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Studies on Chemical Composition and Antinutritional Factors in Three Germplasm Seed Materials of the Tribal Pulse, *Mucuna pruriens* (L.) DC

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Three germplasm seed materials of the Indian tribal pulse, *Mucuna pruriens*, collected from different agroclimatic regions [Begur Reserve Forest and Silent Valley in Kerala State and Mothimahal Campus, Lucknow (Uttar Pradesh)] were analysed for proximate composition, seed protein fractions, amino acid composition, minerals and antinutritional factors. All three germplasm seed materials of *M. pruriens* analysed contained higher contents of crude protein and crude lipid when compared with most of the commonly consumed pulses and other species of *Mucuna*. Albumin and globulin fractions constituted the major bulk of seed proteins in all three germplasms. Profiles of amino acids revealed that the seed proteins contained relatively higher levels of all the essential amino acids except sulpho-amino acids in all the three germplasms of *M. pruriens* and threonine in the germplasms of Silent Valley and Lucknow when compared with the WHO requirement pattern. In addition, the seed proteins of Lucknow germplasm were found to be limiting in isoleucine + leucine also. All three samples of *M. pruriens* were rich in minerals such as, K, Mg and P. The Begur Reserve Forest germplasm was also rich in Fe. Except for L-DOPA, all antinutritional factors detected/quantified were heat-labile and hence could be eliminated by cooking. The albumins of Lucknow germplasm alone exhibited weak agglutination with erythrocytes from 'O' blood group. In all three samples, globulins showed weak agglutination with erythrocytes without any specificity.

INTRODUCTION

Several countries are advocating different nutritional policies to augment available food through the introduction of high-yielding seeds, pest control and preservation. However, one method that has often been neglected is the exploitation of underutilized food sources (Afolabi *et al.*, 1985). *Mucuna pruriens* (L) DC is a tribal pulse found growing even in poor soils and produces abundant seeds. The seeds are eaten by the Indian tribal sects, Mundari and Dravidian groups (Jain, 1981). Hence, in the present study the seeds of this tribal pulse were collected from three different agroclimatic zones in India and their chemical composition was investigated with a view to assessing their nutritional potential.

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MATERIALS AND METHODS

Three germplasms of *M. pruriens* were collected from Begur Reserve Forest and Silent Valley in Kerala State, South India, and Mothimahal Campus, Lucknow, Uttar Pradesh in North India. The moisture content was measured by drying 50 transversely cut mature and dry seeds in an oven at 80°C for 24 h. The seeds were powdered separately in a Willey Mill to 60 mesh size. The fine seed powder so obtained was used for further analysis. The crude protein content was calculated by multiplying the percent Kjeldahl nitrogen (Humphries, 1956) with the factor 6·25. The remaining proximate

Table 1. Proximate and Mineral Composition[a]

	Proximate composition (g 100 g^{-1} seed flour)				Mineral composition (mg 100 g^{-1} seed flour)		
	M. pruriens (Begur)	*M. pruriens* (Silent Valley)	*M. pruriens* (Lucknow)		*M. pruriens* (Begur)	*M. pruriens* (Silent Valley)	*M. pruriens* (Lucknow)
Moisture	3·50 ± 0·04**	2·90 ± 0·73	3·65 ± 1·73	Na	3·72 ± 0·04	3·80 ± 0·02	4·80 ± 0·02**
Crude protein (Kjeldahl N × 6·25)	35·0 ± 0·67	34·0 ± 1·73	34·1 ± 1·70	K	2320 ± 0·47	2790 ± 0·98**	2500 ± 0·47**
Crude lipid	9·06 ± 1·41**	5·97 ± 1·03	8·17 ± 0·10	Ca	218 ± 0·09	268 ± 0·27**	248 ± 0·47**
Crude fibre	7·00 ± 0·03**	2·18 ± 0·11**	1·60 ± 0·31	Mg	68·6 ± 0·02	73·8 ± 0·02	76·4 ± 0·11**
Ash	5·90 ± 0·03	5·07 ± 0·05	3·90 ± 0·17	P	320 ± 0·27	638 ± 0·12**	420 ± 0·27**
Nitrogen free extractives (NFE)	43·0	51·8	52·2	Fe	8·66 ± 0·07**	3·56 ± 0·02	3·36 ± 0·11
Calorific value (kcal 100 g^{-1} DM)	394	385	419	Cu	0·52 ± 0·07	0·44 ± 0·01	0·44 ± 0·01
				Zn	1·68 ± 0·05	1·92 ± 0·04	1·52 ± 0·27
				Mn	0·32 ± 0·30	0·36 ± 0·02	0·24 ± 0·12

