



Variability of L-Dopa and its association with morphological growth and yield traits of *Mucuna pruriens* (L.) germplasm

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ABSTRACT

Fifty eight germplasm of *Mucuna* was analysed for L-Dopa using ultra high performance liquid Chromatography. L-Dopa ranges from 2.94% in IC 332432 to 6.91% in IIHR MP62-1. Low L-Dopa accessions identified IC 332432 (2.94%) and IIHR MP95 (2.98%) was highly beneficial to consume as food with proper processing or boiling. The accessions having high L-Dopa is highly beneficial to the industries for preparation of drugs. High L-Dopa accessions IIHR MP62-1 (6.91%), IIHR MP21 (6.77%), IIHR MP63-1(6.76%) and IIHR MP102 (6.57%) were itchy types and important for drug industry. Hence these high L-Dopa accessions can be utilised in future breeding programme of *Mucuna* to develop high yielding cultivars coupled with high L-Dopa. Correlation coefficient analysis revealed that L-Dopa has significant negative association with pod length indicates selection of smaller pods was a measure of selection index to obtain high L-Dopa accession could be used in breeding programme of velvet bean.

Key words: Germplasm, L-Dopa, *Mucuna pruriens*, Parkinson's disease, UHPLC.

INTRODUCTION

Plants are important source of medicine in addition to food and shelter. Today health care system mainly depends on drugs derived from the plants as raw material due to minimum side effects as compare to that those of allopathic medicines. The WHO has estimated that over 80% of the world population meets primary health care need through traditional medicine (Lambert, 1997). *Mucuna pruriens* (L.) is an important underutilized Indian medicinal plant belonging to the family Fabaceae. It is commonly known as velvet bean, cowhage, kewanch, cowitch and atmagupta (Anonymous, 1985).

It is native to China and India. It is annual herbaceous twining climber grows to a height of 3-18m. The leaves are trifoliate lanceolate leaves 15-30 cm long. The flowers are varied in colour viz., creamy white, purple, deep purple. The long inflorescences are produced in racemes. The pods range in the length of 6-12 cm consist of 4-6 seeds. The seed colour varied from white, black, brown and white colour with mottle. Two types of trichomes are present on the pod. The pod consists of small velvety trichomes which are not causing itchiness and other itchy type pods having long reddish brown stiff trichomes on the pods which cause itching while touch with the skin of human beings. The seeds are important source of L- Dopa (3, 4-Dihydroxyphenylalanine) which was used to treat Parkinson's disease. Parkinson's disease is a progressive age dependent neurodegenerative syndrome, characterised by four main symptoms: tremor, rigidity, bradykinesia and impairment of balance. The drug extracted from the seed of *Mucuna* is more effective than the synthetic counterparts. Velvet bean is used in traditional Ayurvedic system of medicine for the management of male fertility, nervous disorders and as an aphrodisiac. *Mucuna* seeds have been widely used against snakebite in traditional

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medicine in India and West Africa (Haq, 1983; Siddhuraju *et al.*, 1996). It is ingredient of more than 200 indigenous drug formulations. The HP-200 is a commercial preparation of *M. pruriens* derived from the Ayurveda. Manyam (1995) reported that HP-200 was an effective treatment for patients with Parkinson disease. *Mucuna* is pharmacologically important for aphrodisiac, antineoplastic, antimicrobial activities *etc.* (Sathiyarayanan and Arulmozhi, 2007).

