

Deciphering morphological and molecular insights into mothbean genotypes

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Abstract

Mothbean is a drought and heat tolerant pulse crop grown in arid and semi-arid regions of India. The extent of genetic variability, correlation, path analysis for seed yield and nine yield attributes was studied in thirty-eight genotypes during the *kharif*, 2019 in randomized complete block design in four replications. The differences among all the genotypes were significant for all the traits. Days to flowering recorded high genotypic and phenotypic coefficient of variation followed by plant height and low magnitudes were reported for protein content, pod length and the number of seeds per pod. The broad sense heritability was high for days to flowering, days to maturity, the number of pods per plant, hundred seed weight, plant height, seed yield per plant, the number of branches per plant and the number of seeds per pod. A low genetic advance as per cent mean was recorded for protein content and pod length. The trait, seed yield per plant was significantly and positively correlated with the number of pods per plant at both genotypic (0.662) and phenotypic (0.618) levels. The path analysis revealed that the number of pods per plant, days to maturity, the number of seeds per pod and plant height exhibited a high direct effect on seed yield per plant. The microsatellites were used to analyse the polymorphisms among mothbean genotypes. The polymorphic information content value of the microsatellites ranged from 0.23 to 0.57, while the heterozygosity value ranged from 0.26 to 0.64. The cluster diagram revealed a similarity index value ranged from 0.08 to 1.00 and the genotypes were clustered in two main groups. Based on the results it could be concluded that microsatellites are efficient in discriminating among the mothbean genotypes and the study showed a narrow gene base of the genotypes.

Keywords: Mothbean, heritability, genetic advance, correlation and path analysis, molecular study, microsatellites.

INTRODUCTION

The genus *Vigna* is a leguminous group consisting of 104 described species distributed in tropical and subtropical regions of Africa, Asia, America, and Australia. Nine species are domesticated as food crops in Asia, Africa, and America. (Kaewwongwal *et al.*, 2015). Mothbean botanically *Vigna aconitifolia* (Jacq.) Marechal, a self-pollinated crop with 22 diploid chromosomes belongs to the family leguminosae and subfamily papilionaceae. In India, it is mainly grown in Rajasthan which contributes about 75% of the total area and production in the country.

Other important states for cultivation are Maharashtra, Gujarat, Jammu & Kashmir and Punjab (Anonymous, 2016). Kohakade *et al.* 2017^b reported mothbean has a maximum capacity to resist drought conditions among all the *kharif* pulses. The crop grows well between the 24-42°C temperatures but it can also survive at temperatures up to 40-45°C indicating an inducible nature of thermo-tolerance in mothbean (Tiwari *et al.*, 2017). The seed is used either whole or split as a pulse. It is an excellent source of easily digestible protein with low flatulence and

is consumed as 'dal', bean sprouts, noodles, green beans and boiled dry beans. Besides edible uses, mothbean is known as a soil binder. It is principally grown for its protein content rich seeds. Certain anti-nutritional factors like trypsin inhibitors, saponins, phytic acids *etc.*, are found in mothbean. The crop can be grown in various cropping systems like agri-horticultural, silvi-pasture, agro forestry, mixed cropping, inter cropping and sole cropping, which are common in the arid zone. Besides being a multipurpose crop, the productivity of mothbean is still low due to various biotic and abiotic factors. Maximization of yield is the major objective of any crop. For enhancement of yield the knowledge of various genetic variability parameters is required. Genotypic and phenotypic coefficient of variation and heritability coupled with genetic advance is of great importance for the selection and evaluation of germplasm, which is a prime requirement in crop improvement programmes.

Selection based on different components of variability, heritability and genetic advance had been reported for different yield attributing traits in mothbean by several researchers *viz.*, Patel *et al.* (2009), Yogeesh *et al.* (2012), Vir and Singh (2015), Yogeesh *et al.* (2016) Kumar *et al.* (2016) and Kohakade *et al.* (2017^a). Sahoo *et al.* (2019) studied genetic variation, heritability and genetic advance in fifty mothbean germplasm and reported significant variability in all genotypes for various traits. Molecular diversity analysis is a need for the breeder to analyse the genetic base of the available genotypes to design strategies for increasing the efficiency of various breeding programmes. Kaur *et al.* (2018) studied genetic diversity among twenty-three mungbean genotypes using forty Simple Sequence Repeats (SSR) primers of which ten primers showed a good polymorphic rate with 86.66 per cent. Therefore, the present study was conducted with the goal to decipher the polymorphism among mothbean genotypes using quantitative traits and microsatellites markers which will help the breeder to select superior genotypes for an effective breeding programme.

