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Biochemical status of moth bean in various genotypes

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Abstract
A study was conducted to evaluate the Biochemical status of Moth bean in selected genotypes at Agricultural College & Research Institute, Vazhavachanur of Tamil Nadu, India during the period from October 2020 to September 2022. In the present study, fourteen different moth bean genotypes cultivated in several states of India and genotype, DHMB-26 cultivated in Karnataka and also in Maharashtra were included as the test pulses samples in completely randomized design with three replications was tried. Screening and evaluation of protein content in 14 moth bean genotypes were carried out to identify protein rich genotypes. Biochemical studies primarily based on five different traits including contents of albumin (Alb), globulin (Glo), prolamin (Pro), glutelin (Glu), Total protein and Crude protein content were carried out. In addition to that, Total carbohydrate content, Ascorbic acid content, Total phenols, Tannin and phytic acid content along with moisture content were determined. Results showed that the relative contribution of Albumin as 1.74 to 4.135 g/100g, globulin as 14.06 to 20.31 g/100g, prolamin as 0.78 to 2.96 g/100g and glutelin as 0.35 to 1.53 g/100g in Mothbean [FW]. Results revealed a considerable variation also in Total soluble protein contents among fourteen moth bean genotypes ranged from 20.16 to 26.83 g/100g. Total protein contents were higher in CZM-99 and MBS-0605 of Moth bean. The result on status of protein in Moth bean showed that MBS-0605 had the highest Albumin content. CZM-99 exhibited the highest globulin content. The lowest prolamin content was found in RMO 56, whereas the highest content of glutelin was found in TMV-1. The overall results of this study revealed that CZM-99, MBS-0605 and RMO 40 were considered as Top three genotypes suitable for Tamil Nadu state farmers and consumers. The above promising genotypes shall be included in varietal development programme by the breeders in the years ahead.

Keywords: moth bean, biochemical status, protein, ascorbic acid, tannin, total phenol

Introduction
Legumes constitute the main staple foods in many developing countries by providing significant quantities of energy, protein, fibre and selected micronutrients to the animal and human diet. Pulses, a sub-group of legumes, are plant species members of the Pea family that produce edible seeds that are used for human and animal consumption. Only legumes harvested for dry grain are classified as pulses. Significant levels of antioxidants are available in legumes and legume-based products. *Vigna aconitifolia* is generally known as moth bean, mat bean, Matki, Turkish gram (Sushmita Singh and Imtiyaz Ansari, 2018) [16]. Moth bean is underutilized short-season, summer-cultivated legumes. Moth bean is considered as more important because of its drought and heat tolerance. Moth bean belongs to the family Fabaceae, commonly grown in arid and semi-arid regions of India. Moth bean can be cultivated in many different soil types. Reproduction of moth bean is done by seed propogation. Applications of fertilizer to moth bean are rare in India. It has the capacity to fight against soil erosion.
This crop is used as a source of food, feed, fodder, green manuring and green pasture. Green pods are delicious source of vegetables. Being a pulse, it is a cheap source of vegetable protein for balancing nutritional deficiency.
The pods, sprouts and protein rich seeds of this crop are consumed in India. Traditional and Modern utilization of Moth bean flour includes preparation of dal, papad, breads, biscuits, pasta, cakes etc., Utilization of pulses flour is an emerging trend to develop functional foods, depending on nutritional compositions.
Phytochemicals present in pulses and vegetables helps the body to balance between oxidants and antioxidants. Moth bean are good source of protein (19 to 26%) with selected essential amino acids. Moth bean grain/seed contain various protein fractions that include Albumin, globulin, prolamin and glutelin. Major protein fraction of Moth bean is globulin [Salt soluble]. Sprouted and cooked moth bean have decreased protein content.

Major composition of unprocessed moth bean seed are carbohydrate (Approx. 61.5%), protein (Approx. 21.5%) inclusive of major essential amino acids (Leucine-1.5%, Lysine-1.2% and Isoleucine-1.1%), fat (Approx. 1.6%), water (Approx. 9.7%) and fiber (Approx. 4.5%). Moth beans are high in dietary fibers. Moth bean is rich in minerals like Fe (Approx.70ppm), Zn (Approx.38ppm) and Mn (Approx.33ppm). Taking of moth bean in our daily diet regularly may improve human health and nutrition because of their high protein and mineral content. Phytic acid is a natural plant oxidant usually occurs as phytins and considered as Anti-nutritional factor. Phytic acid concentration vary widely in different moth bean seeds between 0.25mg/g and 1.25mg/g. Tannin concentration also vary among various genotypes. negligible amount of anti-nutritional factors has been found in moth bean in contrast with various other common legumes.