Velvet bean seeds are important source of proteins, lipids, minerals and other nutrients. This underutilized tropical plant having rich source of proteins varied from 24.92% to 37.5% (Vadivel and Janardhanan, 2000; Adebowale *et al.*, 2005) which is higher than commonly consumed pulses (Jambunathan and Singh, 1980). Even though velvet bean contains higher levels of proteins and

carbohydrates, their utilization is limited due to the presence of number of antinutrients. Velvet bean has been used as food certain ethnic groups in number of countries viz., India, Philippines, Nigeria, Ghana, Brazil and Malawi (Janardhanan *et al.*, 2003; Emenalom *et al.*, 2004). In India, mature seeds, seeds from unripe pod and young pods of *Mucuna* are soaked and boiled or roasted and eaten as food by north east Indian tribes Khasi, Naga, Kuki, Jaintia, Chakma and Mizo (Arora, 1991) north western parts of Madhya Pradesh tribes Abujh-Maria, Maria Muria, Gond and Halba (Sahu, 1996) south India tribes Mundari Dravidan (Jain, 1981) Kani, Kader and Muthuvan (Radhakrishnan *et al.*, 1996) and Savera Jatapu, Gadebe and Kondadora (Rajyalakshmi and Geervani, 1994). *Mucuna* exhibits more tolerance to adverse environmental conditions such as drought, low soil fertility and high soil acidity (Pugalenthi *et al.*, 2004). Different types of cultivars of *Mucuna* such as early, medium and long duration types comes to harvesting 120, 150 and 180 days of duration, respectively. The seed yield ranges from 1.3 to 2.4 t/ha (Gurumoorthi *et al.*, 2003) and total yield of biomass is 20-30 t/ha dry matter was 7-9 t/ha (Crasky *et al.*, 1998). Hence it is considered to be one of the most productive legumes of the world (Fujii *et al.*, 1991).

The seeds of *Mucuna* collected from wild growing populations in India had reported L-Dopa varying from 1.0 to 4.7% among populations, locations and generations (Siddhuraju *et al.*, 2000 and Vadivel and Janardhanan, 2001). Similar trend of variation of L-Dopa was observed by Krishnamurthy *et al.* (2002) in 17 wild collections from different regions of India and reported that L-Dopa varied from 2.30 to 4.18%. Raina *et al.* (2012) reported that variation of L-dopa ranges from 3.29 to 5.44% in the seeds of *M. pruriens* germplasm collections augmented from various parts of India. Later on Raina *et al.* (2013) also estimated that L-Dopa ranges from 4.20% to 6.11% where eleven *Mucuna pruriens* germplasm collected from three districts of Odisha. Earlier research workers analysis of L-dopa done with few accessions or few accessions collected from particular state only. The present study is analysis of L-Dopa in 58 accessions of *Mucuna* gives more information about variability of L-Dopa and can be useful in future breeding programme of velvet bean. The correlation coefficient gives a measure of the relationship between traits and provides the degree to which various characters of crops are associated with productivity. Knowledge of the correlation between L-Dopa and its component characters is essential for L-Dopa improvement through selection method of breeding programme.

MATERIALS AND METHODS

Plant material

Mucuna accessions were collected from the different parts of India and are maintained at the ICAR-Indian Institute of Horticultural Research, Bengaluru. The germplasm represents from nine Indian states such as, Andhra Pradesh, Bihar, Himachal Pradesh, Gujarat, Kerala, Karnataka, New Delhi and Tamil Nadu. The details of the source of collection

of germplasm presented in the Table 1. The seed coat colour is varied from the creamy white, black, brown and white with mottle colour among the collections. The germplasm consists of both itchy and non-itchy types, where non-itchy types some advance breeding lines and two elite cultivars i.e. *Arka Aswini* and *Arka Dhanwantri* are grown during the year 2014-15 at the experimental plots of medicinal plants at ICAR-IIHR, Bengaluru. Fifty eight accessions of velvet bean were used for estimation of L-Dopa analysis. Correlation studies of L-Dopa with morphological and yield traits were done using SAS software.