MATERIALS AND METHODS

The experimental material used for the study consisted of thirty-eight mothbean genotypes. The investigation was carried out in randomized complete block design using four replications during *kharif*, 2019. The experiment was laid out a single row of four meters with 45 cm x 10 cm spacing. All the recommended agronomic and plant protection measures were followed to raise the normal crop. The data were collected by taking mean values of five randomly selected plants from each accession for the characters *viz.*, plant height (cm), the number of branches per plant, the number of pods per plant, pod length (cm), the number of seeds per pod, 100-seed weight (g), protein content (%) and seed yield per plant (g) and plot basis for days to flowering and days to maturity. The data recorded for all the characters were subjected to analysis of variance with the formula suggested by Panse

and Sukhatme (1985). Analysis of variance permits the estimation of phenotypic, genotypic and environmental variance for various traits calculated as per Johnson *et al.* (1955) while, the genotypic coefficient of variation (GCV) and phenotypic coefficients of variation (PCV) were calculated as per Burton (1952) and categorized as low, moderate and high by Shivasubramanian and Menon (1973). Broad sense heritability and Genetic advance (GA) were calculated by using the formula proposed by Allard (1960) and categorized as demonstrated by Robinson (1966). The phenotypic and genotypic correlations were also estimated from which genotypic correlations were subjected to path coefficient analysis.

For assessment of molecular diversity twenty-four SSR primers were screened. Genomic DNA of all the genotypes was extracted using the CTAB extraction method and purity was checked by 0.8% agarose gel electrophoresis. The extracted DNA was amplified using microsatellites. The PCR mixture contained 1.5 µl 10X PCR buffer, 0.3 µl 10mM dNTPs, 0.1 µl 3U *Taq* DNA polymerase, 1.5 µl 5.0 pmoles/µl primer pair, 1.0 µl 50.0 ng/µl template DNA and 10.6 µl nuclease free water. The amplification was programmed for initial denaturation at 94°C for four minutes followed by 35 cycles of 94°C denaturations for one minute, annealing of primer at annealing temperature for one minute and extension at 72°C for 45 seconds. The cycling programme was terminated by a final extension step at 72°C for ten minutes. The amplified products along with the standard 100 bp DNA marker were separated by electrophoresis and visualized under a gel documentation system. The SSR reproducible bands of DNA fragments were scored as present (1) or absent (0) and was compared with each other and a data matrix was prepared. The data matrix was read by NTSYS-pc version 2.20 (Numerical Taxonomy and Multivariate Analysis System for Personal Computers, Exeter Software) developed by Rohlf (2005) and analysed by SIMQUAL (Similarity for quantitative data) program with Jaccard's similarity coefficient (Jaccard, 1908). The resulted similarity matrix was entered into the SAHN clustering program, a tree matrix was produced and a dendrogram constructed using UPGMA.

RESULTS AND DISCUSSION

The analysis of variance for various traits presented in **Table 1** depicted a significant difference in mean squares and the presence of appreciable inherent genetic variability among all the genotypes for all the traits provides the chance for further improvement of these traits. The magnitude of phenotypic variance for all the yield component was higher than the genotypic variance. Less difference between the values of genotypic and phenotypic coefficient of variation were reported for all the traits which indicate the less environmental influence and more contribution of the genetic factors in phenotypic expression. The result was in accord with the report of Patel *et al.* (2009) and Sahoo *et al.* (2019). GCV and PCV

Table 1. Analysis of variance (mean sum of squares) for different characters in mothbean

Source of variation	df	Mean sum of squares									
		DF	DM	PH	NB	NP	PL	NS	HSW	PC	SY
Replication	3	0.90	2.00	1.99	0.21	4.20	0.08	0.17	0.03	0.18	0.14
Treatment	37	1279.67**	723.32**	508.96**	3.79**	167.04**	0.15**	0.74**	0.77**	0.23**	4.57**
Error	111	1.25	1.28	8.46	0.25	1.60	0.03	0.07	0.01	0.09	0.11

*, ** Significant at 0.05 and 0.01 levels of probability, respectively.

