Studies on the Medicinal Compound L-Dopa in *Mucuna pruriens* Bak.

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Fifty *Mucuna* germplasm accessions were augmented through introduction, exchange and exploration activities of National Bureau of Plant Genetic Resources (NBPGR), New Delhi. L-Dopa contents in *Mucuna* seeds were estimated as per cent/ gram of dry seed on an UV Spectrophotometer at 280 nm. The L-Dopa contents varied significantly in different accessions and ranged between 1.51 to 6.29 %. The minimum L-Dopa contents were recorded in accession number IC471873 and the maximum in IC385843. Out of 50 *Mucuna* accessions analyzed, L-Dopa contents varied significantly between 2-4.5% per gram of dry seeds in 45 accessions. Comparatively higher L-Dopa contents were recorded in the accessions IC385843, IC391885, IC83195, IC471875 and IC396648 with 6.3%, 5.8%, 5.2%, 4.9% and 4.6%, respectively. The present study validates the possible exploitation of five *Mucuna* germplasm for their pharmacological properties mainly the L-Dopa.

Key Words: *Mucuna*, Germplasm, Seeds, L-Dopa contents

Introduction

The genus *Mucuna* has a wide distribution in tropical and subtropical regions of the world. In India, 14 species are widespread over most of the subcontinent in bushes and hedges, dry-deciduous, low forests and throughout the plains of India (Sastry and Kavathekar, 1990; Singh *et. al.,* 1996).

All the parts of *Mucuna* contain valuable medicinal properties (Pandey, 1998, 1999; Caius, 1989) and there is a heavy demand of *Mucuna* in Indian drug market. After the discovery that *Mucuna* seeds contain L-Dopa, an anti-Parkinson's disease drug, its demand in international market has increased many fold (Daxenbichler *et al.,* 1971, 1972; Lorenzetti *et al*., 1998; Farooqi *et al.,* 1999) and demand has motivated Indian farmers to start commercial cultivation. Besides medicinal properties, *Mucuna* fixes nitrogen and is used as a green manure and cover crop.

Mucuna pruriens Bak. belongs to family Fabaceae, commonly known as Velvet bean, is one of the popular medicinal species and is constituent of more than 200 indigenous drug formulations. Seeds contain L-Dopa, glutathione, lecithin, gallic acid, glycosides, nicotine, prurenine, prurenidine, dark brown viscous oil (Ghosal *et al.,* 1971, Szabo and Tebbett, 2002). It is source of minerals (Rastogi and Mehrotra, 1991a, b; Singh *et al*., 1995). According to Ayurveda, seeds are astringent, laxative, anthelmintic, aphrodisiac, alexipharmic and tonic. In particular, the seeds and the roots of *M. pruriens* are used in the system of medicine as effective nerve tonic and aphrodisiac. It has diuretic property and consequently is used in kidney ailments and dropsy. The seeds contain L-Dopa (L-3, 4-dihydroxy phenylalanine), which is the active principle responsible for the treatment of Parkinson's disease and hypertension (Dollery, 1999; Moffat, 1986; Spratto and Woods, 1996; Szabo and Tebbett, 2002). In addition, the seeds have fats, sterols, alkaloids and other amino acids.

Of all these, the L-Dopa is the main active principle. A large number of analytical procedures have been reported in literature for the extraction, isolation and estimation of L-Dopa in the seeds. Considerable research work has also been done to produce L-Dopa through tissue culture (Brain, 1976; Huizing *et al*., 1985; Wichers *et al*., 1985) but it has not gone on commercial scale for industrial purpose. Considering the importance of this genus, attempts have been made throughout the world to screen and identify high L-Dopa yielding lines (Pieris *et al*., 1980; Lubis and Sastrapardha, 1981; Bammi and Gangadhar Rao, 1982) for their commercial cultivation.