Common insect pests of Moth bean are Jassids, White flies, White grubs and pulse beetle. Common diseases associated with Moth bean are YMV & Bacterial Leaf Spot disease.

Hence, the present study was undertaken to assess the Biochemical status of various moth bean genotypes inclusive of Anti nutritional factors, Phytic acid content and Tannin content which are important for breeders, consumers and farmers.

Materials and Methods

Materials: This research was conducted at the Agricultural College & Research Institute, Vazhavachanur-606 753, Tiruvannamalai District, India during the period from October 2020 to September 2022. The Experimental site is geographically located at Altitude 58MSL, Latitude 12°04' N and Longitude 78°59' E in the Thandarampattu block of Tiruvannamalai district. The materials used in this research were fourteen different moth bean genotypes cultivated in several states of India and genotype, DHMB-26 cultivated in Karnataka and also in Maharashtra as the test pulses samples in completely randomized design with three replications was tried. 300g to 1kg sample of each moth bean genotype were procured from different states as sampling locations. Moth bean free from pest and disease were only considered for observation.

Methods

Extraction of Soluble Proteins

Osborne extraction method (1907) [10].

The varietal differences in Total protein contents are mostly contributed by quantitative variations of each fractional protein. Moth bean contains four types of proteins which can be isolated and characterized, mainly according to their solubility properties, using the Osborne extraction method with minor modifications. Moth bean seed storage proteins are grouped into four classes based on solubility properties like albumins (water soluble), globulins (salt soluble), prolamins (soluble in aqueous alcohol solutions), and glutelins (soluble in dilute acid or alkali) (Osborne, 1907) [10]. The fractional proteins are extracted sequentially.

Table 1: The Protein and used Solvent

Sl. No.	Protein name	Solvent used
1	Albumin	Double distilled water
2	Globulin	1M NaCl
3	Prolamin	70% ethanol
4	Glutelin	1% Lactic acid containing 1mmol/L Disodium EDTA. (Jiang *et al*., 2014)

Estimation of Protein by Biuret method (Gornall *et al.* 1949) [6].

Protein content of extracted concentrate from moth bean was determined spectrophotometric ally by Biuret method (*Gornall et al.* 1949) [6]. The amount of protein in sample was calculated from standard curve prepared simultaneously with bovine serum albumin (1-5mg/ml) as standard.

Estimation of Protein (Lowry *et al.* 1951) [9].

Protein content of extracted concentrate from moth bean was determined spectrophotometric ally by Lowry *et al.* (1951) [9]. The amount of protein in sample was calculated from standard curve prepared simultaneously with bovine serum albumin (40-200µg/ml) as standard.

Determination of Crude Protein content (Micro-Kjeldahl method): The crude protein content of the fresh weight samples of moth bean was determined as percent total nitrogen by the Micro-Kjeldahl digestion and distillation procedure. Protein percent was calculated by multiplying the percent nitrogen by the factor 6.25 (AOAC 1980) [1].

Determination of Moisture Content

The percentage of moisture was measured by the methods of Association of Official Analytical Chemists Society (AOAC 2000) [2].

Other Biochemical Estimations

Estimation of Total Carbohydrate by Phenol-sulphuric acid method (Dubois M *et al*, 1956) [5], Total Phenol content of the moth bean samples were estimated with gallic acid as a standard (10 to 50 µg) by Folin-Ciocalteau method (Bray and Thorpe, 1954). Tannin content of moth bean samples was estimated with tannic acid as a standard in alkaline condition by Folin Denis method as described by Sadasivam. S. and Manickam. A(1991) [14]. Phytic acid content in defatted moth bean samples was determined with phytate phosphorus salt as a standard by the method of Davies and Reid (1979) [4].

Statistical Analysis

The experiments, quantification of biochemical parameters were performed in triplicate, using three independent replicates of each moth bean genotype in a completely randomized design. All results were expressed as the mean value. The data obtained were subjected to statistical scrutiny (Steel and Torrie, 1960) [15]. Wherever, the treatment differences were significant, critical differences were worked out manually at five percent probability level.