[a] All values are mean of triplicate determinations expressed on dry weight basis.
± Denotes the standard error.
** Denotes significant difference at $P = 0·01$.

Table 2. Data on Seed Total (true) Proteins and Protein Fractionation (g 100 g^{-1} seed flour)[a]

Name of the fraction	*M. pruriens* (Begur)	*M. pruriens* (Silent Valley)	*M. pruriens* (Lucknow)
Total protein (True protein)	30·7 ± 0·09**	26·0 ± 0·08	27·9 ± 0·05
Albumins	9·62 ± 0·09	10·6 ± 0·09**	5·35 ± 0·04
Globulins	15·2 ± 0·08**	11·7 ± 0·03	17·7 ± 0·21**
Prolamins	0·61 ± 0·01	0·10 ± 0·02	1·09 ± 0·19
Glutelins	5·20 ± 0·14**	3·50 ± 0·07	3·76 ± 0·07

[a] All values are mean of triplicate determinations expressed on dry weight basis.
± Denotes the standard error.
** Denotes significant difference at $P = 0·01$.

constituents were estimated by AOAC (1970) methods. The nitrogen free extractives was calculated by difference (Muller & Tobin, 1980). The energy content was determined by multiplying percentage of crude protein, crude fat and nitrogen free extractives (total crude carbohydrates) with the factors 4, 9 and 4, respectively (Osborne & Voogt, 1978). The total true proteins were extracted by the method of Basha *et al.* (1976) with slight modification (ethanol treatment was omitted to save the prolamin fraction). The extracted proteins were purified by precipitation with cold 20% TCA. The seed protein fractions, albumins and globulins, were extracted following the method of Basha and Beevers (1975). From the residue the prolamin fraction was extracted by treating it with 70% ethanol 1:5 (w/v) overnight. After centrifugation the supernatant containing prolamins was air-dried and dissolved in 0·1N NaOH. To the remaining pellet 0·4N NaOH, 1:10 (w/v), was added, left overnight and centrifuged at 20 000 × *g* for 20 min. The supernatant thus obtained was designated as glutelins. The proteins in the fractions so obtained were estimated (Lowry *et al.*, 1951) after 20% cold TCA precipitation. The purified total seed proteins were acid-hydrolysed with 6N HCl at 110°C for 24 h *in vacuo*. After flash evaporation the dried residue was dissolved in citrate buffer (pH 2·2). Known aliquots were analysed in a LKB-Biochrome Automated Amino Acid Analyser, Model 4151-Alpha Plus. All the minerals excepting phosphorus were analysed by Atomic Absorption Spectrophotometer (Issac & Johnson, 1975). Phosphorus content in the triple acid digested extract was determined colorimetrically (Dickman & Bray, 1940). The antinutritional factors like tannins (Burns, 1971), total free phenols (Bray & Thorne, 1954) and the non-protein amino acid, L-DOPA (3,4-dihydroxyphenylalanine), (Brain, 1976) were quantified. The haemagglutinating activity of albumin and globulin fractions of seed proteins (Liener, 1976) and the trypsin inhibitor activity of the seed meal (Chrispeels & Baumgartner, 1978) were also assayed.

