Genetic Diversity Analysis in Relation to Flowering Behavior in Mungbean (*Vigna radiata)*

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ABSTRACT

Mungbean (*Vigna radiata* L.) is an important food legume that is widely cultivated in tropical and subtropical regions of the world. Asynchrony in pod maturity is the major problem in mungbean cultivation faced by farmers and this is occurred due to flowering over an extended period. This may lead to low yield, time consumption and additional cost. Better understanding of flowering behavior is essential for successful breeding programs designed to develop plants with synchronous pod maturity. Therefore, this experiment was carried out to evaluate the flowering behavior of 20 mungbean accessions. Fourteen flowering related phenotypic characters were measured and their variations and correlations were analyzed. Results revealed that flowering duration which leads to the asynchronous pod maturity is significantly positively correlated with days to first flower, number of pods at maturity, plant height, number of leaves at first flowering, number of pod clusters per plant, number of primary branches, number of flowers and yield per plant at first harvest. Molecular genotyping was conducted with three Simple Sequence Repeat (SSR) markers and a dendrogram was drawn based on their polymorphism using Jaccard's similarity coefficient. It has separated 20 mungbean cultivars into seven clusters, showing suitable parents with maximum intra-cluster distance to be selected for gene mapping studies on synchrony of pod maturity.

KEYWORDS: Diversity, Mungbean, SSR Markers, *Vigna radiata*

INTRODUCTION

Mungbean (*Vigna radiata* L.) is an important legume crop which is widely cultivated in Asia. It is self-pollinating diploid crop (2n=2x=22) with a genome size of 579 Mb/1C (Sehrawat *et al.*, 2014). Seeds, young pods and fresh sprouts are consumed as human food while plant parts are used as forage for livestock and green manure. Mungbean serves as inexpensive vital source of vegetable protein (19.1-28.3%), mineral (0.18-0.21%) and vitamins (Singh *et al.*, 2013).

Mungbean is a tropical crop requiring 90–120 days from planting to maturity which can be grown under low moisture and low fertile soil conditions. Mungbean is one of the important grain legumes in the rain fed farming system in Dry and Intermediate Zones of Sri Lanka (Ranawake *et al.*, 2011). Mungbean is grown about 15,722 ha in Sri Lanka with an average yield of 1.16 mt/ha. Production level of mungbean in the year 2011 was approximately 10,838 mt which is far below the national requirement (Annual performance report, 2012).

Asynchrony in pod maturity is one of the major problems faced by famers in harvesting because of consuming time and adding cost for repeated hand picking of pods. There are three harvesting periods in mungbean and first harvest occurs at 70-75 days after sowing (DAS) giving 65% of pods. About 18% of the pods can be harvested from the second harvest at 75-80 DAS and about 17% of the pods can be harvested from the third harvest at 90-95 DAS (Mondal *et al.*, 2011). If the cultivar produces more than 90% of the total harvest from the first single harvest, the trait of the cultivar could be considered as ideally synchronous. Therefore, flowering behavior and morphological traits related to flowering of the cultivar need to be assessed to predict the pod maturity synchrony and final yield in order to use them in successful breeding programs.

Hence, this study was conducted aiming at understanding the flowering behavior of some Sri Lankan mungbean cultivars leading to synchrony of pod maturity. Also molecular analysis was conducted to assess the genetic diversity among cultivars to identify suitable parental lines for mapping of genes on synchrony of pod maturity.

MATERIALS AND METHODS

Experimental Location

This experiment was conducted in field plots and the laboratory of Department of Biotechnology, Wayamba University of Sri Lanka, Makandura, Gonawila (NWP) from December 2015 to May 2016.

Plant Material

A total of twenty mungbean accessions with genetically diverse background were used in this study including four traditional varieties. These mungbean accessions were obtained from the Plant Genetic Resource Center, Gannoruwa and from Field Crop Research and Development Institute, Mahailluppallama, Sri Lanka.

Experimental Design

The experiment was carried out in a randomized block design containing 15 plants from each accession. Seeds were sown in ridges with the spacing of 30×10 cm. The general management practices were carried out as recommended by the Department of Agriculture, Sri Lanka.

Data Recording

Field was monitored regularly and thirteen flowering related phenotypic characters were assessed from each plant. Flowering duration (FD), pod length (PL), days to first flowering (DFF), number of pods at 80% maturity (NPM), plant height at 1st flowering (PH), number of leaves at 1st flowering (NLFF), number of primary branches per plant (NPB), number of pod clusters per plant (NCPP), number of seeds per pod (NSPP), 100-seed weight (HSW), number of pods per cluster (NPPC), number of flowers per plant (NF) and yield per plant (YPP) were recorded. Yield per plant was measured by dry weight of seeds at the first harvest.

