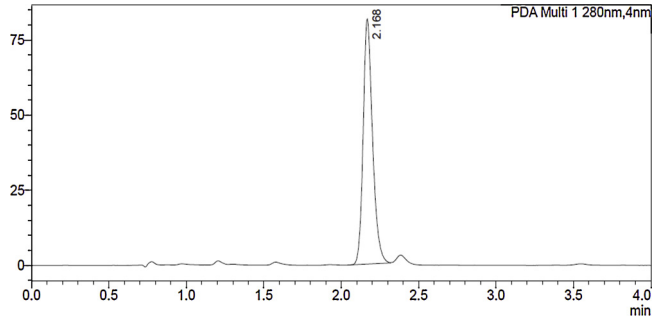


**Fig. 2.** UHPLC chromatogram of L-DOPA in IIHR MP 62-1(maximum6.91%).

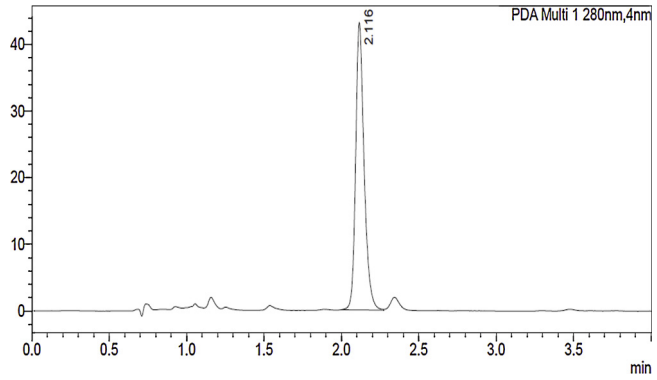


**Fig. 3.** UHPLC chromatogram of L-DOPA content in IC33243 (low 2.94%).

were observed among the genotypes.

L-Dopa content of seed was significantly highest in IIHR MP 62-1 (6.91%) and minimum in IC332432 (2.94%) and these UHPLC chromatograms are shown in Figs. 2 and 3. In a similar study results were reported by Raina et al. (2012) in 38 accession of *Mucuna,* where range of L-Dopa was 3.29–5.44% and significant high L-Dopa content was recorded in IC551549 (5.44%) and minimum in IC385841 (3.29%). Significant variation of L-Dopa in mucuna genotypes could be due to difference between the genetic makeup of genotypes, geographic location and environment effect. In the present study, it was observed that the wild types with itchy trichomes possess high L-Dopa and non itchy types having low L-Dopa in the seed. L-Dopa is important active ingredient for preparation of medicine to relief symptoms of the Parkinson's disease than the synthetic L-Dopa. However, it is toxic if consumed in large quantity without proper processing or boiling.

The total phenol was significantly high in IIHR MP 62-2 (103.50 mg/100 g) whereas lowest phenol content was observed in IIHR MP 89-1 (27.73 mg/100 g). Similar results have been reported by Arivalagan (2014) where the range was observed from 3.62 g to 6.13 g/100 g in a study of 20 accessions of *Mucuna*. The total tannin content was significantly high in IIHR MP 63-1 (0.96 mg/100 g) and lowest in IIHR MP 10 (0.18 mg/100 g). These results are in line of conformity with Vadivel and Janardhanan (2000) and Siddhuraju et al. (2000). As like L-DOPA, polyphenols such as phenolics and tannins are also of main concern as these bio active compounds reported to possesses many favourable medicinal properties including potential antioxidant activities (Siddhuraju and Manian, 2007). These antinutrient factors lower the activity of several digestive enzymes e.g α-amylase, trypsin, chymotrypsin and lipase. Phenolics also form complex with iron and prevent absorption and reduce the absorption of nutrients and cause damage to the mucosa of digestive tract. From this investigation, the genotypes which are having high bioactive compounds were identified and can be used for the purpose of pharma industry. The genotypes having rich source of L-Dopa can be used in future breeding programme of velvet bean.