DF = Days to 50% flowering, DM = Days to maturity, PH = Plant height, NB = Number of branches per plant, NP = Number of pods per plant, PL = Pod length, NS = Number of seeds per plant, HSW = 100-seed weight, PC = Protein content, SY = Seed yield per plant.

were higher for days to flowering followed by plant height. Selection based on traits possessing high GCV and PCV can lead to successful isolation of advantageous genotypes, as the selection is proportional to variability present in the genotypes. This result was in concordance with Patel *et al.* (2009), Yogeesh *et al.* (2012), Vir and Singh (2015), Yogeesh *et al.* (2016), Kumar *et al.* (2016) and Kohakade *et al.* (2017^a). Whereas, moderate estimates were recorded for the number of branches per plant, the number of pods per plant, 100-seed weight, seed yield per plant and days to maturity which signifies the improvement in these traits could be achieved only up to a reasonable extent. While GCV and PCV values were low for protein content, pod length and the number of seeds per pod indicating less range of variation for traits. The relative amount of variation which is heritable or not can be assessed through heritability. Broad sense heritability was high for days to flowering, days to maturity, the number of pods per plant, 100-seed weight, plant height, seed yield per plant, the number of branches per plant and the number of seeds per pod. Heritability was moderate for pod length. The lowest heritability for protein content. Genetic advance provides information about the expected genetic gain for a particular trait. It depends upon the phenotypic variability in population, the heritability of the character and the selection intensity. High genetic advance as per cent of mean was observed for days to flowering, plant height, the number of pods per plant, 100-seed weight, the number of branches per

plant, days to maturity and seed yield per plant whereas, low genetic advance as per cent mean was recorded for protein content and pod length. Moderate genetic advance coupled with high heritability was recorded for the number of seeds per pod suggesting the presence of both additive and non-additive genes. Similar results for high heritability coupled with high genetic advance were reported by Yogeesh *et al.* (2016), Kohakade *et al.* (2017^a) and Sahoo *et al.* (2019). The traits which exhibited high heritability coupled with high genetic advance signifies the role of additive genes and less environmental influence on the character and offering a better chance for a future breeding programme using appropriate breeding methods. Moderate heritability with moderate genetic advance indicates the influence of both additive and non-additive gene effects (Table 2).

The correlation analysis presented in Table 3 signifies the role of other characters in enhancing the seed yield. In general, genotypic correlation coefficients were higher than their respective phenotypic correlation coefficients, indicating the influence of genetic variance in the expression of character. Vir and Singh (2015), Kohakade *et al.*, (2017^a) and Sahoo *et al.*, (2018) reported a similar result. A significant and positive association was found between seed yield per plant and the number of pods per plant at both genotypic and phenotypic levels. Concurrent results for the association between the number of pods per plant with seed yield per plant was observed by

Table 2. Genetic parameters for seed yield and its contributing characters in mothbean

Characters	σ_g^2	σ_p^2	GCV (%)	PCV (%)	h_{bs}^2 (%)	GAM (%)
Days to flowering	319.60	320.85	30.06	30.12	99.60	61.82
Days to maturity	180.51	181.79	15.34	15.40	99.29	31.50
Plant height	125.12	133.58	20.18	20.85	93.66	40.23
Number of branches per plant	0.88	1.13	18.03	20.41	78.00	32.80
Number of pods per plant	41.35	42.96	18.14	18.49	96.26	36.68
Pod length	0.03	0.06	4.67	6.47	52.09	6.95
Number of seeds per pod	0.16	0.23	6.91	8.28	69.77	11.90
100-seed weight	0.18	0.19	17.91	18.35	95.19	36.00
Protein content	0.03	0.13	0.86	1.68	26.10	0.90
Seed yield per plant	1.11	1.22	15.06	15.79	91.00	29.60