Genomic DNA Extraction

Extraction of DNA was done according to the general CTAB extraction method. A 0.125 g of young leaf sample from one week old seedling was collected and homogenized with 800 µL of pre warmed CTAB extraction buffer (1.4 M NaCl, 0.02 M EDTA, 0.1 M Tris HCl, 2% CTAB, 0.2% β-mercapto-ethanol). The contents were incubated in a water bath at 60 °C for 30 min followed by centrifugation at 3,500 rpm for 15 min and Phenol: chloroform: isoamyl alcohol (25:24:1 v/v) extraction was performed by adding with the supernatant and then the DNA was precipitated with cold isopropanol. The contents were incubated at -20 °C for 2 h. The tubes were centrifuged at 13000 rpm for 10 min to pellet out DNA. DNA pellet was washed with 70% ethanol and then the pellet was dissolved in 200 µL TE buffer.

DNA samples were incubated with 2 µL of RNase ONE (10 U/µL) at 37 °C for 1 h. Phenol: chloroform: isoamyl alcohol (25:24:1 v/v) extraction was performed and the DNA was precipitated with cold isopropanol as mentioned above followed by washing with 70% ethanol. The Tubes were air dried and the pellet was dissolved in 100 µL of 1/10th TE buffer.

Selection of Microsatellite Markers

The SSR markers namely, CEDAAG002 and CEDG037 which are near to the flowering time QTL, qDFL2.1 and qDFL6.1, respectively reported by Somta *et al.*, 2015 and another SSR marker CEDG044 reported by Bajracharya *et al.*, 2008 were used for the present study.

Polymerase Chain Reaction (PCR) Amplification

Amplification was carried out in 12 µL reaction mixture by using BioRad (My Cycler™). Reaction mixture contained 2 µL (15 ng/µL) of DNA, 1.2 µL of 10X PCR buffer, 1.2 µL of 2.5 mM dNTP, 1.1 µL of 20 pmol/µL primer and 0.2 µL of 5 U/µL Taq DNA polymerase. Amplification conditions were, initial denaturation at 95 °C for 5 minutes followed by 35 cycles of denaturation at 95 °C for 1 minute, annealing optimum temperature for 30 seconds and extension 72 °C for 1 minute and a final extension at 72 °C for 5 minutes. PCR amplified products were subjected to gel electrophoresis in a 3.5% agarose gel stained with ethidium bromide.

Statistical Analysis

Analysis of variance (ANOVA) was done to study the variations on phenotypic traits among the genotypes. Correlation between pairs of each trait was estimated by using the Pearson correlation coefficient. Cluster analysis was conducted with phenotypic DNA alleles using unweighted pair group method with arithmetic mean (UPGMA) (between group linkages) to investigate distance, similarity and relatedness of genotypes by SPSS 16.0 version.

RESULTS AND DISCUSSION

Field Data Analysis and Correlation between Traits

The analysis of variance showed significant differences among the genotypes for all the measured characters at $P<0.001$ level, except DFF ($P<0.05$) and NPB.

Most of the correlation coefficients between traits were significant (Table 1). Asynchrony in flowering is indicated by higher flowering duration. Flowering duration showed strong positive correlation ($r=0.824$) with number of primary branches and number of flowers per plant ($r=0.849$). It also showed significant positive relationship ($r=0.521$) with the yield at the first harvest. This information revealed that plants which have extended flowering period were bearing branches with

higher number of flowers. Days to first flowering is strongly positively correlated with number of pods at maturity (r=0.820), plant height (r=0.610), number of leaves at first flowering (r=0.814) and number of flowers (r =0.709). However hundred seed weight was negatively correlated (r=-0.576) with the days to first flowering significantly.

Similar relationships were reported in the study conducted on identifying QTL for seed weight and days to flowering in mungbean by Somta *et al.* (2015).

As indicated in Table 2, phenotypic data revealed that most of the accessions produced 80% of the total flowers within 10 days after first flowering and ceased the number of flowering after 15 days. Accession AC0099 produced 98% of the total flowers within 10DAF while AC3022, AC8416, AC8433, MI5 (a recommended variety) and AC0993 (a traditional variety) produced >90% of total flowers within 10 days.