Results and Discussion

Cultivation of pulse crop is very ancient. Moth bean is the neglected crop from the evolution point of view. Moth bean is the secondary choice of farmers. Rajasthan is the major moth bean cultivating state in India and RMO-40 is the commonly cultivated genotype. Maharashtra and Gujarat are the important states next to Rajasthan concerned with surface area. The moth bean fodder forms an important feed supplier for large and small ruminants in the desert regions of India. Moth bean is the most drought tolerant (can survive with little moisture) pulse crop grown majorly and commonly consumed by people in India. All pulses are legumes but all legumes are not pulses. Moth bean is considered as body building pulses for poor socio-economic groups. Protein malnutrition exists even in developing countries. Pulses are meant for protein.

Pulses or legume seeds or grain legumes are nutritionally 2.5 fold richer in proteins than cereal grains. Moth bean is grown for its protein rich seeds, dry seeds and sprouts. Moth bean is the basic source for easily digestible proteins with low flatulence. The cooking time for moth bean ranges from 15 min to 18 min. Soak solution consists of 0.8% citric acid may decrease the cooking time. Under cooked or partially cooked beans may be avoided. Sprouted seeds can be roasted with suitable spices. When moth bean consumed along with wheat, balanced essential amino acids may be received. Pearl millet and Moth bean combination can be recommended for Roti preparation. Black gram and Moth bean combination for vada preparation may decrease constipation. Trypsin inhibitors activity can be reduced maximum by cooking of sprouted seeds of Moth bean.

Table 2: Quantification of Storage Proteins in Moth bean [FW] by Biuret method

Sl. No.	Moth bean Genotype	Albumin Content [g/100g]	Globulin Content [g/100g]	Prolamin Content [g/100g]	Glutelin Content [g/100g]	Total Protein [g/100g]	Moisture content [g/100g]
1	CZM-99	3.081	20.312	2.188	1.250	26.831	10.00
2	MBS-0605	4.135	18.400	2.970	1.040	26.514	10.00
3	MBS-856-1	3.060	17.498	2.742	0.800	24.100	9.97
4	TMV-1	3.310	17.200	2.040	1.530	24.080	9.90
5	DHMB-26** Karnataka	3.200	16.800	1.979	1.510	23.489	9.89
6	Telungana local variety	3.125	17.76	1.666	0.417	22.968	9.93
7	DHMB-26* Maharashtra	2.083	17.708	1.736	1.388	22.915	10.00
8	MBS-722	3.150	16.909	1.327	0.663	22.049	9.96
9	DHMB-31	2.777	15.972	2.430	0.694	21.873	10.00
10	IC 36392	2.917	14.792	2.031	0.417	20.157	9.84
11	IC 39702	2.966	15.000	2.187	0.781	20.934	9.67
12	RMO 72	3.869	15.473	2.232	0.744	22.318	9.80
13	RMO 56	1.736	18.055	0.781	0.347	20.919	9.75
14	RMO 257	2.669	14.063	2.539	1.367	20.638	9.70
15	RMO 40	3.487	18.333	1.980	0.990	24.790	9.00
Statistical analysis	S.Em	0.105	0.154	0.062	0.040		0.072
	SEd	0.149	0.218	0.087	0.057		0.101
	CD1%	0.411	0.604	0.241	0.156		0.272
	CD5%	0.305	0.447	0.179	0.116		0.204

- Mean values of three replications
- Where, S.Em: Standard Error Mean, SEd: Standard Error Deviation, CD: Critical Difference.