Extraction and estimation of L-Dopa

The matured pods are harvested and seeds were separated from the pods. The seed material which made fine powder through 60 mesh size was used for analysis of biochemical traits during 2015 in the plant physiology and biochemistry laboratory at ICAR-IIHR, Bengaluru. L-Dopa estimated according to protocol developed by Shivananda *et al.*, (2003) by using analytical instrument ultrahigh performance liquid chromatography (UHPLC). The pure L-Dopa was obtained from Sigma. A stock solution of L-Dopa was prepared by dissolving an accurately weighed 100 mg of L-Dopa standard in 100 ml of 0.1M H₃PO₄ in a volumetric flask. From the stock solution prepared different concentration of 10, 20, 30, 50, 100 and 200 ppm (Fig 2) solution and standard curve was prepared and depicted in Fig 1. Quantitative estimation of L-Dopa was done by Shimadzu Nexera X₂ 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 (NaH₂PO₄ 2H₂O), flow rate: 0.4 mL/min, injection volume: 5 µL, Retention time of L-Dopa: 1.94 to 1.96 min.

The percentage of L-Dopa was calculated using the following formula:

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

RESULTS AND DISCUSSION

Fifty eight accessions of *Mucuna pruriens* received from Section of Medicinal Crops, ICAR-IIHR, Bengaluru was utilized for variability studies. Seed colour ranges from creamy white, black, brown and mottled and the size of the seed ranges from small size in itchy type and medium to bold size in non-itchy types. L-Dopa content of 58 accessions of *Mucuna* is varied significantly between 2.94% (Fig 4) and 6.91% (Fig 3). The range of L-Dopa lowest (2.94%) to highest (6.91%) was recorded with mean value of 4.26%. This variation of L-dopa is due to genetic potential of germplasm. Similar results were reported by Prakash and Tewari (1999) and L-Dopa varied from 3.6% to 4.2% in three accessions collected from India. The present findings were in agreement with Laurent *et al.*, (2002) reported that L-Dopa varied from 4% to 6% in analysis of L-Dopa among 38 accessions of

Table 1: List of velvet bean germplasm used for L-Dopa estimation.

