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Short Communication

Genetic divergence studies in mungbean (*Vigna radiata* L. Wilczek) using morpho-physio and molecular markers to identify drought tolerant genotypes

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Abstract

Sixty mungbean genotypes were evaluated for genetic divergence under limited moisture condition during summer season. Based on 19 phenotypic markers, hierarchical cluster analysis identified nine clusters containing one to 15 genotypes. Principal factor analysis reduced the nineteen variables into six principal components explaining 74.66% of the total variability. Seed yield per plot, harvest index and number of seeds per pod loaded highly on the PC 1; photochemical efficiency and canopy temperature depression on PC 4 and membrane stability index, total chlorophyll content and 100 seed weight on PC 5. Genotypes MH 810, MH 721, MH 736, M 395, SML 668, Pusa 9972 were found promising with regard to yield and drought tolerance traits. Out of 31 SSR primers used, only 18 were polymorphic and amplified 74 alleles. Primer CEDG067 exhibited maximum PIC value (0.84). The NTSYS-pc UPGMA cluster analysis divided the sixty genotypes into nine distinct groups.

Key words: Genetic diversity, cluster analysis, drought, seed yield and SSR primers

Mungbean (*Vigna radiata* L. Wilczek) is grown as sole as well as inter and multiple crop in different crop seasons. The regions that grow mungbean traditionally most often face water scarcity at one or the other stages. Rainy season mungbean doesn't require irrigation generally, however, it often encounters water stress either due to long dry spell or early withdrawal of monsoons. Spring/summer crop requires assured irrigation due to high temperature during crop season. For yield improvement and yield stability under water stress conditions, development of drought tolerant varieties is the best option (Siddique et al. 2000). Therefore, one of the major objectives of mungbean improvement program is to develop cultivars with stable grain yield under water stress. Drought tolerance is a complex trait, involving interactions of many metabolic pathways. Exploitation of genetic variability in the germplasm for traits to be improved is considered to be critical for making further genetic improvement in desired traits. Therefore, the study was planned to work out genetic divergence in mungbean genotypes using phenotypic and molecular markers.

The experiment was carried out with 60 mungbean genotypes (Table 1) derived from different sources during summer season of 2013 at CCS Haryana Agricultural University, Hisar. The experiment was conducted in two sets (Set I-irrigated and Set II-with pre-sowing irrigation only) in RBD with three replications. Each genotype was having a plot size of 2rows x 2m and spacing 30 x 10 cm. Observations were recorded on five plants for flower retention, plant height, number of pods per plant, number of branches per plant, number of seeds per pod, pod weight per plant, 100-seed weight, photochemical efficiency, membrane stability index and total chlorophyll content while, days to flowering, days to first pod initiation, days to maturity, biomass, seed yield, harvest index, incidence of Mungbean Yellow Mosaic Virus (MYMV), canopy temperature depression and necrosis were

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Table 1. Cluster membership profile of mungbean genotypes based on phenotypic observations under limited irrigation and molecular markers