Velvet bean seeds contain protein in addition to L-Dopa. Significantly high amount of protein was found in IIHR MP-74 (38.18%) and low in EC 17827 (19.08%). The results are in line of conformity reported by Ezeagu et al. (2003) earlier in *Mucuna*, Adebowale et al. (2007) and Fathima et al. (2010). These results are also in agreement with the Balogun and Olatidoye (2012) who reported biochemical analysis of velvet bean. The crude fat content significantly highest recorded in IIHR MP 95 (7.54%) and lowest in IIHR MP 62-3 (3.10%). The results are in accordance with the findings reported in velvet bean by Vadivel and Janardhanan (2000); Ezeagu et al. (2003); Kala and Mohan (2010) and contradictory to results reported by Sridhar and Rajeev Bhat (2007) where high amount of fat observed in seeds of velvet bean. These differences may be due to genetic constitution of materials used in the study and also influence of environment. The total carbohydrate was significantly maximum in IIHR MP 62-3 (64.36%) and minimum in IIHR MP 74 (47.81%). Similar findings were reported by Ezeagu et al. (2003) carbohydrate in the range of 59.20%–64.88% in 12 accessions of *Mucuna*. The carbohydrate content obtained in the present study is consistent with the value reported by Sridhar and Rajeev Bhat (2007) and contradictory to the low levels of carbohydrate reported by Adebowale et al. (2005) and Balogun and Olatidoye (2012). This difference may be due to species and genetic makeup of genotypes used in the study.

## 4. Genetic divergence

### 4.1. Clustering pattern of genotypes

The analysis of variance exhibited highly significant difference among 58 genotypes for six biochemical traits and thus indicated that presence of substantial amount of diversity among the genotypes. The 58 genotypes of velvet bean were grouped into eight distinct clusters as evident from the Dendrogram. The distribution of genotypes into eight distinct clusters was shown in Table 2. Out of the eight clusters formed, cluster II was the largest comprising of 25 genotypes followed by cluster V with 13 genotypes, cluster I with 7 genotypes, cluster III and VI with 5 genotypes, whereas cluster IV, VII and VIII consisted of single entity

**Table 2**
Clustering pattern of 58 genotypes of *Mucuna* sp.

| Group K | n | Cluster Members |
|---|---|---|
| Cluster I | 7 | IIHR MP 62-2, IIHR MP 102, IIHR MP 105, IIHR MP 21, IIHR MP 74, IIHR MP 63-1, IIHR MP 62-1, |
| Cluster II | 25 | IIHR MP 9, IIHR MP 104, IC332432, IIHR MP 5, IIHR MP 10, Arka Aswini, IC 21998, IIHR Selection 8, IIHR MP 87, IIHR MP 93, IIHR MP 11, IIHR MP 88, IIHR MP 91, IIHR Selection 9, IC 202969, IIHR Selection 2, IIHR MP 85, IIHR MP 92, IIHR MP 89, IIHR Selection 3, IIHR MP 98, IIHR MP 95, EC 17827, IC 2199, IC 33243, |
| Cluster III | 5 | IIHR MP 45, IIHR MP 74-3, IIHR MP 62-3, IIHR MP 63, IIHR MP 44, |
| Cluster IV | 1 | IIHR MP 101, |
| Cluster V | 13 | IIHR MP 84, IIHR MP 96, IIHR MP 90, IIHR Selection 1, IIHR Selection 10, IIHR Selection 4, IIHR Selection LP, EC 2533A, EC 25334, IIHR MP 90-1, IIHR MP 99, IIHR MP 89-1, IIHR MP 22, |
| Cluster VI | 5 | IC 2534, IIHR MP 17, IC 83195, Arka Dhanawantari, IIHR MP 7-1, |
| Cluster VII | 1 | IIHR MP 82 |
| Cluster VIII | 1 | IIHR MP 47 |

**Table 3**
Inter (above diagonal) and intra (diagonal and bold) Cluster distance ($D^2$ values) among the genotypes of velvet bean.

| | 1 Cluster | 2 Cluster | 3 Cluster | 4 Cluster | 5 Cluster | 6 Cluster | 7 Cluster | 8 Cluster |
|---|---|---|---|---|---|---|---|---|
| 1 Cluster | **41.64** | 1123.33 | 182.09 | 971.95 | 2014.94 | 728.52 | 425.20 | 353.37 |
| 2 Cluster | | **61.74** | 634.96 | 106.25 | 332.50 | 208.10 | 281.89 | 408.15 |
| 3 Cluster | | | **60.56** | 647.54 | 1212.42 | 348.84 | 255.65 | 198.78 |
| 4 Cluster | | | | **0.00** | 614.32 | 283.40 | 161.27 | 350.21 |
| 5 Cluster | | | | | **124.65** | 532.98 | 961.13 | 1105.29 |
| 6 Cluster | | | | | | **167.51** | 288.45 | 233.34 |
| 7 Cluster | | | | | | | **0.00** | 155.94 |
| 8 Cluster | | | | | | | | **0.00** |

