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Genetic diversity analysis among Yellow Mosaic Virus (YMV) resistant and susceptible varieties in mungbean (*Vigna radiata* L.) using SSR markers

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Abstract **– Mungbean *(Vigna radiata* L Wilczek), is a self pollinated diploid (2n = 22) pulse crop, with a genome size of 515 Mb/1C, popularly known as greengram and is native to the Indian subcontinent. Mungbean is an excellent source of proteins (19-28%), mineral (0.18-0.21%) and vitamins which complements the staple rice diet in Asia and particularly in India. Among the various diseases limiting the mungbean productivity, Yellow Mosaic Virus YMV or Yellow Mosaic Disease (YMD) transmitted through the white fly, *Bemisia tabaci* Genn, is wide spread. In the present investigation, an attempt was made to study the genetic diversity analysis among 17 varieties of mungbean, collected from different geographical regions of India using Simple Sequence Repeat (SSR) markers. Molecular polymorphism was 58.8% with 29 SSR primers indicating the low level of genetic variation among the varieties. The polymorphic bands were scored visually as present (1) or absent (0) on a binary matrix. Genetic similarity between the varieties was estimated using Jaccords Coefficient of similarity. Dendrogram was performed using the Unweighted Pair Group Method with an Arithmetic mean (UPGMA) algorithm and the NTSYS software. The mungbean variety WGG-2 is appeared to be more divergent with 42.1% similarity, while high similarity of 81.7% was recorded between two susceptible varieties KM 14-34 and KM 14-61 and also in between one resistant variety (ESM 14-1413) one susceptible variety (KM 14-57). In one cluster all the resistant varieties (ESM 14-1312, KM14-43 and KM 14-62) and all the susceptible varieties (KM 14-34, Km-14-61 and MGG-295) were grouped together based on their phenotypic disease reaction for YMV resistance, except one resistant variety WGG-42 is grouped with all these susceptible varieties. The study clearly indicates that SSR marker profiles were best-suitable for assessing genetic relationships among Yellow Mosaic Virus (YMV) resistant and susceptible varieties.**

Keywords **– Mungbean, Genetic Diversity, SSR Markers, Cluster Analysis, Yellow Mosaic Virus (YMV).**

I. INTRODUCTION

Mungbean (*Vigna radiata* L. Wilczek), (2n = 22) is an economically important pulse crop, popularly known as greengram and is an excellent source of easily digestible proteins with low flatulence, which complements the staple rice diet in Asia and particularly in India. Mungbean is the third most important short-duration pulse crop after chickpea and pigeonpea. Mungbean requires special significance in crop intensification, diversification and conservation of natural resources and sustainability of production systems because of its short duration, photo insensitivity and dense crop canopy (Gunjeet *et al.* 2016). Extensive cultivation around the year leads to heavy disease and pest infestation, resulting in low productivity. Being a legume, it has the ability to fix atmospheric nitrogen (30–50 kg/ha) (Chadha, 2010). India is the leading mungbean cultivator, with up to 55% of the total world acreage and 45% of total production (Rishi, 2009, Singh N et al. 2013). However, the per capita availability of pulses has declined from 60.7g per day in 1951 to 41.9g per day in 2013 as against the FAO/WHO's recommendation of 80 g per day (Economic survey 2014-15) (Sai Rekha *et al*. 2016).

Among the various diseases limiting the mungbean productivity, Yellow Mosaic Virus (YMV) or Yellow Mosaic Disease (YMD) transmitted through the white fly, *Bemisia tabaci* Genn (Nariani 1960, Nene 1972), is wide spread. YMV also affects *V. mungo* (urdbean), *V. unguiculata* (cowpea) and *Glycine max* (soybean). In mungbean YMV causes irregular chlorotic yellow patches on the leaf lamina and with spread of the virus the entire leaf lamina turns yellow, affecting the photosynthetic efficiency. Yield loss due to YMV may reach up to 10 to 100% depending on the severity of the disease (Nene 1972, Chenulu *et al*. 1979, Marimuthu *et al.* 1981). The most efficient and eco-friendly approach to reduce YMD damage in mungbean is the use of host plant resistance. Keeping in view of the severity of the YMV it is highly essential to develop YMV resistant varieties in mungbean otherwise the crop may extinct from the region due to more competition from other commercial crops which may lead to severe scarcity of pulses in the state/country, hence, most essential.