Accession AC0219 produced only 50% of the total flowers within 10DAF. However, maximum yield was recorded by the accession, AC0219. This might be due to production of higher number of flowers.

According to the previous studies, genotypes which produced maximum opened flowers at 10th day and ceased flowering within 15th day are considered as synchrony in pod maturity (Mondal *et al.*, 2013 and Fakir *et al.*, 2011). The air temperature is the critical factor for the duration of the flowering and for pod filling of mungbean (Roknuzzaman *et al.*, 2007). Therefore, this study should be carried out in different seasons to confirm the results.

PCR Amplification and Genotyping

Quality of the DNA is crucial for the amplification of PCR and success of genotyping.

Our DNA extraction protocol was able to produce successful amplification and scorable polymorphic alleles from twenty mungbean accessions. All three SSR markers exhibited polymorphism among the genotypes (Figure 1) and number of alleles ranged from two to three.

Cluster Analysis

Unweighted pair group method with arithmetic mean dendrogram constructed based on Jaccard's similarity coefficient grouped 20 mungbean genotypes into 7 clusters with 100% similarity (Figure 2). This polymorphic allele based grouping indicated distantly related genotypes which would be useful to select parents for mapping studies on flowering gene and related traits.

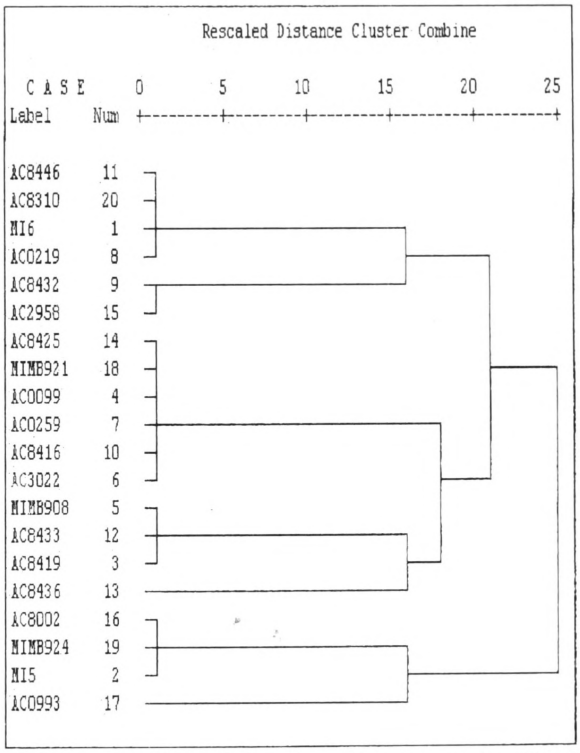

Figure 2. Unweighted pair group method with arithmetic mean (UPGMA) dendrogram drawn among twenty mungbean genotypes based on three SSR marker

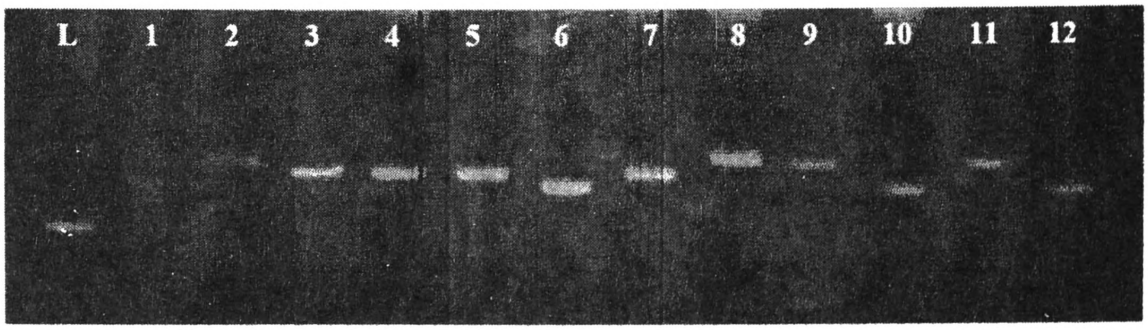

Figure 1. Polymerase Chain Reaction (PCR) amplification of twelve mungbean accessions using CEDG044 DNA marker. *Lane L- 100bp ladder, Lane 1- MI6, Lane 2- MI5, Lane 3- AC8419, Lane 4- AC8432, Lane 5- AC8436, Lane 6- AC8425, Lane 7- AC2958, Lane 8- AC8002, Lane 9- AC0993, Lane 10- MIMB921, Lane 11- MIMB924, Lane 12- AC8310*