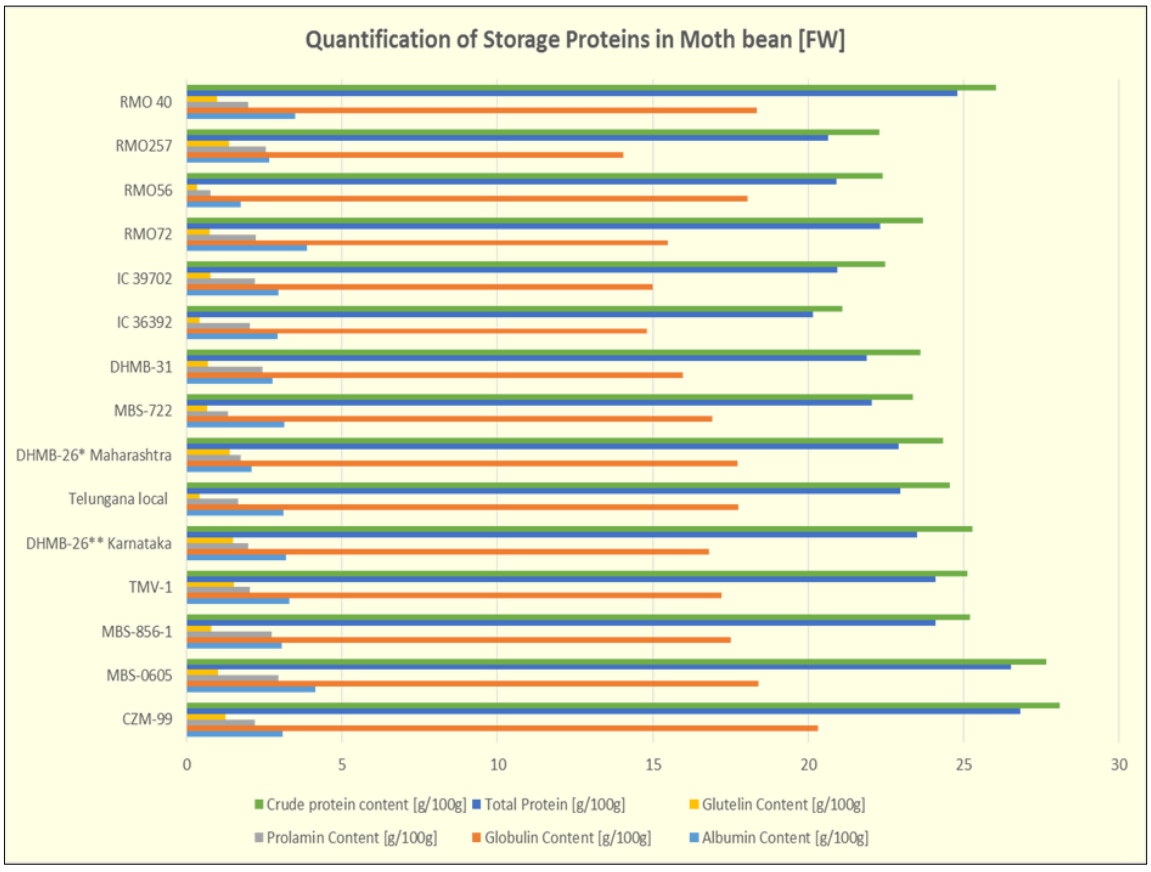

Fig 1: Quantification of Storage Proteins in Moth bean [FW]

Table 3: Biochemical Estimations in Moth bean [FW]

Sl. No.	Moth bean Genotype	Crude protein content [g/100g]	Ascorbic acid content [mg/100g]	Total Carbohydrate content [g/100g]	Total phenol content [mg/100g]	Tannin content [mg/100g]	Phytic acid content [mg/100g]
1	CZM-99	28.083	60.00	56.4	176	24.83	21
2	MBS-0605	27.647	56.66	56.9	278	38.83	26
3	MBS-856-1	25.208	54.10	59.0	170	24.17	77
4	TMV-1	25.125	53.56	59.2	270	38.16	16
5	DHMB-26** Karnataka	25.271	52.66	59.8	263	35.33	72
6	Telungana local variety	24.562	51.66	60.2	221	31.17	31
7	DHMB-26* Maharashtra	24.337	51.40	60.4	249	36.16	67
8	MBS-722	23.350	49.36	61.1	257	74.83	58
9	DHMB-31	23.594	48.33	61.5	242	35.00	35
10	IC 36392	21.100	48.40	63.0	228	32.16	63
11	IC 39702	22.475	48.53	62.4	236	33.16	53
12	RMO 72	23.675	49.30	61.0	194	27.67	39
13	RMO 56	22.400	48.73	62.5	182	26.00	48
14	RMO 257	22.291	48.63	62.8	188	26.66	43
15	RMO 40	26.041	55.30	58.5	200	14.33	11
Statistical analysis	S.Em	0.414	0.554	0.419	4.867	0.496	1.513
	SEd	0.585	0.783	0.592	6.882	0.702	2.140
	CD1%	1.616	2.107	1.594	18.529	1.889	5.760
	CD5%	1.198	1.578	1.194	13.87	1.415	4.312

- Mean values of three replications
- Where, S.Em: Standard Error Mean, SEd: Standard Error Deviation, CD: Critical Difference.

