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Analysis of Variability in Seed Storage Proteins of *Mucuna pruriens* var. *utilis* by using SDS-PAGE

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ABSTRACT The influence of agro-ecological conditions of different growing locations on the seed proteins of a tropical under-utilized legume, *Mucuna pruriens* var. *utilis* (Wall ex Wight) Baker ex Burck (velvet bean) was investigated. A wide variation was observed in the total seed protein content (22.8-26.1 g/100 g seed flour) and different protein fractions with relation to different growing locations. However, the major seed protein fractions, the albumins and globulins of all the accessions exhibited similar kind of banding pattern and same molecular weight protein sub units when subjected to SDS-PAGE. indicating that the environmental conditions of the growing locations influence only the quantity of the proteins not their molecular characteristics in velvet bean seeds.

Key words: *Mucuna pruriens* var. *utilis*, total proteins, protein fractions, SDS-PAGE

In recent years, efforts are being made to identify and evaluate the under-utilized/non-conventional legume food sources as alternative protein crops for the future [1].

Among the various under-utilized pulses, the velvet bean [*Mucuna pruriens* van *utilis* (Wall ex Wight) Baker ex Burck] seeds merit a wide use in South Asian countries and other parts of the tropics as food legume and experiments have also been conducted on its utilization as a dietary protein source in animal feeds [2]. The velvet bean seeds have a nutritional quality comparable to soybeans and other conventional legumes as it contains similar proportions of protein and carbohydrates along with adequate concentration of minerals and other nutrients [3]. The velvet bean leaves are used as fodder and the seed meal is used as cattle feed along with cotton seed meal.

The available information shows that the food constituents of the legume seeds are largely influenced by different environmental factors such as soil type, climate, location, soil fertility etc. [1, 4]. The environment of different locations plays an important role in the determination of quantity and quality of seed proteins [5, 6]. In velvet beans also the existence of diversity among the plants growing at different locations with respect to the agro-botanical characters [7] and chemical composition [3] was already reported. However, the effect of agro-ecological conditions of different growing locations on the seed protein profiles of velvet bean has not been addressed. Hence, the present study was carried out with a view to analyze and characterize the seed storage proteins by implementing the SDS-PAGE technique to evaluate the impact of climatic conditions of growing locations on the seed protein profiles of velvet beans collected from different agro-ecological regions of South India.

MATERIALS AND METHODS

Eleven accessions of seed samples of velvet bean were collected from different agro-ecological regions of western Ghats, South India from the natural stands (Table 1). Collected seed samples were dried in the sun light for 24 h. After removing immature and damaged seeds, the dried seeds were powdered in a Willey Mill to 60-mesh size. The

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Table 1. Agroecological regions of eleven accessions of *Mucuna pruriens* var. *utilis* seeds

S.No.	Name of the accession	Place of collection	Ecological region
1	Thachenmalai (white coloured seed coat)	Kanyakumari, Tamil Nadu, India	Deciduous forest, slightly elevated, sandy soil, near riverside
2	Thachenmalai (black coloured seed coat)	Kanyakumari, Tamil Nadu, India	Deciduous forest, slightly elevated, sandy soil, near riverside
3.	Mundanthurai (white coloured seed coat)	Thirunelveli, Tamil Nadu, India	Ever-green forest, red soil, altitude 500 m
4.	Mundanthurai (black coloured seed coat)	Thirunelveli, Tamil Nadu, India	Ever-green forest, red soil, altitude 500 m
5.	Kailasanadu (white coloured seed coat)	Idukki, Kerala, India	Semi ever-green forest, red soil, altitude 500-700 m
6.	Valanad (black coloured seed coat)	Thiruvananthapuram, Kerala, India	Moist deciduous forest, black clay soil, altitude 600-800 m
7.	Mylaru (white coloured seed coat)	Thirunelveli, Tamil Nadu, India	Ever-green forest, red soil, altitude 500 m
8.	Mylaru (black coloured seed coat)	Thirunelveli, Tamil Nadu, India	Ever-green forest, red soil, altitude 500 m
9.	Keriparai (white coloured seed coat)	Kanyakumari, Tamil Nadu, India	Deciduous forest, slightly elevated, sandy soil, near riverside
10	Nedumangadu (black coloured seed coat)	Thiruvananthapuram Kerala, India	Moist deciduous forest, black clay soil, altitude 600-800m
11	Naiyar (black coloured seed coat)	Thiruvananthapuram Kerala, India	Moist deciduous forest, black clay soil, altitude 600-800m

powdered samples were stored in plastic containers for further use.