Table 3. Genotypic and phenotypic correlation coefficient for different characters in mothbean

S.No.	Characters		DF	DM	PH	NB	NP	PL	NS	HSW	PC	SY
1	DF	G	1.000	0.918**	0.551**	0.733**	-0.115	-0.350**	-0.113	-0.092	-0.345**	-0.162*
		P	1.000	0.914**	0.532**	0.644**	-0.112	-0.251**	0.095	-0.090	-0.179*	-0.155
2	DM	G		1.000	0.533**	0.611**	-0.158	-0.320**	-0.073	-0.059	-0.217**	-0.094
		P		1.000	0.517**	0.537**	-0.154	-0.231**	-0.067	-0.055	-0.125	-0.087
3	PH	G			1.000	0.547**	0.110	-0.068	-0.216**	-0.060	-0.235**	0.143
		P			1.000	0.478**	0.104	-0.025	-0.188*	-0.054	-0.150	0.130
4	NB	G				1.000	0.065	-0.070	0.099	-0.036	-0.356**	-0.058
		P				1.000	0.060	-0.007	0.092	-0.057	-0.167*	-0.075
5	NP	G					1.000	0.012	-0.197*	-0.083	-0.155	0.662**
		P					1.000	-0.001	-0.176*	-0.087	0.078	0.618**
6	PL	G						1.000	0.475**	-0.221**	0.200**	0.030
		P						1.000	0.381**	-0.151	0.063	0.055
7	NS	G							1.000	-0.000	0.172*	0.094
		P							1.000	0.008	0.082	0.101
8	HSW	G								1.000	-0.055	-0.002
		P								1.000	-0.041	0.005
9	PC	G									1.000	-0.175*
		P									1.000	-0.065
10	SY	G										1.000
		P										

*, ** Significant at 0.05 and 0.01 levels of probability, respectively.

DF = Days to 50% flowering, DM = Days to maturity, PH = Plant height, NB = Number of branches per plant, NP = Number of pods per plant, PL = Pod length, NS = Number of seeds per plant, HSW = 100-seed weight, PC = Protein content, SY = Seed yield per plant

Patel *et al.* (2009) and Sahoo *et al.* (2018). This indicating the seed yield per plant is mutually associated with the other traits, which is useful for the plant breeder to select a desirable genotype from diverse plant genotypes. The direct and indirect contribution of various traits for improving seed yield is studied through correlation and mutual association among the different traits. However, a significant and negative association was recorded between seed yield per plant and days to flowering and protein content at the genotypic level. Therefore, improving these characters can inversely affect the other characters and the net yield can be affected. Path analysis (Fig.1) based on genotypic correlation revealed that the number of pods per plant, days to maturity, the number of seeds per pod and plant height exhibited a high direct effect on seed yield per plant in the desirable direction which confirms their strong association with seed yield per plant and thus suggesting that selection based on these characters will be beneficial. The residual value (0.591) indicated that other factors influencing the seed yield were not included in the present study and requires further study. The information gained from correlation and path analysis in mothbean will help the breeder to find out the functional yield component that can be appropriately used in developing diverse genotypes and direct selection can be based on the number of pods per plant which can increase the seed yield in mothbean. Kumar *et al.* (2016) and Sahoo *et al.* (2018) observed similar results for seed yield per plant.

Twenty-four SSR primers were screened for molecular characterization of thirty-eight different mothbean genotypes of which only twelve primers were found to be polymorphic among the genotypes. The list of SSR primers along with the melting temperature and citation is presented in Table 4. The results obtained by SSR primers indicating the presence of genetic diversity and the transferability of the SSR primers among *Vigna* species. A total of 27 alleles with a mean of 2.25 alleles per locus were detected from twelve primers among the genotypes. The amplified fragments ranged from 104 bp (with primer CEDC050) to 270 bp (with primer AGB16) indicating the presence of considerable amount of variation in the number of repeats between the different alleles. The PIC (Polymorphism Information Content) value of microsatellite locus, is a measure of the allelic diversity of the genotypes. The PIC values of these SSR markers varied from 0.23 (primer CEDGAG001) to 0.57 (primer VuUGM62) with an average of 0.36 and heterozygosity values was ranged from 0.26 to 0.64. Limited availability of polymorphism and low amount of heterozygosity is due to the highly self-pollinated nature of the crop which leads to the narrow genetic base of mothbean. However, some new length variants may occur due to natural crossing or residual heterozygosity. The total number of alleles at each locus, observed range of fragment size of alleles, PIC value and heterozygosity value of each locus are given in Table 5. The allele size of various locus ranged from 104 bp (CEDC050) to 270 bp (AGB16) while, the