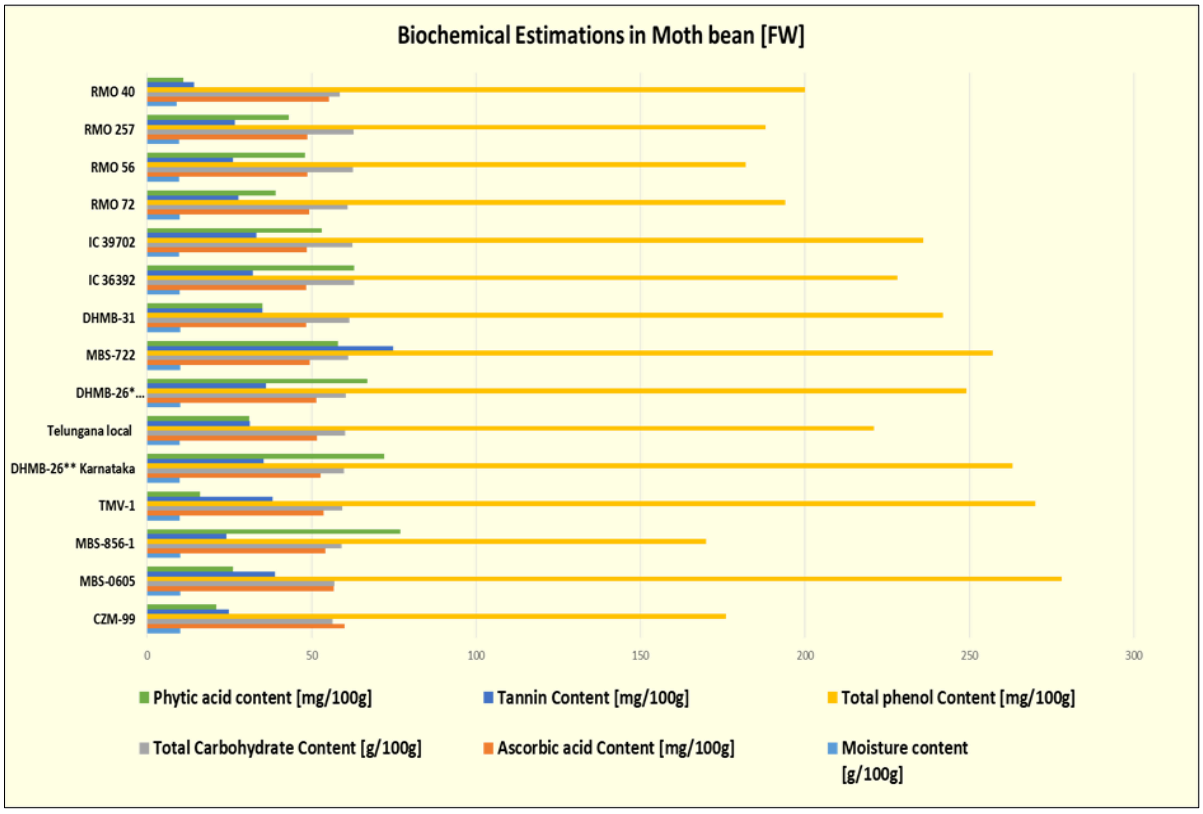

Fig 2: Biochemical Estimations in Moth bean [FW]

In the present study, significant differences were observed in all biochemical composition among the varieties ($p \leq 0.01$). Moisture content ranged between 9.0 and 10.0%. CZM-99, MBS-0605, DHMB-26 (Maharashtra) and DHMB-31 has recorded highest moisture content (10%) and least was recorded by RMO 40 (9%). Crude protein content varied from 21% (IC 36392) to 28% (CZM-99). The Total protein content among fourteen genotypes varied from 20.16% to 26.83%. Total protein contents were higher in CZM-99 (26.83%) and MBS-0605 (26.51%); Lower in IC 36392 (20.16%). Biochemical studies on four different traits comprising contents of albumin (Alb), globulin (Glo), prolamin (Pro), glutelin (Glu) were also reveals that the relative contribution of Albumin as 1.74 to 4.135 g/100g, globulin as 14.06 to 20.31 g/100g, prolamin as 0.78 to 2.96 g/100g and glutelin as 0.35 to 1.53 g/100g in Mothbean [FW]. The result on status of protein in Moth bean showed that MBS-0605 had the highest Albumin content (4.135%). CZM-99 exhibited the highest globulin content (20.31%). The lowest prolamin content was found in RMO 56(0.78%), whereas the highest content of

glutelin (1.53%) was found in TMV-1.