Cluster No.	Genotypes	No. of genotypes
	Based on phenotypic observations	
I	BDYR-1 (1), IPM 3-2 (12), IPM 06-5 (13), GP149 (15), ML 818 (21),MH 125 (23), MH 421 (24), MH 709 (29), MH 729 (30), EC – 581523 (54), EC – 30400 (49), EC 399223 (48), Pusa 105 (45), Pusa 9071 (43), IPM 02-03-3 (39)	15
II	BDYR-2 (2), HUM 7 (7), M 605 (18), M 839 (19), MH 98-1 (22), PM 99-3 (33), EC 393410 (51)	7
III	COGG 912 (3)	1
IV	EC 251552 (4), HC 682 (6), IC15276 (8), MH 521 (25), EC – 393407 (50), Pusa Vishal (35)	6
V	Ganga 8 (5), IPM 02-17 (10), KM 2328 (16), ML 776 (20), MH 909 (46), ML 1108 (47), MH 934 (52), MH 805 (57)	8
VI	IPM 02 -3 (9), IPM 02-19 (11), KM 2241 (14), MH 560 (27), PDM 96-262 (32), HUM-12 (34), IPM 205-4 (37), MH 421-1 (38), IPM 05-3-6 (40), IPM 06-LS-1 (42), IPM 409-4 (55)	11
VII	M 395 (17), SML 668 (36), Pusa 9972 (44)	3
VIII	MH 534 (26), MH 565 (28), MH 318 (31), IPM 05-3-21 (41), EC 470096 (53), IPM 205-7 (56)	6
IX	MH 810 (58), MH 721 (59), MH 736 (60)	3
	Based on molecular markers	
I	BDYR-1, KM2328, MH98-1, HC682, MH421, MH534, MH565, IPM05-3-21, Pusa9972, IC15276, IPM06-5, HUM-12, PDM96-262	13
II	M839, MH729, EC393407	3
III	EC251552, MH934, Pusa9071, IPM205-7, SML668, MH421-1, MH909, GP149, ML776, EC581523, MH810, IPM02-03-3	12
IV	BDYR-2, Ganga8, MH709, P. Vishal, IPM205-4, MH521, HUM7, IPM02-17, MH318, IPM3-2, M605	11
V	KM2241, IPM409-4, EC30400, ML1108	4
VI	COGG912, MH805, MH736	3
VII	MH560, IPM05-3-21, Pusa105, MH721	4
VIII	IPM02-3, M395, IPM05-3-6	3
IX	IPM02-19, ML818, MH125, EC393410, EC470096, PM99-3, EC399223	7

Figures in parenthesis denote genotype number assigned in figures

recorded on plot basis. For canopy temperature depression, infrared thermometer (IRT), model AG-42, Tele temp crop, Fullerton (CA), was used between 12:00 h to 14:00 h. Photochemical efficiency was measured by chlorophyll a florescence meter (Maxwell and Johnson, 2000). Total chlorophyll content was measured using Single Photon Avalanche Diode chlorophyll meter (Dwyer et al. 1991). To measure membrane thermo stability, method of Sullivan (1972) was followed. Hierarchical Cluster and Principal Factor analysis was done using SPSS software (Version 20). UPGMA with City Block distance was used for clustering the genotypes. Principal component method of factor extraction with Varimax rotation was used for extraction of factors.

For molecular analysis, a set of 31 SSR markers of mungbean and adzuki bean were used. Genomic DNA was isolated from the leaves by CTAB method (Murray and Thompson, 1980, Saghai-Maroof et al. 1984 and Xu et al. 1994). PCR was conducted in a reaction volume of 20 μl containing 2 μl of 1X PCR buffer, 100 μM dNTPs, 0.5 μl primer, 1.5 unit Taq DNA polymerase and 25 ng genomic DNA. PCR amplified DNA products were resolved by submerged horizontal electrophoresis in 2.5% (w/v) agarose gel. The molecular data 0/1 matrix was used to calculate similarity index, genetic distance using 'simqual' sub-program of software NTSYS-PC.

Hierarchical cluster analysis identified nine clusters (Table 1) containing one to 15 genotypes. Cluster I was having maximum number of genotypes (15) and cluster III the minimum (1). Maximum inter-cluster distance was observed between clusters IV and IX (471.21) followed by clusters VIII and IX (413.32) (Table 2). The crosses between the genotypes belonging to distantly located clusters are likely to produce better transgressive segregants. Hossain et al. (2010) characterized mungbean genotypes under water stress condition and recorded wide diversity among the genotypes for physio-morphological characters. Similarly, Katiyar and Dixit (2011), Abna et al. (2012), Shweta (2013) and Divyaramakrishnan and Savithramma (2014) assessed genetic divergence in mungbean genotypes and grouped them into well characterized clusters.