**Table 4**
Cluster means for biochemical parameters.

| | L-Dopa (%) | Total Phenols (mg/100 g) | Total Tannin (mg/100 g) | Protein (%) | Fat (%) | Carbohydrate (%) |
|---|---|---|---|---|---|---|
| Cluster I | 6.61 | 97.40 | 0.84 | 32.36 | 3.40 | 53.76 |
| Cluster II | 3.53 | 64.00 | 0.33 | 26.79 | 6.07 | 56.47 |
| Cluster III | 5.90 | 81.39 | 0.87 | 27.29 | 3.45 | 58.63 |
| Cluster IV | 3.50 | 75.41 | 0.24 | 28.74 | 7.21 | 53.42 |
| Cluster V | 3.91 | 40.10 | 0.32 | 27.01 | 5.88 | 56.60 |
| Cluster VI | 4.15 | 67.22 | 0.58 | 33.39 | 4.39 | 51.52 |
| Cluster VII | 4.64 | 85.10 | 0.48 | 21.95 | 5.88 | 61.54 |
| Cluster VIII | 3.65 | 85.33 | 0.68 | 31.62 | 3.33 | 54.43 |

genotype. The genotypes belonging to same cluster indicates that are to be more closely related than those belonging to different clusters. The monogenotypic cluster shown that such genotypes might have completely different genetic makeup from the remaining genotypes and each other thus leading to the formation of separate cluster. There were no distinct clusters according to species, geographical origin, morphological and biochemical traits. From the clustering pattern it is obvious that selection of different diverse genotypes have played a greater role in total divergence between the clusters than the geographical diversity *i.e* the genotypes have grouped into different clusters irrespective of their geographical origin, morphological and biochemical traits which means that genetic constitution of the genotypes are more dominant than the geographical, morphological and biochemical traits. These results are conformity with findings reported by Rai et al. (2010) in pole type of French bean.

### 4.2. Average intra and inter cluster distances

Average intra and intercluster $D^2$ values are presented in Table 3 providing information on the nature of genetic divergence at intra and intercluster levels, respectively. The $D^2$ values computed among 58 genotypes ranged from 106.25 to 2014.94. The group constellation showed that intracluster and intercluster $D^2$ values ranged from 0.00 (cluster IV, VII and VIII) to 167.51(cluster VI) and 106.25 (between cluster II and IV) to 2014.94 (between cluster I and V), respectively. Among eight clusters intra cluster distance was maximum in cluster VI (167.51) followed by cluster V (124.65), cluster II (61.74) and minimum was in cluster III (60.56) and zero in cluster IV, VII and VIII. The intra cluster distance values indicate the closeness of the genotypes forming in the same cluster. The clusters showing an intra cluster distances of 0.00 reveal to be monogenotypic and ultimately less heterogeneous whereas high intra cluster $D^2$ values indicate more genetic divergence between genotypes belonging to the same cluster and more heterogeneous. As per Murthy and Arunachalam (1966) the success of hybridization followed by selection is largely depends on the choice of parents showing high genetic diversity of the character of the interest. The inter cluster distance was maximum in between cluster I and V (2014.94) followed by cluster III and V (1212.42) while minimum in between cluster II and IV (106.25) followed by VII and VIII (155.94). It indicated that these cluster pairs were most divergent or on the other hand, the genotypic constituent of these cluster pairs consisting the genes from most distantly related in respect of the characters studied. Win et al. (2011) reported that intra and inter cluster distances might arises due to differential genetic makeup of genotypes. The genotypes belonging to different clusters separated by high statistical distance may be used in the hybridization programme for crop improvement as well as for studying of the inheritance pattern of different traits in velvet bean. The cluster with high intercluster distance was between I and V and these divergent clusters can be used for selecting the parents for hybridization.