Germplasm	State / Source of collection	Species type	L-Dopa (%)
IIHR MP 17	New Delhi	<i>M. pruriens</i> var. <i>utilis</i>	3.81
IIHR MP 11	Tamil Nadu	<i>M. pruriens</i> var. <i>utilis</i>	3.76
IIHR MP 5	Karnataka	<i>M. pruriens</i> var. <i>utilis</i>	4.09
<i>Arka Dhanwantari</i>	IIHR, Bengaluru	<i>M. pruriens</i> var. <i>utilis</i>	4.31
IIHR MP 101	Andhra Pradesh	<i>M. pruriens</i> var. <i>utilis</i>	3.50
IIHR MP 99	NBPGR, New Delhi	<i>M. pruriens</i> var. <i>utilis</i>	4.73
IIHR MP 82	Gujarat	<i>M. pruriens</i> var. <i>utilis</i>	4.64
IIHR MP 91	NBPGR, New Delhi	<i>M. pruriens</i> var. <i>utilis</i>	3.45
IIHR MP 84	NBPGR, New Delhi	<i>M. pruriens</i> var. <i>utilis</i>	3.26
IIHR MP 89	NBPGR, New Delhi	<i>M. pruriens</i> var. <i>utilis</i>	3.15
IIHR MP 89-1	IIHR, Bengaluru	<i>M. pruriens</i> var. <i>utilis</i>	3.18
IIHR MP 7	Karnataka	<i>M. pruriens</i> var. <i>pruriens</i>	4.90
IIHR MP 88	NBPGR, New Delhi	<i>M. pruriens</i> var. <i>utilis</i>	3.08
IIHR MP 90	NBPGR, New Delhi	<i>M. pruriens</i> var. <i>utilis</i>	3.13
IIHR MP 92	NBPGR, New Delhi	<i>M. pruriens</i> var. <i>utilis</i>	3.68
IIHR MP 95	NBPGR, New Delhi	<i>M. pruriens</i> var. <i>utilis</i>	2.98
IIHR MP 96	NBPGR, New Delhi	<i>M. pruriens</i> var. <i>utilis</i>	3.58
IC33243	NBPGR, New Delhi	<i>M. pruriens</i> var. <i>utilis</i>	2.94
IIHR Selection 1	IIHR, Bengaluru	<i>M. pruriens</i> var. <i>utilis</i>	4.22
IIHR Selection 9	IIHR, Bengaluru	<i>M. pruriens</i> var. <i>utilis</i>	3.09
IIHR MP 104	Bihar	<i>M. pruriens</i> var. <i>utilis</i>	3.43
IIHR Selection 2	IIHR, Bengaluru	<i>M. pruriens</i> var. <i>utilis</i>	4.02
EC 25334	NBPGR, New Delhi	<i>M. pruriens</i> var. <i>utilis</i>	3.92
IC202969	NBPGR, New Delhi	<i>M. pruriens</i> var. <i>utilis</i>	3.61
IIHR MP10	Tamil Nadu	<i>M. pruriens</i> var. <i>utilis</i>	4.03
IIHR Selection 8	IIHR, Bengaluru	<i>M. pruriens</i> var. <i>utilis</i>	3.10
IIHR Selection 3	IIHR, Bengaluru	<i>M. pruriens</i> var. <i>utilis</i>	3.11
<i>Arka Aswini</i>	IIHR, Bengaluru	<i>M. pruriens</i> var. <i>utilis</i>	3.96
IIHR Selection LP	IIHR, Bengaluru	<i>M. pruriens</i> var. <i>utilis</i>	3.94
IC 2199	NBPGR, New Delhi	<i>M. pruriens</i> var. <i>utilis</i>	3.17
EC17827	NBPGR, New Delhi	<i>M. pruriens</i> var. <i>utilis</i>	3.37
IC2534	NBPGR, New Delhi	<i>M. pruriens</i> var. <i>utilis</i>	3.30
IC 332432	NBPGR, New Delhi	<i>M. pruriens</i> var. <i>utilis</i>	3.62
IIHR MP 44	Tamil Nadu	<i>M. pruriens</i> var. <i>pruriens</i>	6.45
IIHR MP 47	Karnataka	<i>M. pruriens</i> var. <i>pruriens</i>	3.65
IIHR MP 63	Kerala	<i>M. pruriens</i> var. <i>pruriens</i>	6.36
EC2533A	NBPGR, New Delhi	<i>M. pruriens</i> var. <i>utilis</i>	3.10
IIHR MP 90-1	IIHR, Bengaluru	<i>M. pruriens</i> var. <i>utilis</i>	3.54
IC 21998	NBPGR, New Delhi	<i>M. pruriens</i> var. <i>utilis</i>	3.61
IIHR MP 87	NBPGR, New Delhi	<i>M. pruriens</i> var. <i>utilis</i>	3.63
IC 83195	NBPGR, New Delhi	<i>M. pruriens</i> var. <i>prurita</i>	4.43
IIHR MP 85	NBPGR, New Delhi	<i>M. pruriens</i> var. <i>utilis</i>	4.44
IIHR MP 98	NBPGR, New Delhi	<i>M. pruriens</i> var. <i>utilis</i>	3.65
IIHR MP 93	NBPGR, New Delhi	<i>M. pruriens</i> var. <i>utilis</i>	3.61
IIHR MP 22	Gujarat	<i>M. pruriens</i> var. <i>utilis</i>	5.62
IIHR Selection 4	IIHR, Bengaluru	<i>M. pruriens</i> var. <i>utilis</i>	4.62
IIHR Selection 10	IIHR, Bengaluru	<i>M. pruriens</i> var. <i>utilis</i>	4.06
IIHR MP 9	Karnataka	<i>M. pruriens</i> var. <i>utilis</i>	3.64
IIHR MP 62-1	Kerala	<i>M. pruriens</i> var. <i>pruriens</i>	6.91
IIHR MP 62-2	Kerala	<i>M. pruriens</i> var. <i>pruriens</i>	6.46

Table 1: Continue...