Progress in MYMV-resistant mungbean breeding is hampered because the vector transmitting MYMV is neither sap nor seed transmissible. Hence, disease incidence depends on the vector population, which in turn depends on the environmental conditions and cannot be created as and when required and lack of uniform screening procedures (Haq *et al*. 2010). In such circumstances, indirect selection using molecular markers linked to resistance genes should be an effective approach as they enable marker-assisted selection (MAS) to overcome the inaccuracies in the field evaluation (Tanksley *et al*. 1989) (Xu *et al.* 2012). Novel genotypes can be characterized and identified for uses in the crop

breeding process by using molecular techniques (O' Neill *et al.* 2003). DNA finger printing is a routine method employed to study the extent of genetic diversity across a set of genotypes or cultivars and group them into specific categories (Gunjeet *et al*. 2016). An extent of genetic diversity across a set of genotypes or cultivars can be studied and group them into specific categories by using routine method of DNA fingerprinting technique (Gunjeet *et al.* 2016). Progress in mungbean is not significant, since it is a highly self- pollinated crop and lacks diverse gene pools (Sai Rekha *et al*. 2015). In order to enhance genetic potential, there must be a comprehensive understanding of the amount and pattern of genetic diversity that exists within and between the available cultivars. The genetic knowledge regarding the variation available in the germplasm helps to compare each germplasm and to choose diverse parents for hybridization. Simple sequence repeat (SSR) or microsatellite marker is one of the most useful genetic marker systems that uses PCR technique to identify differences in microsatellite repeat units. SSR markers are widely used because of its co-dominant, multi allelic, high polymorphism, reproducibility, abundant informativeness, convenience of assay by PCR and distribution throughout the genome, independent of environments, independent of tissue effects and providing more precise characterization of genotypes and measurement of genetic relationships than other markers (Litt & Luty, 1989; Souframanien & Gopalakrishna, 2004, Singh *et al*. 2015). The use of germplasm with distinct DNA profiles helps to generate breeding populations with broad genetic base. Keeping in view, the present investigation was carried out with an objective of identifying genetic diversity among yellow mosaic virus resistant and susceptible varieties using SSR markers.

II. Materials and Methods

Plant Material:

Seventeen mungbean varieties including 9 YMV resistant and 8 YMV susceptible varieties were used for genetic diversity analysis in the present study and details of plant material used in the present study were given in the Table 1.

DNA Isolation and PCR Analysis:

For isolation of Genomic DNA from leaf samples, we followed the standard protocol as per the procedure described by Lin *et al.* (2001) with few modifications. Final concentration of 30 ng/µl of genomic DNA was used for PCR (Eppendorf) amplification. The PCR conditions were performed at Initial denaturation of 94^0 C for 4 minutes, 35 cycles of denaturation at 94^0 C for 1 minute, annealing temperature (Table 2) for 1 min, extension at 72^0 C for 1 min, final extension at 72^0 C for 7 min, storage at 4ºC. The PCR amplified products were resolved on 4% agarose gel and Gel documentation was done using SYNGENE system.