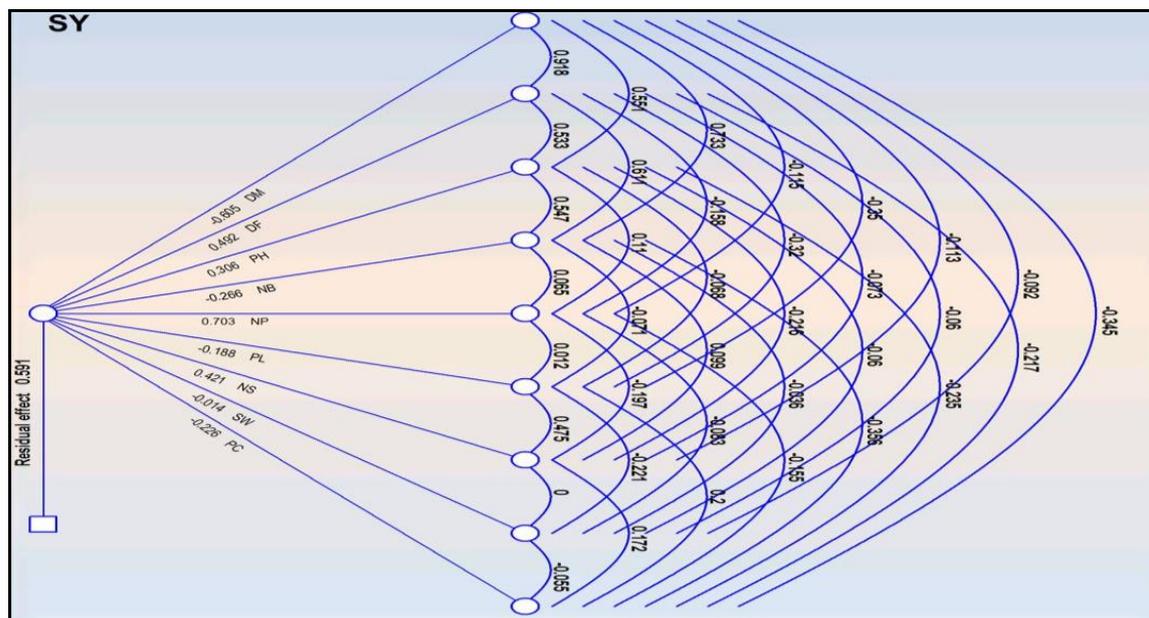


Fig. 1. Path diagram for seed yield per plant based on genotypic correlation

Table 4. Details of polymorphic SSR markers used in the study

S. No.	Primer	Sequence	Tm value (°C)	
1	VuUGM07	F TGTTTCCAACAGGATTAGCC	55.25	Gupta and Gopalakrishna, (2010)
		R AAGGCCAATAATTGCACAAG	52.20	
2	VuUGM33	F AAAGGTGGGGGATTATGAGG	57.30	
		R TGTCCAATCCTGATGGATGA	55.25	
3	VuUGM62	F TTGTCAAAAATCAGAAGCTC	51.15	
		R CTTGCATTCACTTCTACCAC	55.25	
4	CEDG037	F GGCTGAAGGTGATGACAGAAG	57.30	Dikshit <i>et al.</i> (2012)
		R GGCCTGGTTTTCTAAGTTGTTG	57.30	
5	CEDC050	F TCCCACTTCTCCATTACCTCCAC	62.43	
		R GAGATTATCTTCTGGCAGCAAGG	62.72	
6	CEDG149	F GGCTGAAGGTGATGACAGAAG	59.82	
		R GGCCTGGTTTTCTAAGTTGTTG	61.01	
7	CEDG100	F CCCATCAAGTAACTACATAACA	54.66	Sangiri <i>et al.</i> (2007)
		R ATGTGGGACTGGACAAATAAAA	54.66	
8	CEDG174	F GAGGGATCTCCAAAGTTCAACGG	62.43	
		R GAAGGCTCCGAAGTTGAAGGTTG	62.43	
9	VR040	F TGACAACATGGGAAGAAGAAGA	56.53	Sharma and Sirohi, (2018)
		R ACACCAACACAAAAGCAAACAC	56.53	
10	AGB16	F GCATCACAAAGGACTGAGAGC	59.82	Gioi <i>et al.</i> (2012)
		R CCCAAGCAAAGAGTCGATTT	55.25	
11	CEDGAG001	F CTCATCAGGGACATCCTCCC	61.40	Suman <i>et al.</i> (2017)
		R GATCGTGATCGATCCAACGGTC	62.12	
12	CEDAAG002	F GCAGCAACGCACAGTTTCATCG	62.12	
		R GCAAACTTTTCACCGGTACGACC	62.72	

PIC value and heterozygosity of markers were minimum for CEDGAG001 as 0.23 and 0.26 and maximum in VuUGM62 as 0.57 and 0.64, respectively. Out of twelve markers only three markers revealed three alleles and the rest markers amplified with two alleles.