Table 1.Correlation coefficient among traits

TRAIT	FD	PL	NSPP	DFF	NPM	PH	NLFF	NPPC	NPB	NCPP	HSW	NF	YPP
FD	1	-.085	.354	.531*	.566**	.444*	.571**	-.092	.824**	.751**	-.331	.849**	.521*
PL			.191	-.371	-.387	-.151	-.295	-.315	-.246	-.295	.348	-.339	-.168
NSPP				.472*	.397	.596**	.405	.344	.346	.472*	-.548*	.349	.513*
DFF					.820**	.610**	.814**	.421	.633**	.842**	-.576**	.709**	.679**
NPM						.680**	.844**	.616**	.698**	.895**	-.413	.825**	.804**
PH							.594**	.540*	.443	.656**	-.403	.669**	.943**
NLFF								.451*	.773**	.730**	-.303	.726**	.759**
NPPC									.142	.395	-.411	.257	.540*
NPB										.784**	-.235	.845**	.612**
NCPP											-.461*	.898**	.758**
HSW												-.403	-.232
NF													.755**
YPP													1

** Significant at 5% and ** Significant at 1% level. Flowering duration (FD), Pod length (PL), Number of seeds per pod (NSPP), Days to first flowering (DFF), Number of pods at 80% maturity (NPM), Plant height at 1st flowering (PH), Number of leaves at 1st flowering (NLFF), Number of pods per cluster (NPPC), Number of primary branches per plant (NPB), Number of pod clusters per plant (NCPP), 100-seed weight (HSW), Number of flowers per plant (NF) and Yield per plant at first harvest(YPP)*

Table 2. Flowering behavior and seed yield of mungbean accessions

Genotype	1-5 DAF	6-10 DAF	Cumulative flowers in 10DAF	Percentage of flowers till 10DAF	11-15 DAF	16-20 DAF	21-25 DAF	Open flowers /plant	Seed yield per plant at first harvest (g)
MI6	8.54	6.00	14.54	78.74	3.64	0.29	0.00	18.46	2.60
MI5	8.50	8.50	17.00	93.10	1.26	0.00	0.00	18.26	3.20
AC8419	10.94	10.78	21.72	87.68	3.05	0.00	0.00	24.77	2.05
AC0099	14.10	12.78	26.88	98.61	0.38	0.00	0.00	27.26	1.13
MIMB908	9.47	5.28	14.75	74.38	1.88	1.30	1.90	19.83	2.03
AC3022	11.20	7.09	18.29	91.84	1.48	0.15	0.00	19.92	2.62
AC0259	9.18	5.26	14.44	77.66	3.56	0.59	0.00	18.60	2.50
AC0219	9.80	7.94	17.74	50.56	6.90	5.31	5.14	35.08	4.66
AC8432	7.40	6.08	13.48	76.89	2.55	1.50	0.00	17.53	1.62
AC8416	9.55	8.45	18.00	92.07	1.55	0.00	0.00	19.55	2.72
AC8446	8.50	7.61	16.10	81.52	3.65	0.00	0.00	19.75	2.96
AC8433	6.97	7.18	14.15	95.41	0.68	0.00	0.00	14.83	1.91
AC8436	9.24	9.95	19.19	84.11	3.63	0.00	0.00	22.81	3.98
AC8425	8.99	9.24	18.23	83.77	3.53	0.00	0.00	21.76	2.02
*AC2958	14.53	13.76	28.29	68.54	8.14	3.30	1.55	41.27	3.26
*AC8002	8.07	9.62	17.68	71.86	6.74	0.18	0.00	24.60	3.99
*AC0993	8.81	8.27	17.08	93.87	1.12	0.00	0.00	18.20	1.75
MIMB921	9.13	10.20	19.33	82.05	4.23	0.00	0.00	23.56	4.55
MIMB924	8.87	7.09	15.95	85.53	2.70	0.00	0.00	18.65	2.62
*AC8310	7.23	8.05	15.28	81.36	3.50	0.00	0.00	18.78	2.32

*DAF-Days after first flowering * Traditional varieties*

CONCLUSIONS

Analysis of variance revealed significant variations among mungbean accessions for most of the observed characters with P<0.001. Mungbean accession AC0099 showed the least flowering duration while AC0219 showed longest flowering duration. Although shorter flowering duration was favorable for synchronous pod maturity, it has given a low yield in this study. Successful PCR amplification was found with three SSR makers, indicating polymorphism among 20 accessions, thereby indicating distantly related accessions to be selected as parents in gene mapping studies.

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