### 4.3. Cluster means of the biochemical characters

Genetic diversity is the basic prerequisite for any crop improvement programme. Hence the improvement of seed yield and biochemical quality traits is the basic objective in the breeding programme of velvet bean, mean value of cluster for biochemical traits along with seed yield and its contributing traits need to be considered while selection of genotypes for hybridization. The cluster means for different biochemical characters indicated considerable diversity among the clusters (Table 4). From the data, it can be seen that significant variability exists for characters under study. The value of cluster means for L-Dopa varied from 3.50 to 6.61. L-Dopa content is highest in cluster I (6.61%), followed by cluster III (5.90%), whereas lowest was observed in cluster IV (3.50%). For improvement of L-dopa in seed of velvet bean to extract the high L-Dopa by pharmaceutical industry, it is ideal that plant breeders select genotypes from cluster I. The mean value for phenolics varied from 97.40 mg/100 g to 40.10 mg/100 g. Maximum total phenol content recorded in cluster I (97.40 mg/100 g) followed by cluster VIII (85.33 mg/100 g) whereas minimum was found in cluster V (40.10). The total tannin content maximum recorded in cluster III (0.87 mg/100 g) followed by Cluster I (0.84 mg/100 g) and low tannin content recorded in cluster IV (0.24 mg/100 g). The protein content was maximum in cluster VI (33.39%) followed by cluster I (32.36%) while minimum was found in cluster VII (21.95). Highest fat content was recorded in cluster IV (7.21%) followed by cluster II (6.07%), whereas minimum was observed in cluster VIII (3.33%). The highest carbohydrate content was recorded in cluster VI (61.54%) followed by cluster III (58.63%), cluster V (56.60%) and lowest was observed in cluster VI (51.52%). The result of the clusters mean values indicates that there is no cluster containing genotypes with all the desirable biochemical traits which could be directly selected and utilized. This study reveals that the

**Table 5**
Per cent contribution of different biochemical traits towards divergence of velvet bean genotypes.

| Divergence | L-Dopa (%) | Total phenols (mg/100 g) | Total tannin (mg/100 g) | Protein (%) | Fat (%) | Carbohydrate (%) |
|---|---|---|---|---|---|---|
| Number of times ranked first | 161 | 1108 | 86 | 113 | 184 | 1 |
| Contribution towards divergence (%) | 9.74 | 67.03 | 5.2 | 6.84 | 11.13 | 0.06 |

**Table 6**
Eigen vectors and eigen values for first three principal components (PC1, PC2 and PC3) in *Mucuna* genotypes.

| | PCA1 | PCA2 | PCA3 |
|---|---|---|---|
| Eigen value | 4.188 | 0.842 | 0.511 |
| Percentage of variance | 69.806 | 14.040 | 8.524 |
| Cumulative percentage of variance | 69.806 | 83.847 | 92.372 |
| **Trait** | **Eigen vectors** | | |
| L-Dopa (%) | 0.418 | 0.045 | 0.579 |
| Total Phenols(mg/100 g) | 0.415 | −0.183 | −0.537 |
| Total Tannin (mg/100 g) | 0.450 | −0.015 | 0.255 |
| Protein (%) | 0.269 | 0.864 | −0.347 |
| Fat (%) | −0.460 | −0.041 | −0.233 |
| Carbohydrate (%) | 0.405 | −0.463 | −0.366 |

**Table 7**
Estimates of genetic parameters for biochemical characters in 58 genotypes of velvet bean.

| Characters | Mean | Range | | Genotypic variance | Phenotypic variance | GCV | PCV | Heritability (%) | GA | GA as % mean |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Min | Max | | | | | | | |
| L-Dopa (%) | 4.26 | 2.94 | 6.90 | 1.42 | 1.43 | 27.97 | 28.08 | 99.20 | 2.45 | 57.38 |
| Total phenols (mg/g) | 65.38 | 27.73 | 103.50 | 348.35 | 349.14 | 28.54 | 28.57 | 99.80 | 38.40 | 58.73 |
| Total tannin(mg/g) | 0.46 | 0.18 | 0.96 | 0.05 | 0.05 | 49.83 | 50.19 | 98.60 | 0.47 | 101.94 |
| Protein (%) | 28.15 | 19.08 | 38.18 | 19.84 | 20.61 | 15.81 | 16.12 | 96.30 | 9.00 | 31.97 |
| Fat (%) | 5.31 | 3.10 | 7.54 | 1.69 | 1.72 | 24.49 | 24.72 | 98.20 | 2.65 | 50.00 |
| Carbohydrate (%) | 55.93 | 47.54 | 64.36 | 16.44 | 17.35 | 7.25 | 7.44 | 94.80 | 8.13 | 14.53 |

most of the maximum and minimum cluster mean values are distributed in relatively distant clusters.