Data Analysis:

Allele number was given and scored according to its presence or absence, based on difference in molecular weight. Only the clear and unambiguous bands were scored. Markers (29 SSR primers) were scored for the presence (1) and absence (0) of the corresponding band among the genotypes. Consequently, a data matrix comprising '1' and '0' was formed and subjected to further analysis. Further processing of data was done by carrying out sequential agglomerative hierarchical non-overlapping clustering (SAHN), on squared Euclidean distance matrix. Similarity matrix was done using Jaccard's coefficient (Supplementary Table 1) in which similarity matrices were utilized to construct the UPGMA (Unweighted Pair Group Method with Arithmetic average) dendrogram. Data analysis was done using NTSYs PC (Rohlf, 1998).

III. Results

The present investigation identifies the degree of genetic diversity based on DNA bands data in mungbean Yellow Mosaic Virus (YMV) resistant and susceptible varieties (Fig. 1). A total of 25 alleles were detected with 29 SSR primers (Table 2) with an average of 3 alleles per primer pair. The number of alleles ranged from 2 (CEDG 133) to 5 (CEDG 014). Molecular polymorphism was 58.8% with 29 SSR primers indicating the low level of genetic variation among the varieties (Supplementary Table 1). The polymorphic bands were scored visually as present (1) or absent (0) on a binary matrix. Genetic similarity between the varieties was estimated using Jaccords Coefficient of similarity. Dendrogram (Fig. 2) was performed using the Unweighted Pair Group Method with an Arithmetic mean (UPGMA) algorithm and the NTSYS software. The mungbean variety WGG-2 is appeared to be more divergent with 42.1% similarity, while high similarity of 81.7% was recorded between two susceptible varieties KM 14-34 and KM 14-61 and also in between one resistant variety (ESM 14-1413) one susceptible variety (KM 14-57) (Fig. 2). In one cluster all the resistant varieties (ESM 14-1312, KM14-43 and KM 14-62) and all the susceptible varieties (KM 14-34, KM-14-61 and MGG-295) were grouped together based on their phenotypic disease reaction for YMV resistance, except one resistant variety WGG-42 is grouped with all these susceptible varieties (Fig. 2). The study clearly indicates that SSR marker profiles were best-suitable for assessing genetic relationships among Yellow Mosaic Virus (YMV) resistant and susceptible varieties. The short genetic distances between KM 14-34 and KM14-61, ESM1413-13 and KM 14-57 permit to not recommend these species as donors of valuable agronomic traits for improving mungbean breeding program (Fig. 2). Based on similarity coefficients and cluster analysis mungbean varieties WGG-37, GP8 and WHH 2 (Fig. 2) were genetically more distant from other mungbean varieties and these varieties can be used for their desirable characteristics in breeding programs for mungbean improvement.

IV. Discussion

Genetic diversity is the basis for genetic improvement. Information on the levels and distribution of genetic diversity of any plant species may contribute to the knowledge of their evolutionary history, potential and is critical to their conservation and management (Schaal *et al*. 1991, Hamrick and Godt, 1996). Efficient use of conserved biodiversity requires information about the degree and distribution of genetic diversity. The genetic variation within and between species, generated by the process of mutation, sexual reproduction and selection ensure its capacity in evolutionary change and ecological adaptations. Unraveling genetic diversity is quintessential to plant breeding, as the development of new varieties depends on the existing diversity of parent genotypes (Vishalakshi *et al*. 2017).