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IIHR MP 62-3	Kerala	<i>M. pruriens</i> var. <i>pruriens</i>	5.99
IIHR MP 63 -1	Kerala	<i>M. pruriens</i> var. <i>pruriens</i>	6.76
IIHR MP 45	Karnataka	<i>M. pruriens</i> var. <i>pruriens</i>	5.24
IIHR MP 74-3	Karnataka	<i>M. pruriens</i> var. <i>pruriens</i>	5.49
IIHR MP 74	Karnataka	<i>M. pruriens</i> var. <i>pruriens</i>	6.53
IIHR MP 105	Bihar	<i>M. pruriens</i> var. <i>pruriens</i>	6.26
IIHR MP 21	Himachal Pradesh	<i>M. pruriens</i> var. <i>pruriens</i>	6.77
IIHR MP 102	Andhra Pradesh	<i>M. pruriens</i> var. <i>pruriens</i>	6.57
Mean			4.27
Minimum			2.94
Maximum			6.91
S.E			0.06
C.D. 5%			0.18
CV (%)			2.56

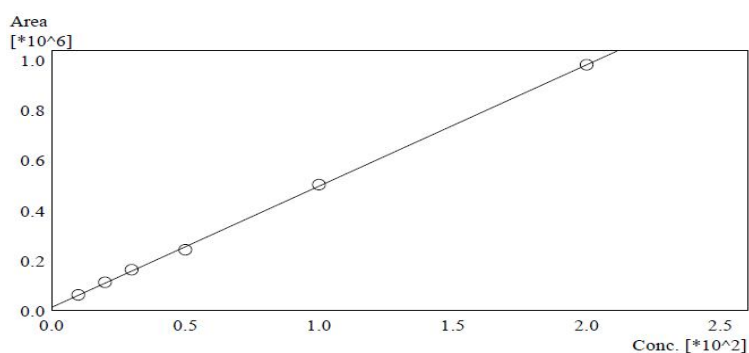


Fig 1: Calibration curve prepared using different concentration of standard L-Dopa.

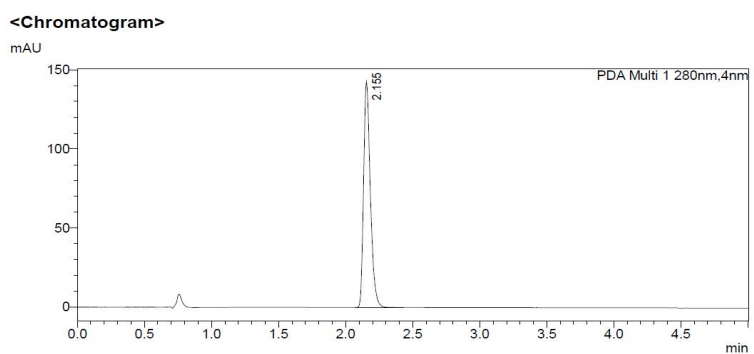


Fig 2: UHPLC chromatogram of 100 ppm standard L-Dopa.

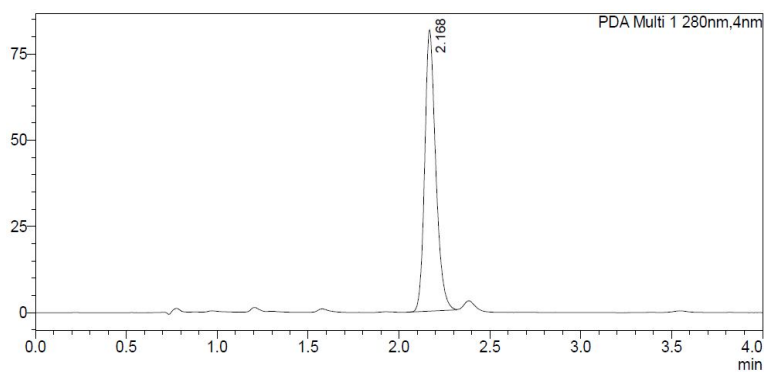


Fig 3: UHPLC chromatogram of L-DOPA in IIHR MP62-1 (maximum 6.91%).