Materials and Methods

Plant Material

Fifty *Mucuna* germplasm accessions were selected from exotic and indigenous collections of National Bureau of Plant Genetic Resources (NBPGR), New Delhi and through explorations. The germplasm accessions represented 12 Indian states and eight exotic collections representing four species of *Mucuna*, namely, *M. utilis* Wall. ex. Wight, *M. panaba, M. pruriens, M. prurita*

and others as *Mucuna* species. The details of NBPGR accession numbers, *Mucuna* species, and place of collection are given in Table 1.

L-Dopa Estimation in Mucuna Seeds

L-Dopa contents in *Mucuna* seeds were estimated following the method described by Brain (1976) with some modifications. Standard curve of L-Dopa was plotted using pure L-Dopa AR (Sisco Research Lab) concentration 10 to 100 µg/ml on an UV/Vis Spectrophotometer (Hitachi U1500). The stock solution of L-Dopa was prepared using 15 mg of L-Dopa AR in a test tube and 3 ml of 0.1 N HCl was added to it. The solution was heated in a boiling water bath for 15 minutes. Cooled down to room temperature and added 3 ml of ethanol. Shaked for 10 minutes with hand and centrifuged at 2000 rpm for 10 minutes. Supernatant was aspirated and volume raised up to 15 ml. Finally, the solution was filtered through Whatman micro-filter (0.22 micron porosity) and diluted with ethanol so as to obtain 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 µg/ml concentrations of L-Dopa.

The absorption was read at 280 nm and standard curve was plotted taking L-Dopa concentration on X-axis and optical density at Y-axis.

Extraction and Estimation of L-Dopa

The *Mucuna* seeds were crushed in pastel mortar and seed coats were removed. Further, the seeds contents were powdered and taken in to 18 x 150 mm test tubes. The L-dopa was extracted from the 0.5 g powdered seed. Three replications were maintained for each germplasm accession. To 0.5 g powdered seed, 3 ml of 0.1 N HCl was added and kept in boiling water bath for 15 minutes. The test tubes were allowed to cool down to room temperature before adding 3 ml of absolute ethanol. The test tubes were then vigorously shaken for 10 minutes and centrifuged at 2000 rpm for 10 minutes. Supernatant was aspirated using 1000 µl micropipette. Finally, the volume was raised up to 15 ml. The solution was then filtered through Whitman membrane filter (0.22 micron porosity). The solution was diluted 1:10 with ethanol before reading A_{280}.

Calculation

The OD for 100 µg was taken as standard.

$$\text{L-Dopa /100 µl solution (A)} = \frac{100 \times X}{Y} \text{ (in µg)}$$

So, L-Dopa / ml solution (B) = A x 10 (in µg)

So, L-Dopa in 15 ml Solution (0.5 g seeds) (C) = B x 15 (in µg)

So, L-Dopa content in 1 g seed (D) = C x 2 (in µg) = C x $2/10^{-6}$ g

So, Per cent L-Dopa content = 100 x D (in g)

Where,

$X = A_{280}$ for sample

$Y = A_{280}$ for 100 µg L-Dopa

Results and Discussion

The standard curve prepared using pure L-Dopa with 10 to 100 µg/ml for quantification of L-Dopa in *Mucuna* seeds is shown in Figure 1. Almost straight line of the standard curve at recommended wave length of 280 nm validated the precise quantification of L-Dopa in seeds of *Mucuna* accessions analyzed. The L-Dopa contents were finally estimated as percent/gram of dry seed. The L-Dopa contents varied significantly in different accessions and ranged between 1.51 to 6.29%. The minimum L-Dopa contents were recorded in accession number IC471873 and the maximum in IC385843.

Of the 50 *Mucuna* accessions analyzed, L-Dopa contents varied significantly between 2-4.5 % per gram of dry seeds in 46 accessions. Comparatively higher L-Dopa contents were recorded in the accessions IC385843, IC391885, IC83195, IC471875 and IC396648 with 6.3%, 5.8%, 5.2%, 4.9% and 4.6%, respectively (Table 1, Fig. 2). All these five high L-Dopa yielding *Mucuna* accessions can be exploited for extraction of L-Dopa contents.