Table 2. Inter and Intra cluster distances in mungbean genotypes under limited irrigation

Cluster No.	I	II	III	IV	V	VI	VII	VIII	IX
I	74.395								
II	120.15	60.76							
III	228.232	252.08	0.00						
IV	217.10	176.58	370.96	70.68					
V	172.72	228.08	120.49	334.25	72.07				
VI	114.20	109.89	284.02	176.55	227.99	68.06			
VII	115.27	170.13	183.87	271.17	126.15	172.92	60.80		
VIII	165.21	147.49	337.90	127.42	285.19	115.10	212.23	58.51	
IX	303.15	376.28	213.24	471.21	187.58	360.28	245.93	413.32	46.81

The first six principal components (PC) having eigen values more than one cumulatively explained 74.66 % variability (Table 3). Factor loading of different characters clearly indicated high loading of seed yield (0.867), harvest index (0.749) and number of seeds (0.673) on the first PC and this can be regarded as seed yield factor. The second PC showed high loadings of days to flowering (–0.827), days to maturity (0.815), days to pod initiation (0.779), number of pods (0.639) and pod weight (0.700) and can be regarded as phenological and pod factor. MYMV (0.847), necrosis (-0.597), plant height (0.647), number of branches (0.612) and total biomass (0.841) had high loading on PC 3 while, PC 4 exhibited high loadings of photochemical efficiency (0.664) and canopy temperature depression (0.794). The PC 5 showed high loadings for membrane stability index (0.826), total chlorophyll content (0.636) and 100 seed weight (0.606). PC 4 and 5 can be construed as physiological factors. PC 6 exhibited high loading only for flower retention (0.751). High loadings of different traits in a PC indicated strong association among them. These parameters could be used as selection criteria in

Table 3. Total variance explained by different principal components

Principal component	Extraction sums of squared loadings (without selection)			Rotation sums of squared loadings (without selection)		
	Total	% of variance	Cumulative % variance	Total	% of variance	Cumulative % variance
1	4.917	24.583	24.583	4.005	20.026	20.026
2	4.035	20.176	44.759	3.178	15.888	35.914
3	2.081	10.407	55.166	2.216	11.082	46.996
4	1.649	8.245	63.411	2.184	10.919	57.915
5	1.202	6.009	69.419	1.722	8.612	66.527
6	1.048	5.241	74.660	1.627	8.133	74.660

breeding programmes aiming to improve yield coupled with drought tolerance.

Principal factor scores (PF scores) for all the genotypes were estimated in all the six PCs. Using these PF scores, all the genotypes were plotted for PC 1 and 4 and then for PC 1 and 5 which cumulatively accounted for the most important seed yield and physiological characters. The genotypes Ganga 8, IPM 06-5, IPM 02-03-3, MH 810, MH 721, MH 736, MH 534, MH 318, SML 668, EC 30400, EC 393407 and IPM 205-7 had higher yield and better drought tolerance. The two plots of PC 1 and PC 4 and PC 1 and PC 5 accounting for about 40 % variation, shows clear differentiation of genotypes according to their cluster membership denoted by different markers. The superior genotypes MH 810, MH 721, MH736, M 395, SML 668 and Pusa 9972 were also found to be members of the best performing clusters *i.e.* cluster VII and IX. Such confirmatory results were also obtained in green gram by Bisht et al. (1998). Tripathy et al. (2016) also screened a set of 292 mungbean germplasm accessions and identified eight for drought tolerance.

Genetic diversity was also assessed using 31 SSR primer pairs out of which 18 showed polymorphism revealing total 74 bands (Fig. 1 and

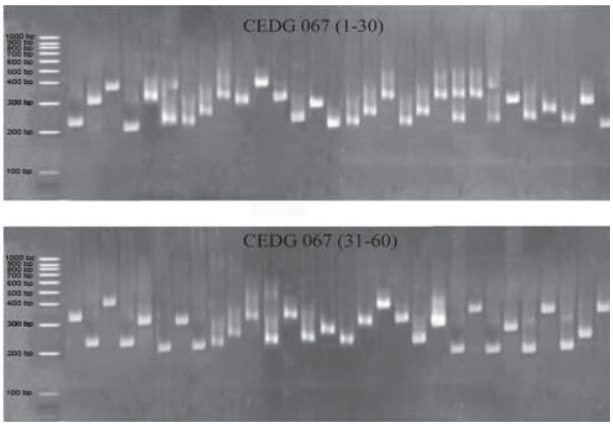

Fig. 1. Polymorphism in sixty genotypes of mungbean by using primer CEDG067

Table 4). The overall size of PCR amplified products ranged from 200 bp (CEDG180) to 400 bp (CEDG204 and CEDG024). Polymorphic information content (PIC) value ranged from 0.42 to 0.83 with an average of 0.69. The maximum polymorphism was shown by primer CEDG067 with highest PIC value (0.84. The SSR diversity data used to determine genetic relationship among the sixty genotypes formed nine