DNA-based markers are effective and reliable tools for measuring genetic diversity in crop germplasm and studying evolutionary relationship. Molecular genetic techniques using DNA polymorphism is increasingly used to characterize and identify a novel germplasm for uses in the crop breeding process (Collard and Mackill 2008) (Gunjeet *et al*. 2016). Earlier, assessment of the genetic variation in mungbean has been carried out using different types of molecular markers including random amplified polymorphic DNA (RAPD) (Santalla *et al*. 1998, Lakhanpaul *et al*. 2000), amplified fragment length polymorphism (AFLP) (Bhat *et al*. 2005), inter-simple sequence repeat (ISSR) (Reddy *et al*. 2008) and simple sequence repeat (SSR) (Gwag *et al*. 2010). As compared to the earlier successful reports, the number of markers (i.e. 29 SSR markers) used for genetic diversity analysis in the present investigation is more. The mungbean variety WGG-2 is appeared to be more divergent with 42.1% similarity, while high similarity of 81.7% was recorded between two susceptible varieties KM 14-34 and KM 14-61 and also in between one resistant variety (ESM 14-1413) one susceptible variety (KM 14-57) (Fig. 2). In one cluster all the resistant varieties (ESM 14-1312, KM 14-43 and KM 14-62) and all the susceptible varieties (KM 14-34, KM-14-61 and MGG-295) were grouped together based on their phenotypic disease reaction for YMV resistance, except one resistant variety WGG-42 is grouped with all these susceptible varieties. The study clearly indicates that SSR marker profiles were best-suitable for assessing genetic relationships among Yellow Mosaic Virus (YMV) resistant and susceptible varieties.

The genetic similarities obtained from the present investigation can be used for selection parents to generate mapping populations and for breeding purposes. The narrow genetic base revealed in the present study emphasizes the need to exploit large germplasm collections having diverse morpho agronomic traits in cultivar improvement. Earlier, Palaniappan & Murugaiah (2012), Gupta *et al*. (2013) reported similar type of polymorphism by using SSR markers in black gram and Chattopadhyay *et al*. (2005) by using ISSR markers.

V. Conclusion

Characterization and assessment of genetic diversity among the mungbean genotypes have great significance in designing breeding strategies; both for qualitative and quantitative traits. SSR markers are useful in the assessment of mungbean diversity and the selection of a core collection to enhance the efficiency of genotype management for use in green gram breeding and conservation. In the present investigation, we have successfully assessed the levels of inter and intraspecific diversity relationships among different YMV resistant and susceptible varieties. Results obtained from the present investigation would be highly useful in mungbean breeding programs and may be used for further crop improvement using advance marker systems. We are currently in the process of hybridizing and analyzing genetic diversity of the crosses and predicting a completely YMV resistant mungbean variety.

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Fig. 1. PCR amplification of 17 mungbean varieties with SSR marker CEDG008

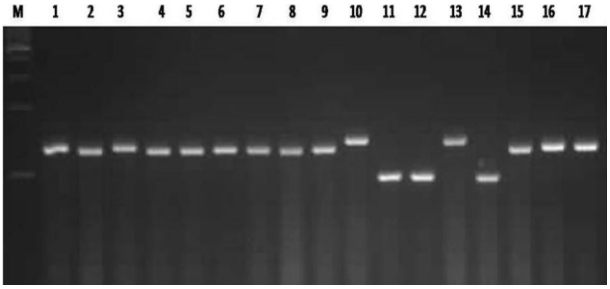

Fig. Legends: The label M is 100 bp ladder, the lane numbers written on the top of the gel corresponds to the list of mungbean varieties as given in table 1.

Fig. 2. Dendrogram showing genetic relationship among yellow Mosaic Virus (YMV) resistant and susceptible mungbean varieties.