Table 2: Correlation coefficients among various growth and yield traits of *Mucuna* germplasm.

N	DF	IL	NFI	PL	Pwd	PW	NBP	NPB	DPYP	NSP	SYPP	SP	DM	TW	LD
DF	1.000														
IL	0.814**	1.000													
NFI	0.870**	0.905**	1.000												
PL	-0.519**	-0.603**	-0.584**	1.000											
Pwd	-0.633**	-0.558**	-0.574**	0.463	1.000										
PW	-0.776**	-0.707**	-0.719**	0.613**	0.518**	1.000									
NBP	-0.450	-0.552**	-0.42510	0.162	0.441	0.375	1.000								
NPB	0.903**	0.905**	0.941**	-0.495**	-0.589**	-0.702**	-0.476**	1.000							
DPYP	0.47441**	0.398	0.522**	0.042	-0.193	-0.169	0.033	0.601**	1.000						
NSP	0.139	0.071	0.079	0.390	-0.194	0.171	0.058	0.187	0.433	1.000					
SYPP	0.476**	0.399	0.510**	0.060	-0.183	-0.171	-0.004	0.589**	0.918**	0.488**	1.000				
SP	0.017	-0.031	-0.091	0.265	-0.045	0.031	0.062	-0.011	-0.075	0.423	0.051	1.000000			
DM	0.760**	0.826**	0.813**	-0.576	-0.487**	-0.756**	-0.388	0.771**	0.293	-0.007	0.284	-0.036	1.000		
TW	-0.850**	-0.757**	-0.840**	0.378	0.653**	0.747**	0.412	-0.838**	-0.410	-0.269	-0.435	-0.162	-0.765**	1.000	
LD	0.328	0.280	0.354	-0.578**	-0.252	-0.313	-0.226	0.261	0.032	-0.447	0.000	-0.365	0.140	-0.151	1.000

**Significant at 1% level of probability.

Pwd= Pod width.

PW= Pod weight.

NBP= Number of bunches per plant.

NPB= Number of pods per bunch.

DPYP= Dry pod yield per plant.

SP= Shelling percentage.

DM = Days to maturity.

TW= Test weight.

LD= L-Dopa.

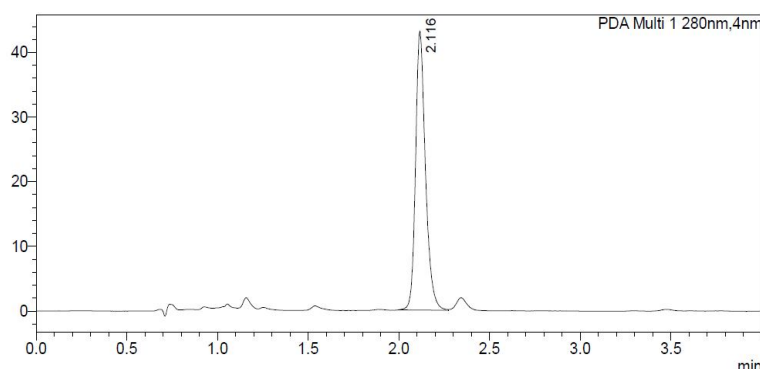
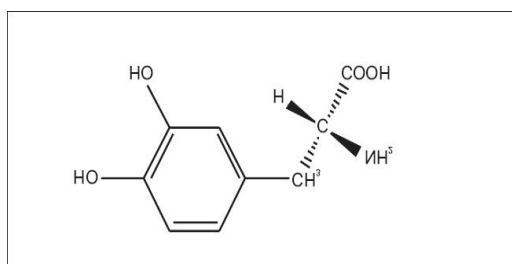


Fig 4: UHPLC chromatogram of L-DOPA content in IC33243 (low 2.94%).