Table 4. List of SSR primers showing polymorphism in mungbean genotypes

S.No.	Polymorphic primers	Annealing temp. (°C)	No. of alleles	PIC
1	CEDG204	59	5	0.77
2	CEDG008	55	4	0.70
3	CEDG092	55	3	0.61
4	CEDG024	54	5	0.76
5	CEDG198	50	3	0.62
6	DMB-SSR182	54	4	0.70
7	DMB-SSR186	52	4	0.72
8	CEDG141	60	5	0.77
9	CEDG225	60	2	0.42
10	CEDG127	61	3	0.66
11	CEDG020	56	5	0.79
12	CEDG067	62	6	0.84
13	CEDG245	53	5	0.76
14	CEDG059	60	3	0.64
15	CEDC011	59	4	0.73
16	CEDG056	61	5	0.78
17	CEDG180	55	6	0.83
18	CEDG044	58	2	0.49
	Range	50-62	2-6	0.42-0.84
	Total		74	12.59
	Mean		4.11	0.69

groups. Genotypes per group varied from 3 to 13 Cluster I was the largest one comprising 13 genotypes followed by cluster III (12), cluster IV (11) and cluster IX (7). The similarity index varied from 0.78 to 0.99.

Genetic relationships as determined by NTSYS-PCA 3-dimensional scaling of 60 genotypes shown in Fig. 2. Two-dimensional PCA analysis showed that the lines were scattered in two major groups, which were further divided into different subgroups. Some of the genotypes overlapped in their positions. Somta et al. (2008) studied more than 200 SSRs to analyze 17 mungbean accessions, however, only 12 markers showed polymorphism. Similarly, Yuliasti and Reflinur (2015) analyzed genetic diversity among mungbean mutant lines using SSRs and identified PSJ31 as the most tolerant to drought.

Clustering pattern obtained through molecular and morphological analysis the genotypes grouped were not similar to each other. It may be because of the

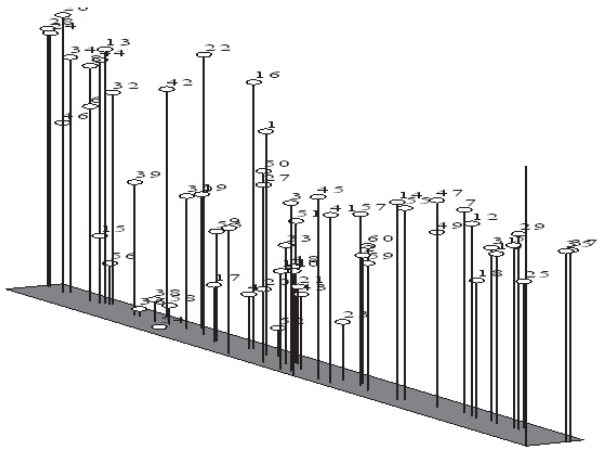

Fig. 2. Three dimensional PCA scaling 60 mungbean genotypes based on SSR

reason that the morphological traits were more affected by the environment and further the clustering was based only on a set of traits related to yield and drought, however, molecular analysis diversified the genotypes based on entire variability and there is no effect of environment. The second possible reason for this is that 31 SSR primers were able to scan only 74. This meagre screening of genome and information thus obtained is not sufficient to define actual genetic distinctness for genotypes. However, both types of analysis revealed wide range of divergence among these genotypes.

Authors' contribution

Conceptualization of research (RY, MSP); Designing of the experiments (RY, MSP); Contribution of experimental materials (RY); Execution of field/lab experiments and data collection (RY, S); Analysis of data and interpretation (S, RY, R); Preparation of manuscript (S, R, RY).

Declaration

The authors declare no conflict of interest.

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