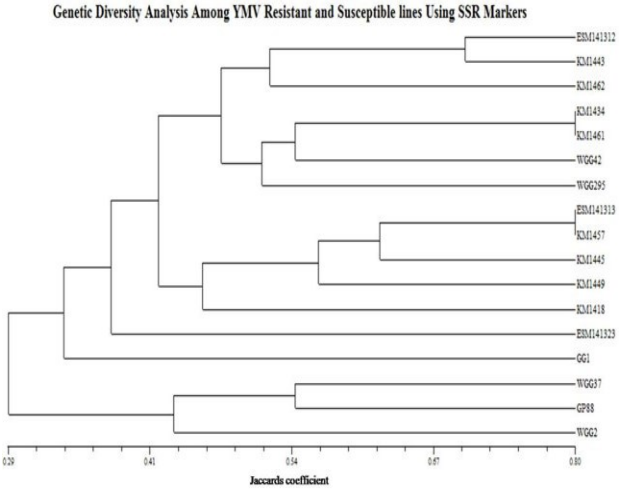

Table 1. List of 17 Mungbean Varieties used for molecular analysis

S. No.	Name of The Variety	YMV Resistance/Susceptible
1	ESM-14-1312	Resistance
2	ESM-14-1313	Resistance
3	ESM-14-1323	Susceptible
4	KM-14-18	Resistance
5	KM-14-34	Susceptible
6	KM-14-43	Resistance
7	KM-14-45	Resistance
8	KM-14-49	Resistance
9	KM-14-57	Susceptible
10	KM-14-61	Susceptible
11	KM-14-62	Resistance
12	GG-1-H	Susceptible
13	WGG-42	Resistance
14	WGG-37	Susceptible
15	WGG-2-H	Susceptible
16	MGG 295	Susceptible
17	GP-88	Resistance

Table 2. List of primers used and their Tm as standardized in the present study

S. No	Primer Name	Primer Sequence	Annealing temp in ° C
1	CEDG 204 F	CCTTGGTTGGAGCAGCAGC	60
2	CEDG 204 R	CACAGACACCCTCGCGATG	
3	CEDG 139 F	CAAACTTCCGATCGAAAGCGCTTG	65
4	CEDG 139 R	GTTTCTCCTCAATCTCAAGCTCCG	
5	CEDG 268 F	CATCTCCCTGAAACTTGTG	55
6	CEDG 268 R	GCTATCAATCGAGTGCAG	
7	CEDG 030 F	TGAGGGAATGGGAGAGAGGC	60
8	CEDG 030 R	TCCGCAGATAGAGGCTCACG	
9	CEDG 092 F	TCTTTTGGTTGTAGCAGGATGAAC	65
10	CEDG 092 R	TACAAGTGATATGCAACGGTTAGG	
11	CEDG 022 F	AGGAATGTGAGATTTG	55
12	CEDG 022 R	AATCGCTTCAAGGTCAAGCC	
13	CEDG 024 F	CATCTTCCTCACCTGCATTC	60
14	CEDG 024 R	TTTGGTGAAGATGACAGCCC	
15	CEDG 198 F	CAAGGAAGATGGAGAGAATC	60
16	CEDG 198 R	CCTTCTAAGAACAGTGACATG	
17	DMB-SSR182 F	TAGAGCCTTCTGGTTTTTCACA	60
18	DMB-SSR182 R	AGGAGGAGGATTTTGATGATGA	
19	DMB-SSR186 F	GAGAGAGAAGGAGAGGGAGA	60
20	DMB-SSR186 R	ATTCTTTCTCCACCACAATG	
21	CEDG 133 F	GCATACATAATGTGGTGAGATG	60
22	CEDG 133 R	GTCTCGTGCCTTTCACAC	
23	CEDG 141 F	CCAGGCATCCATGATGACC	65
24	CEDG 141 R	GAAGTTGTTGGTAATGGTTGCCTC	
25	CEDG 225 F	GAGGAAGTGTTGCAGCACC	60
26	CEDG 225 R	GTAGACTCTGCAGAGGGATG	
27	CEDG 284 F	GGTGCTAACGTTGGAAACTGAG	65
28	CEDG 284 R	CACTCCATTCTGAGGATCAATCC	
29	CEDG 077 F	ATCCCGTGACCCTTCTTCCT	60
30	CEDG 077 R	GCTCAAGCGAAAACCCAGCA	
31	CEDG 127 F	GGTTAGCATCTGAGCTTCTTCGTC	65
32	CEDG 127 R	CTCCTCACTTGGTCTGAAACTC	
33	CEDG 014 F	GCTTGCATCACCCATGATTC	60
34	CEDG 014 R	AAGTGATACGGTCTGGTTCC	
35	CEDG 020 F	TATCCATACCCAGCTCAAGG	60
36	CEDG 020 R	GCCATACCAAGAAAGAGG	
37	CEDG 067 F	AGACTAAGTTACTTGGGCAACCAG	65
38	CEDG 067 R	TGACGGCCCGGCTCTCC	
39	CEDG 059 F	AGAAAAGGGTGGCCTCGTTG	60
40	CEDG 059 R	GCAGGCATTTCCATCGCAG	
41	CEDG 112 F	GCAATATTCGCATTATTCATTCA	60
42	CEDG 112 R	GTGTTTCAAAGCACTATACTTAA	
43	CEDG 269 F	CTGTTACGGCACCTGGAAAG	65
44	CEDG 269 R	GCAGAGACACACCTTAACCTTG	
45	CEDC 011 F	GTCCGACTTTATGTGTGGAG	60
46	CEDC 011 R	TTTCTAGTTCCAGCCCCGAC	
47	CEDG 056 F	TTCCATCTATAGGGGAAGGGAG	65
48	CEDG 056 R	GCTATGATGGAAGAGGGCATGG	
49	CEDG 180 F	GGTATGGAGCAAAACAATC	55
50	CEDG 180 R	GTGCGTGAAGTTGTCTTATC	
51	CEDG 044 F	TCAGCAACCTTGCATTGCAG	60
52	CEDG 044 R	TTTCCCGTCACTCTTCTAGG	
53	CEDG 104 F	TATGGCCCGAGCAAACCTTG	60
54	CEDG 104 R	CCGTTCGGTCTTCGGTTGAA	
55	VrCS SSR3 F	GCAGACACAACCATAAATCC	60
56	VrCS SSR3 R	GGTCTTTGACGGCAATCTC	
57	CEDG 008 F	GCTCAAGCGAAAACCCAGCA	60
58	CEDG 008 R	CTCCTCACTTGGTCTGAAACTC	