RESULTS AND DISCUSSION

The crude protein contents of all the three germplasms of *M. pruriens* (Begur, Silent Valley and Lucknow) show little variation and contain higher crude protein (Table 1) when compared with other *Mucuna* species reported earlier (Janardhanan & Lakshmanan, 1985; Afolabi *et al.*, 1985; Rajaram, 1990; Rajaram & Janardhanan, 1991; Arulmozhi, 1990). Of the three germplasms of *M. pruriens* investigated, the Begur germplasm seems to have a higher crude lipid content compared with the other two germplasms (Lucknow and Silent Valley) and the other *Mucuna* species such as *M. solanei* (Afolabi *et al.*, 1985); *M. atropurpurea* (Rajaram, 1990) and *M. monosperma* (Arulmozhi, 1990). Among the three germplasms investigated (Table 1) Lucknow germplasm has the highest calorific value, a value greater than the other *Mucuna* species/germplasms (Rajaram & Janardhanan, 1991; Rajaram, 1990; Arulmozhi, 1990).

Total (true) protein content of *M. pruriens* germplasms in the present study has been found to be higher (Table 2) than the other *Mucuna* germplasms reported from our laboratory (Janardhanan & Lakshmanan, 1985; Rajaram & Janardhanan, 1991; Rajaram, 1990; Arulmozhi, 1990). The data on protein fractions reveal (Table 2) that, in all three germplasms, albumins and globulins constitute the major bulk of seed proteins as in most of the legumes and the other *Mucuna* species reported earlier (Boulter & Derbyshire, 1976; Duranti & Cereletti, 1979; Janardhanan & Lakshmanan, 1985; Rajaram & Janardhanan, 1991; Rajaram, 1990; Arulmozhi, 1990).

The data on amino acid profiles (Table 3) indicate that the contents of phenylalanine + tyrosine and valine in the seed proteins of Lucknow germplasm and isoleucine + leucine in Begur germplasm are higher when compared with the values reported earlier in *M. pruriens* (Prakash & Mishra, 1987). The seed proteins of Lucknow and Silent Valley germplasms investigated

Table 3. Amino Acid Profiles of Acid-Hydrolysed, Purified Total Seed Proteins

	M. pruriens (Begur) (g 100 g^{-1} protein)	*M. pruriens* (Silent Valley) (g 100 g^{-1} protein)	*M. pruriens* (Lucknow) (g 100 g^{-1} protein)	FAO/WHO (1973) requirement pattern (g 100 g^{-1} protein)
Glutamic acid	13·1	13·5	6·20	—
Aspartic acid	12·2	11·9	14·2	—
Serine	2·94	3·10	3·03	—
Threonine	4·00	3·56	3·44	4·0
Proline	9·36	7·34	9·51	—
Alanine	5·29	4·61	5·25	—
Glycine	4·44	3·13	7·20	
Valine	5·70	5·77	9·94	5·0
Cystine	Trace	Trace	Trace	3·5
Methionine	Trace	Trace	Trace	
Isoleucine	14·3	4·60	7·40	4·0
Leucine		7·76		7·0
Tyrosine	5·04	5·52	5·35	6·0
Phenylalanine	5·90	5·37	6·35	
Lysine	5·81	5·68	5·24	5·5
Histidine	2·62	2·96	3·42	
Tryptophan	ND	ND	ND	1·0
Arginine	7·04	6·42	4·35	

ND — Not Detected.

Table 4. Data on Antinutritional Factors

Component		*M. pruriens* (Begur)	*M. pruriens* (Silent Valley)	*M. pruriens* (Lucknow)
Total free phenols (g 100 g^{-1})				
Seed coats		11·8 ± 0·07°	15·2 ± 0·09°**	7·60 ± 0·04°
Seed kernel		7·42 ± 0·02°	7·36 ± 0·03°	5·14 ± 2·72°
Tannins (g 100 g^{-1})				
Seed coats		0·48 ± 0·02°	0·44 ± 0·03°	0·14 ± 0·02°
Seed kernel		0·02 ± 0·002°	0·06 ± 0·003°	0·01 ± 0·001°
L-DOPA (g 100 g^{-1})		6·97 ± 0·04°	8·18 ± 0·03°	9·16 ± 0·17°**
Phytohaemagglutinating activity[a]				
Name of the protein fraction	Erythrocytes from the human blood group			
Albumins	A	−	−	−
Albumins	B	−	−	−
Albumins	O	−	−	+
Globulins	A	+	+	+
Globulins	B	+	+	+
Globulins	O	+	+	+
Half inhibition of trypsin/mg protein[a]		51·1 units	50·1 units	46·3 units
Protein content of the extract used for trypsin inhibitor assay (mg/ml)		16·4	16·2	15·4