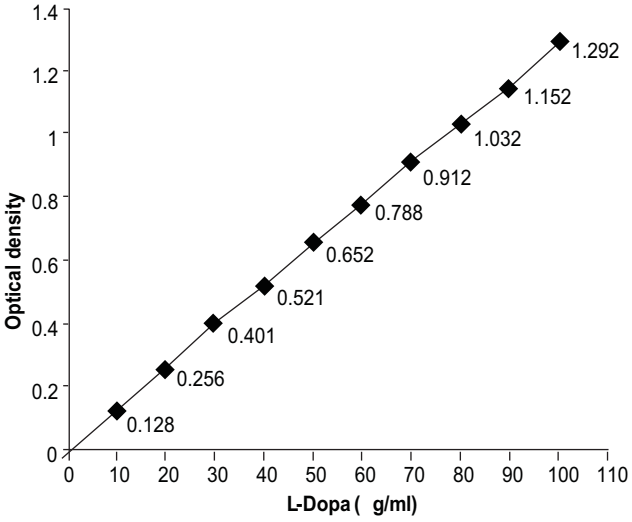

Fig. 1: Standard curve of L-Dopa

Table 1. *Mucuna* germplasm accessions with L-Dopa content per gram of seed

S. No.	Accession No.	Species	Collection Place	L-Dopa content per gram seed
1	EC030	*M. pruriens*	Australia	3.05
2	EC13047	*M. pruriens*	USSR	2.21
3	EC144945	*M. pruriens*	Italy	3.61
4	EC169813	*M. pruriens*	Nigeria	3.02
5	EC1842	*M. pruriens*	Australia	2.07
6	EC1842-A	*M. pruriens*	Australia	3.63
7	EC25334	*M. pruriens*	Kenya	2.49
8	EC4475	*M. pruriens*	Australia	3.48
9	IC127363	*M. pruriens*	NBPGR, HQ	3.21
10	IC15809-A	*M. pruriens*	Palaman/Bihar	3.04
11	IC21992	*M. utilis*	Madhya Pradesh	3.85
12	IC21996-A-1	*M. utilis*	Madhya Pradesh	3.15
13	IC21998	*M. utilis*	Madhya Pradesh	3.26
14	IC241679	*M. pruriens*	NBPGR,HQ	2.98
15	IC241680	*M. pruriens*	Kerala	2.99
16	IC241682	*M. pruriens*	Palaghat/Kerala	3.35
17	IC25333	*M. pruriens*	Mizoram	3.29
18	IC25333-2	*M. pruriens*	NBPGR, HQ	3.20
19	IC25333-A-1	*M. pruriens*	NBPGR, HQ	3.01
20	IC25334-2	*M. pruriens*	NBPGR, HQ	2.91
21	IC2534-2	*M. pruriens*	NBPGR, HQ	2.53
22	IC260046	*M. pruriens*	Kerala	4.12
23	IC260707	*M. pruriens*	Salem/Tamil Nadu	2.52
24	IC265577	*M. pruriens*	Kerala	4.11
25	IC296846	*M. pruriens*	Karnataka	3.64
26	IC296847	*M. pruriens*	Karnataka	2.67
27	IC326953	*M. utilis*	Solan/Himachal	4.17
28	IC33243	*M. pruriens*	Madhya Pradesh	3.38
29	IC369144	*M. pruriens*	Jharkhand	3.01
30	IC385841	*M. prurita*	Jharkhand	2.43
31	IC385843	*M. prurita*	Jharkhand	6.29
32	IC385844	*M. pruriens*	Godda/Jharkhand	3.10
33	IC385925	*M. prurita*	Jharkhand	2.39
34	IC385926	*M. prurita*	Jharkhand	2.44
35	IC391885	*M. pruriens*	Orissa	5.76
36	IC391899	*M. pruriens*	Orissa	4.38
37	IC391941	*M. pruriens*	Orrisa	3.45
38	IC392241	*M. prurita*	Jharkhand	4.22
39	IC392835	*M. pruriens*	Orrisa	4.51
40	IC392850	*M. pruriens*	Orrisa	3.60
41	IC396648	*M. prurita*	Narmada/ Gujarat	4.60
42	IC43993	*M. pruriens*	Kerala	2.85
43	IC471869	*M. pruriens*	NBPGR, HQ	2.56
44	IC471870	*M. pruriens*	NBPGR, HQ	3.33
45	IC471872	*M. pruriens*	NBPGR, HQ	2.42
46	IC471873	*M. pruriens*	NBPGR, HQ	1.51
47	IC471875	*M. pruriens*	NBPGR, HQ	4.87
48	IC83195	*M. prurita*	Narmada/ Gujarat	5.12
49	IC83298	*M. pruriens*	NBPGR,HQ	3.37
50	IC16993-A	*M. pruriens*	Manipur	3.25
	CD			0.30