### 4.4. Relative contribution of characters towards diversity

An assessment of relative contribution of six biochemical characters towards genetic divergence was presented in Table 5 revealed that the total phenol content had contributed highest (67.03%) by taking 1108 times first ranking followed by fat content (11.13%) by 184 times, L-Dopa (9.74%) by 161 times, protein (6.84%) by 113 times, tannin (5.20%) by 86 times and least contribution towards the carbohydrates (0.06%) by one time only. The results are in agreement with Rekha et al. (2011) who reported that maximum divergence contribution in phenols (52.72%) and for protein (3.91%) in pigeon pea. Arun Kumar et al. (2014) reported that similar results by contribution of protein (11.52%) to the total divergence in French bean germplasm. In a similar study in fenugreek Patahk et al. (2014) reported that protein content contributed maximum account (53.85%) of total divergence. Barh et al. (2014) reported that contribution of fat (6.22%) and protein content (3.10%) to the total divergence in soybean. In case of maize, Showemimo (2004) reported that contribution of protein content (7.7%), fat (0.9%) and carbohydrate (1.3%) to the total divergence. These results were also in agreement with Sutariya et al. (2011) and Bekele et al. (2012).

The biochemical characters under this study exhibited high variability as evident from the estimates of mean, range, coefficients of variation, heritability and genetic advance are presented in Table 7. In general the phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) in the studied six biochemical characters were almost equal. Highest PCV and GCV recorded in total tannin (50.19, 49.83) followed by total phenols (28.57, 28.54) and L-Dopa (28.08, 27.97) whereas lowest PCV and GCV observed in carbohydrate (7.44, 7.25), respectively. High heritability was exhibited for phenols, L-Dopa and low heritability was recorded for carbohydrate. But overall, six biochemical compounds recorded high percentage of heritability. The estimates of genetic advance as percent of mean were high and recorded in total tannin (101.94%), followed by total phenols (58.73%) and L-Dopa (57.38%) and least GAM recorded in carbohydrate (14.53%). Similar GCV, PCV and genetic advance results were reported in soybean by Barh et al. (2014) for protein and fat content. Parhe et al. (2014) reported low GCV and PCV values for protein in chickpea. The heritability of the protein and fat content contradict to results reported by Bekele et al. (2012) in soybean where it showed very low heritability (8.45% and 39%).

In the Principal Component Analysis, Principal components (PCs) are the linear combinations of the biochemical contents obtained in the experiment. The first three principal components together explained the 92.37% of the total variance (Table 6). PC1, PC2 and PC3 explained 69.80%, 14.04%, and 8.52% of the total variance respectively. The eigen values indicated that first two or three good summery of the data, two components accounting for 83.84% the total variance and three components explaining the 92.37%. The first component is the measures of overall biochemical traits except fat which have negative loading. The second eigen vector has high positive loading on variable of protein; low positive loading on L-Dopa and remaining were negative loadings. The third component is a measure of L-Dopa and total tannin and remaining traits are negative loading.

## 5. Conclusions

Biochemical analysis revealed that the presence of wide variability among the germplasm of *Mucuna pruriens* in respect of the bioactive compounds. The mucuna seeds are good source of L-DOPA, total phenols and tannins in addition to protein, fat and carbohydrate and used as drug to the treatment of Parkinson disease. The present finding revealed that itching types having high amount of bioactive compounds particularly high L-Dopa in the germplasm IIHR MP 62-1(6.91%), IIHR MP 21 (6.77%), IIHR MP 63-1(6.76%) and IIHR MP 102 (6.57%) were identified, but these cannot be grown on commercial scale due to itching trichomes on the pods causing intense irritation, dermatitis and difficult to harvest. Hence these genotypes can be used in the hybridization programme to get higher seed yield along with high content of L-Dopa in the seed and non itchy type to meet the demand of pharma industry.

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