The Ascorbic acid content ranged from 48.33 mg/100g to 60 mg/100g. Highest was observed in CZM-99 (60 mg/100g) followed by MBS-0605 (56.66 mg/100g) and RMO 40 (55.3 mg/100g) and least was measured in DHMB-31(48.33 mg/100g). Ascorbic acid is mainly meant for Antioxidant and antiviral property.

Moth bean contains 56 to 63 percentage of carbohydrate. Carbohydrate comprises starch, non-starchy polysaccharides, oligosaccharides and tracer amount of few monosaccharide. In moth bean, starch is the main carbohydrate. In the present study, the total carbohydrate content varied from 56.4g/100g to 63g/100g. Highest carbohydrate content was observed in the genotype IC 36392 (63%) followed by RMO 257 (62.8%) and RMO 56 (62.5%) and the least content was observed in the genotype CZM-99 (56.4%). Pallavi Badami *et al*., 2019 [11] recorded slightly lower values i.e 55 to 60.5g/100g in other genotypes when compared to present study.

Total phenol content varied significantly among the varieties ($p \leq 0.01$). The total phenol content varied from 170 mg/100g to 278 mg/100g. MBS-0605 had highest total phenol content (278 mg/100g) followed by TMV-1 (270 mg/100g) and MBS-856-1 had least content of total phenol (170 mg/100g). In moth bean, phenolic compounds are majorly distributed in the seed coat region. General recommendation of phenolic compound consumption is minimum 1g/day through dietary sources because of their health promoting properties. Phenolic compounds acts as non-vitamin antioxidant as well as non-enzyme antioxidants for nullifying the harmful effects of free radicals (S.Pandarinathan and S. Geethanjali, 2023) [12]. Certain phenolic compounds are essential for human cell rejuvenation and face glow. Certain phenolic compounds are essential for prevention of constipation also.

Anti-nutritional factors like phytic acid are able to decrease palatability, protein digestibility and bioavailability of nutrients (Jain *et al*., 2009) [7]. Quantity of phytic acid can be decreased by germination of seed. Phytic acid content varied among seeds of various moth bean genotypes between 11mg/100g and 77mg/100g. Tannin belongs to phenolic compounds category. Tannin possess dual role as anti-nutritional, decrease the digestibility property of major primary metabolites and nutritional, since protects the gastrointestinal system. Tannin content was also varied among seeds of various moth bean genotypes i.e. between 14.33mg/100g and 38.83mg/100g. Negligible amount of anti-nutritional factors has been found in moth bean in contrast with various other common legumes.

Moth bean can be used for intercropping with coconut. Moth bean can be planted between rows of sunflower for weed control and forage crop production. (Pantipa Na chiangmai *et al*., 2012) [13].

Conclusion

Indians are the largest consumers of pulses in the world. Pulses are major part of human diet. Grain legumes like moth bean play a significant role in meeting the demands of dietary proteins by vegetarian population. Moth bean should not be consumed raw. Legumes are essential source of protein and carbohydrates. Consumption of legumes may reduce the risk of colon cancer and body weight. Moth bean is considered as body building pulses for poor socio-economic groups. Diets comprising of tubers (excluding potato), multiple legumes and cereals like wheat may be the best solution for undernourishment. Legumes can be combined with millets in daily intake to balance the essential amino acids. There was a significant variation in the nutritional profile of moth bean genotypes. Variation in levels of Biochemical traits in moth bean variety is due to their nature and also based on the genotypes. The overall results of this study revealed that CZM-99, MBS-0605 and RMO 40 were considered as Top three genotypes suitable for Tamil Nadu state farmers and consumers.

Future Scope

This study may provide detailed information about the content of selected biochemical constituents of Moth bean in various genotypes and would be very much useful for consumers. The outcome of the present research project envisages the promising moth bean entries having high protein values. These valuable findings would be helpful for further preparation of value added products by multination companies involved in the production of branded Health Mix and also would be utilised the Self Help Groups for value addition of their products so as to benefit the consumers and in turn, they could fetch higher net return. On the other hand, scientific point of view, Top three genotypes of Moth bean would be very much useful for pulse breeders for evolving improved moth bean variety possessing higher protein content coupled with other favourable attributes like drought tolerant, pests and disease tolerant with high yield capacity. In a nutshell, the outcomes of this project would be useful in future moth bean research and development.

Author contributions

S. Pandarinathan: Research proposal preparation, design, Sample collection, Biochemical analysis, writing, editing and review.

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Conflict of Interest

The author declare no conflict of Interest

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