The cluster analysis was performed using genetic similarity values and a dendrogram was generated showing genetic relationships among all mothbean genotypes. Similarity index values ranged from 0.08 to 1.00, which indicates the presence of the moderate amount of variation among all the genotypes. The highest similarity index value of 1.00 was found between IC-33742 and IC-399037,

IC-39418 and IC-399037 followed by 0.93 value between IC-28792 and IC-33742, IC-28792 and IC-39418. This suggested that these genotypes may have similar parentage in their ancestors. While, the least similarity index value of 0.08 was recorded between IC-9109 and IC-28792, IC-33741, IC-33742, IC-39642, IC-415127 and IC-415139 suggesting that they were divergent. The clustering pattern of the dendrogram generated by pooled SSR data of twelve markers constructed using UPGMA showed two major clusters A and B formed at a similarity coefficient of 0.28 (Fig. 2). The similarity coefficient was ranged from 0.28 to 0.98 which is indicative of the presence of moderate variation among the genotypes.

Table 5. Microsatellite marker analysis in mothbean genotypes

S.No.	Marker name	Allele size range (bp)	Number alleles amplified	PIC	Heterozygosity (H)
1	VuUGM07	169 - 223	2	0.37	0.50
2	VuUGM33	126 - 158	2	0.36	0.47
3	VuUGM62	175 - 214	3	0.57	0.64
4	CEDG037	113 - 121	2	0.31	0.38
5	CEDC050	104 - 120	2	0.35	0.45
6	CEDG149	186 - 225	2	0.33	0.41
7	CEDG100	181 - 237	2	0.36	0.48
8	CEDG174	176 - 214	3	0.36	0.41
9	VR040	163 - 199	2	0.35	0.45
10	AGB16	231 - 270	2	0.30	0.37
11	CEDGAG001	169 - 201	2	0.23	0.26
12	CEDGAG002	123 - 145	3	0.44	0.53