° Denotes mean of triplicate determinations expressed in percentage on dry weight basis.
[a] Values of two independent experiments.
\+ Some clumping, pellet disperses partially.
− No clumping, pellet disperses easily.
± Denotes the standard error.
** Denotes the significant difference at $P = 0·01$.

in the present study are distinctly deficient in sulphoamino acids and threonine when compared with the WHO requirement pattern (FAO/WHO, 1973). Similarly the seed proteins of Begur germplasm are markedly deficient only in methionine and cystine. The seed proteins of Lucknow germplasm are also found to be deficient in isoleucine + leucine when compared with the WHO requirement pattern. The levels of all the other essential amino acids are comparable with the WHO requirement pattern. When compared with the earlier data on profiles of amino acids of seed proteins of other *Mucuna* species/germplasms (Janardhanan & Lakshmanan, 1985; Afolabi *et al.*, 1985; Rajaram & Janardhanan, 1991; Arulmozhi, 1990), the essential amino acids, proline, valine and histidine, are found to be relatively higher in all three germplasms of *M. pruriens* investigated in the present study.

The data on mineral analysis reveal (Table 1) that the three investigated germplasm seed materials do not show significant differences in the contents of Zn, Mn and Cu, whereas the mineral profiles of the three germplasms of *M. pruriens* of the present study show much variation compared with the other related species such as *M. utilis* (Janardhanan & Lakshmanan, 1985; Ravindran & Ravindran, 1988), *M. solanei* (Afolabi *et al.*, 1985), and *M. gigantea* (Rajaram & Janardhanan, 1991). The minerals K, Mg and P in all the three germplasms and Fe of the Begur germplasm investigated in the present study occur in adequate concentrations when compared with RDA values (NRC/NAS, 1980).

The data on antinutritional factors (Table 4) reveal that the tannin content does not show significant variation among the three germplasms of *M. pruriens* investigated and is negligible when compared with the other *Mucuna* species reported earlier (Janardhanan & Lakshmanan, 1985; Rajaram & Janardhanan, 1991; Rajaram, 1990; Arulmozhi, 1990). A significant difference is observed in the occurrence of total free phenols in the seed coats among the three germplasms. In general, seed coats contain more free phenols compared with the kernels. Among the three germplasms, the Lucknow sample has a relatively lower phenolic content in both seed coats and kernel. The content of L-DOPA in *M. pruriens* (Begur) is slightly lower than that of the other two germplasms. The Lucknow germplasm of *M. pruriens* has the lowest trypsin inhibitor activity and this is similar to that of *M. utilis* (Janardhanan & Lakshmanan, 1985). Without any specificity, the globulin proteins alone weakly agglutinated the erythrocytes from the human ABO system as in *M. utilis* (Janardhanan & Lakshmanan, 1985) and *M. monosperma* (Arulmozhi, 1990). Besides this, the albumin proteins of Lucknow germplasm alone weakly agglutinated (specifically) the erythrocytes from the human 'O' blood group as in *M. flagellipes* (Mbadiwe & Agogbua, 1978) in which the globulin fraction has been found to agglutinate the erythrocytes from 'B' blood group specifically.

This study reveals that the chemical composition of all the germplasm seed material of *M. pruriens* seems to be similar to that of the other *Mucuna* species/germplasms reported earlier. Since most of the antinutritional factors, except L-DOPA reported in the present study are heat-labile, their harmful effects can be eliminated by a cooking process. Repeated soaking and boiling of seeds of *M. utilis* result in considerable reduction in content of L-DOPA (See Rajaram & Janardhanan, 1991). Since the Silent Valley germplasm exhibits robust growth with high yield and seems to be resistant to pests and pathogens, this particular germplasm can be further exploited to improve the yield-based parameters and develop resistance in the common pulses in future.

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