(Source: Raina *et al.*, 2012).

Fig 5: Chemical structure of 3, 4-dihydroxyphenyl-L-alanine (L-DOPA).

Mucuna. The variation of L-Dopa reported between 5.60% to 5.93% in five accessions collected from Tamil Nadu (Janardhanan *et al.*, 2003). High amount of L-Dopa reported by Kala and Mohan (2010) where 7.55g/100g and 7.93g/100g in *Mucuna pruriens* var *utilis* in white coloured seed coat and black coloured seed coat, respectively. Whereas in Nigeria Ezeagu *et al.* (2003) found that higher L-Dopa (8.34%) in black seed of *Mucuna veracruz* in study of 12 accessions of *Mucuna* (4.00% to 8.34%). Earlier Josephine and Janardhanan (1992) reported a higher value of L-Dopa for some germplasm of *Mucuna pruriens* (6.97-9.16%). This indicated that L-Dopa is not only the genetic makeup of germplasm and it's also greatly influenced by the environmental conditions.

Significantly low L-Dopa accession was identified IC 332432 (2.94%) and IIHR MP95 (2.98%) was highly beneficial to consume as food with proper boiling or processing which were collected from NBPGR, New Delhi. The accessions having high L-Dopa is highly beneficial to the industries for preparation of the drugs for the treatment of Parkinson's disease. Significantly high L-Dopa accessions IIHR MP62-1(6.91%), IIHR MP21 (6.77%), IIHR MP63-1(6.76%) and IIHR MP 102 (6.57%) were collected from Kerala, Himachal Pradesh, Kerala and Andhra Pradesh, respectively. Itchy types are important for drug industry to extraction of L-Dopa and its cultivation is very difficult due to the presence of itchy trichomes. Hence these high L-Dopa accessions can be utilised in breeding programme of *Mucuna* to develop high yielding cultivars coupled with high L-Dopa and non-itchy nature.

Correlation coefficients revealed that non-significant positive correlation of L- Dopa with days to 50% flowering, inflorescence length, number of flowers per inflorescence, number of pods per bunch, dry pod yield per plant and days to maturity. L-Dopa has non-significant negative association with pod width, pod weight, number of bunches per plant, number of seeds per pod, shelling percentage and test weight of the seed. Our results corroborated with Raina *et al.*, (2012) who reported that L-Dopa was negatively correlated with seed traits indicating that seed size may not be selection criterion for identifying a genotype with high L-Dopa. Whereas the pod length has significant negative association with L-Dopa (Table 2) indicates that the selection of smaller pods rather than long pods could be useful to develop high L-Dopa accessions which can be utilized in future breeding programme of velvet bean.

CONCLUSION

Mucuna seeds are rich source of protein and natural source of L-Dopa for preparation of drugs to the treatment of the Parkinson disease. On the basis of the present studies considerable variation for L-Dopa exists among accessions collected from different parts of India. The low content of L-Dopa accessions IC 332432 (2.94%) and IIHR MP95 (2.98%) were highly beneficial to consume as food with proper boiling or processing as an alternative source of protein to overcome the problem of protein deficient malnutrition. High L-Dopa accessions IIHR MP62-1(6.91%), IIHR MP21 (6.77%), IIHR MP63-1(6.76%) and IIHR MP102 (6.57%) were itchy types and important for drug industry for extraction of L-Dopa could be exploited for their pharmacological properties especially L-Dopa content. L-Dopa significantly and negatively correlated with pod length indicated that selection of shorter pod was a marker trait for high L-Dopa in selection programme of *Mucuna*. Hence these itchy types can be used in crop improvement programme of velvet bean for desirable trait of high L-Dopa with non-itchy nature for commercial cultivation to address the demand of domestic and international market of L-Dopa.

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