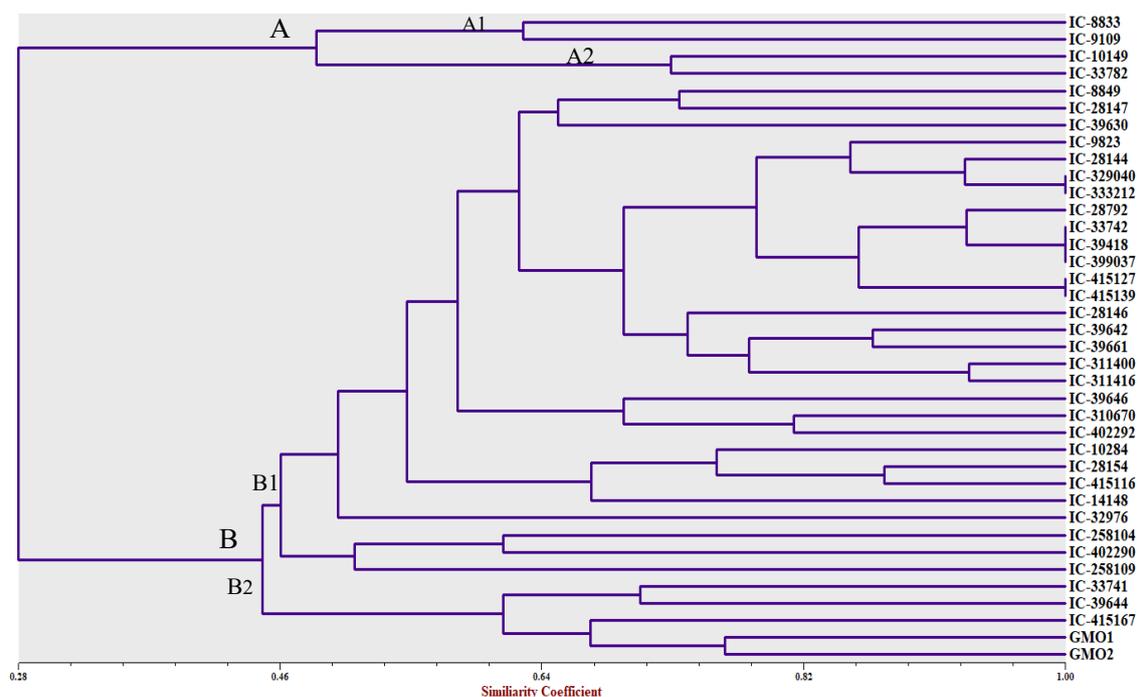


Fig. 2. Dendrogram showing clustering of thirty-eight mothbean genotypes constructed using UPGMA based on Jaccard's coefficient obtained from microsatellite marker analysis

Cluster A was divided into two sub clusters A1 and A2. Four genotypes were grouped in one major cluster 'A'. Sub-cluster A1 included mothbean genotypes viz., IC-8833 and IC-9109. Sub-cluster A2 comprises genotypes IC-10149 and IC-33782. Cluster B was also divided into two sub-clusters B1 and B2. Thirty-four genotypes were grouped in major cluster 'B'. Sub-cluster B1 consists of genotypes viz., IC-8849, IC-28147, IC-9823, IC-28144, IC-329040, IC-333212, IC-28792, IC-33742, IC-39418, IC-399037, IC-415127, IC-415139, IC-28146, IC-39642, IC-39661, IC-311400, IC-311416, IC-39646, IC-310670, IC-402292, IC-10284, IC-28154, IC-415116, IC-14148, IC-258104 and IC-402290; whereas genotypes IC-33741, IC-39644, IC-415167, GMO 1 and GMO 2 were different from those grouped into B2. Clustering pattern based on SSR marker data suggesting the presence of low genetic variation in the mothbean genotypes clustered in 'B' cluster. Three genotypes viz., IC-39630, IC-32976 and IC-258109 were grouped out from all the clusters thus indicating that they were genetically diverse. Gupta and Gopalakrishna (2009) successfully amplified the primers VuUGM07, VuUGM33 and VuUGM62 in cowpea genotypes. Dikshit *et al.* (2012) reported a good PIC value for the SSR markers CEDG037 and CEDG149 in mungbean and different *Vigna* species. Thus, the present study revealed that the SSR marker is more efficient in detecting genetic variability among all *Vigna* species and can be further used for crop improvement.

The overall results revealed that there was a significant difference among the genotypes for all plant growth and yield related traits which suggested a considerable amount of genetic variability. The traits viz., days to flowering, days to maturity, plant height, the number of branches per plant, the number of pods per plant, the number of seeds per pod and 100-seed weight could be utilized an appropriate direction while selecting the suitable genotypes for effective futuristic breeding programmes. The present study shows the efficiency of SSR markers in detecting the genetic variation among mothbean genotypes. In general, the study using microsatellite markers detected a moderate level of genetic variation in mothbean representing the narrow genetic base of the genotypes.

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