The total proteins, prolamin and glutelin fractions were extracted by following the method of Rajaram and Janardhanan [8], whereas, albumins and globulins were extracted using the method of Murray [9]. The protein content of both total proteins and different solubility classes of proteins were estimated after cold 20 per cent TCA precipitation by following the method of Lowry *et al.* [10] in a spectrophotometer at 660 nm. The samples were mixed with equal volume of sample buffer and kept in a boiling water bath for 90 sec and then transferring in an ice bath till loading. Before loading, samples were centrifuged and the supernatant was used for loading in the wells. 30 ml of each sample was loaded in the wells and a mixture of molecular marker proteins consists of Myosin from rabbit muscle (205,000 Da), Phosphorylase b (97,000 Da), Bovine Serum Albumin (68,000 Da), Ovalbumin (43,000 Da) and Carbonic anhydrase (29,000 Da) [Range 29-205 kD; Cat. No. PMW-H. procured from Genei, Bangalore] was loaded in a separate well. The albumin and globulin protein fractions of different accessions of velvet bean were separated through Sodium Dodecyl Sulphate- Polyacrylamide Gel Electrophoresis (SDS-PAGE) by following the method of Laemmli [11] in a vertical slab gel electrophoresis system. The electrophoresis was carried out at 150 V DC current until the marker dye reaches near the lower edge of the slab. The

gels were stained with Coomassie brilliant blue overnight and destained with acetic acid:methanol: water (1:3:6) solution.

Results were expressed as mean values ± standard deviations of three determinations. The data was subjected to a one-way analysis of variance (ANOVA) and the significance of difference between means at 5 per cent was determined by Duncan's Multiple Range Test (DMRT) using Irristat software (version 3/93).

RESULTS AND DISCUSSION

The electrophoretic banding pattern of albumin and globulin proteins have been employed to justify the diversity among the velvet bean accessions growing at different locations, since the seed protein profiles are highly stable and species specific and they are hardly affected by growing conditions or seasonal fluctuations [12]. The total protein content in different accessions of velvet bean seeds falls between 22.8 and 26.1 g/100 g seed flour with Nedumangadu germplasm having the highest level (26.1 %) (Table 2). The total protein content of velvet beans of the present study is comparable with the earlier studies [2, 13] in the same species (18.5-25.7 %) and higher than the values reported for some other under-utilized legumes such as *Cassia floribunda* [14] (15.5-17.7 %); *Mucuna monosperma* [15] (18.5-20.6 %); *Sesbania bispinosa* [16] (21.8 %). A significant variation ($p < 0.05$) exhibited in the total protein content among different accessions of velvet bean seeds of the present study is probably due to the different ecological conditions of growing locations and this is in agreement with an earlier report in *Cassia hirsuta* [17]. The interaction of genotype and location on protein content in the cultivated legumes is reported earlier and the location effect is relatively more important than that of cultivar effect on the protein content [5, 6].

Protein fractionation studies show that the globulins and albumins together constitute the major bulk of the seed proteins (Table 2) as in many other legumes [9]. From nutritional point of view, the high globulin content (51-55 %) of velvet bean is desirable because, the globulins were mainly constituted by legumin and vicilin and represent 60-80 per cent of the extractable proteins. The albumin fraction, less abundant than globulins, represents 34-39 per cent of the cotyledonary proteins. The albumin fraction include most of the enzymatic and metabolic proteins and have high content of lysine and sulphur containing amino acids. The quantity of prolamins (6.1-7.4 %) and glutelins (3.6-7.3 %) were meager. The ratio of albumin and globulin was quite high compared with several other legumes such as chickpea [18]. The ratio of albumins: globulins: prolamins: glutelins in the presently investigated velvet bean is also in agreement with that of earlier results in the same crop [2, 13] and other wild legumes like *Mucuna monosperma*; *Erythrina indica* and *Sesbania bispinosa* [15, 16].

The electrophoretic banding pattern of albumin of different accessions of velvet bean separated into-seven different molecular weight protein sub units. The first band had approximately more than 205 KD molecular weight, second and third bands had apparently 68 and 43 KD molecular weight and were less intensive. The fourth band had the molecular weight of 29 KD (approximately) and the following three thick bands of albumin fraction were below 29 KD in molecular weight. The molecular weight of sub units of albumin fraction of velvet bean is similar with that of common pulses like *Phaseolus vulgaris* [19]. There was no variation in the banding pattern and molecular weight of the albumin protein sub units among the different accessions of velvet bean.