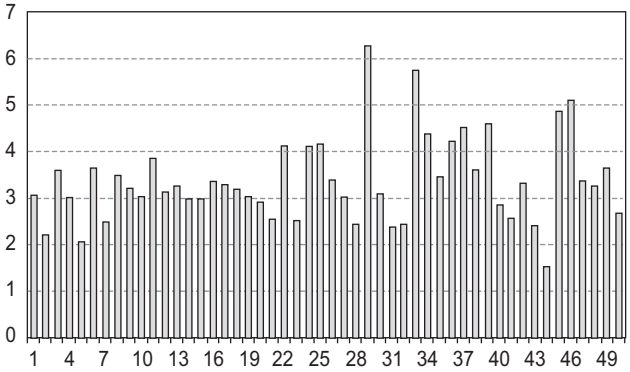

Fig. 2: L-Dopa per cent per gram of seed in fifty accessions of *Mucuna*

In a survey of over 1000 plant species from 160 families, only *Mucuna* seed was found to contain L-Dopa at > 0.5% with concentrations between 3.1 and 6.7% indicated (Daxenbichler *et al.,* 1971, 1972). Other studies have reported as much as 9% L-Dopa contents in some *Mucuna* seeds, and as little as 1.5% in the seeds of *M. gigantea* which grows wild in southern India (Rajaram and Janardhanan, 1991). Less than 1% of the administered dose of L-Dopa actually enters the brain where it is converted to dopamine in the basal ganglia (Dollery, 1999; Moffat, 1986). Currently in the treatment of Parkinson's disease L-Dopa is almost always prescribed in a drug regimen with a peripheral deoxycarboxylase inhibitor to prevent loss of L-Dopa by the enzyme L-aromatic amino acid decarboxylase (LAAD) that begins in the internal mucosa. The presence of LAAD increases the amount of L-Dopa available to the brain by 75-80% (Jancovic and Caine, 1987), thereby decreasing the dosage necessary to achieve therapeutic effect.

L-Dopa itself has very little pharmacological effect, since it is rapidly converted to dopamine. Most side effects arise directly from dopamine's activity as a neurotransmitter involved in the regulation of heart, vascular system, digestive tract, and excretory system, rather than from its well-known effect on receptors in the brain (Dollery, 1999; Standaert and Young, 1996). With the limitation in mind, the inadvertent ingestion of L-Dopa as a result of consuming *Mucuna* that has not been adequately prepared could have serious consequences (Infante *et al.,* 1990).

Therefore, the possibility of raising *Mucuna* as a cash crop for its L-Dopa contents must be addressed and it may prove to be an affordable alternative for patients suffering from Parkinson's disease (Kempster

and Wahlqvist, 1994; Manyam and Sanchez-Ramos, 1999). The present study validates the possible exploitation of five promising *Mucuna* germplasm accessions for their pharmacological properties especially L-Dopa content.

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