The globulin fraction of eleven different accessions of velvet bean separated into three distinct bands. The first and second bands had approximately 68 and 43 KD molecular weight and lighter in intensity. The third band was prominent and broad and below 29 KD in molecular weight. The relative molecular mass of about 45-66 KD in globulin fraction of velvet bean corresponds to the typical molecular mass of 7S storage proteins [20]. The molecular weight of the globulin of velvet bean is similar with that of globulin protein of some commercial pulses like *Vigna unguiculata*, *Phaseolus vulgaris* and *Vicia faba* [20, 21]. All the germplasm of velvet bean of the present study exhibited similar kind of banding pattern for globulin fraction also.

From this study, it is clear that in all the

Table 2. Total protein and protein fractions of eleven different accessions of *Mucuna pruriens* var. *utilis*

Name of the accession	g/100 g seed flour				
	Total proteins	Albumin	Globulin	Prolamin	Glutelin
Thachenmalai (White seed)	$23.86^{e}\pm0.09$	$8.61^{c}\pm0.17$	$12.77^{d}\pm0.11$	$1.52^{a}\pm0.01$	$0.91^{c}\pm0.05$
Thachenmalai (Black seed)	$23.03^{b}\pm0.24$	$8.47^{b}\pm0.06$	$12.78^{e}0.08$	$1.62^{e}\pm0.00$	$0.91^{c}\pm0.02$
Mundanthurai (White seed)	$24.45^{h}\pm0.57$	$8.74^{e}\pm0.12$	$12.84^{f}\pm0.20$	$1.60^{c}\pm0.02$	$1.04^{f}\pm0.07$
Mundanthurai (Black seed)	$23.48^{c}\pm0.12$	$8.42^{a}\pm0.02$	$12.55^{a}\pm0.07$	$1.61^{d}\pm0.02$	$0.94^{e}\pm0.00$
Kailasanandu (White seed)	$24.31^{g}\pm0.10$	$8.72^{d}\pm0.03$	$12.85^{g}\pm0.07$	$1.61^{d}\pm0.02$	$0.87^{b}\pm0.00$
Valanadu (Black seed)	$22.88^{a}\pm0.10$	$9.07^{k}\pm0.04$	$12.73^{c}\pm0.12$	$1.69^{g}\pm0.02$	$0.84^{a}\pm0.02$
Mylaru (White seed)	$23.61^{d}\pm6.26$	$9.05^{j}\pm0.09$	$12.67^{b}\pm0.04$	$1.73^{i}\pm0.00$	$0.91^{c}\pm0.04$
Mylaru (Black seed)	$25.46^{j}\pm0.17$	$8.86^{f}\pm0.11$	$13.05^{i}\pm0.09$	$1.56^{b}\pm0.01$	$1.45^{h}\pm0.11$
Keriparai (White seed)	$24.09^{f}\pm0.06$	$8.94^{i}\pm0.23$	$12.96^{h}\pm0.06$	$1.66^{f}\pm0.01$	$0.93^{d}\pm0.04$
Nedumangadu (Black seed)	$26.10^{k}\pm0.77$	$8.89^{g}\pm0.06$	$13.28^{k}\pm0.11$	$1.74^{j}\pm0.06$	$1.91^{i}\pm0.02$
Naiyar (White seed)	$25.26^{i}\pm0.09$	$8.92^{h}\pm0.00$	$13.12^{j}\pm0.15$	$1.72^{h}\pm0.01$	$1.43^{g}\pm0.02$

Values are means of three determinations ± standard deviation.

Mean values in same columns sharing different letters are statistically different ($p < 0.05$).

accessions, the albumins and globulins form a constant number of bands. The protein spectrum in general showed stable profile. The actual meaning of stability of seed protein profile of a species is that lines or species from different geographical areas still show nearly the same protein profile, the variation most often detected is of a quantitative nature i.e., colour intensity of various bands.

These findings revealed that the total protein content of velvet bean seeds collected from different agro-ecological regions of South India is greatly influenced by climatic and ecological conditions of different growing locations. However, the major seed protein fractions were found to contain same molecular weight protein sub units and do not indicate any difference in the banding pattern when subjected to SDS-PAGE, which shows that the environmental conditions of the growing locations have influence only on the quantity of the proteins in velvet bean seeds and do not affect the protein molecular characteristics.

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