Supplementary Table 1. Genetic similarity coefficients among 17 mungbean YMV resistant and susceptible resistant varieties

Sl. No	Name of the variety	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	ESM 14-1312	1.00																
2	ESM 14-1313	0.38	1.00															
3	ESM 14-1323	0.23	0.34	1.00														
4	KM 14-18	0.28	0.38	0.45	1.00													
5	KM 14-34	0.50	0.50	0.46	0.38	1.00												
6	KM 14-43	0.70	0.54	0.36	0.42	0.55	1.00											
7	KM 14-45	0.42	0.55	0.50	0.55	0.42	0.60	1.00										
8	KM 14-49	0.42	0.55	0.25	0.42	0.55	0.60	0.45	1.00									
9	KM 14-57	0.50	0.80	0.34	0.50	0.64	0.70	0.70	0.70	1.00								
10	KM 14-61	0.38	0.50	0.45	0.38	0.80	0.54	0.41	0.54	0.66	1.00							
11	KM 14-62	0.50	0.38	0.34	0.20	0.50	0.55	0.30	0.30	0.38	0.38	1.00						
12	GG-1	0.38	0.28	0.34	0.38	0.38	0.30	0.42	0.30	0.38	0.38	0.28	1.00					
13	WGG 42	0.42	0.55	0.50	0.22	0.55	0.60	0.34	0.34	0.42	0.55	0.55	0.22	1.00				
14	WGG 37	0.30	0.21	0.15	0.14	0.31	0.14	0.14	0.14	0.21	0.21	0.21	0.31	0.14	1.00			
15	WGG 2	0.55	0.31	0.15	0.14	0.31	0.34	0.23	0.23	0.31	0.21	0.42	0.21	0.21	0.45	1.00		
16	WGG 295	0.38	0.28	0.34	0.20	0.50	0.55	0.31	0.31	0.38	0.50	0.38	0.28	0.55	0.21	0.31	1.00	
17	GP 88	0.38	0.38	0.23	0.38	0.50	0.42	0.42	0.42	0.50	0.38	0.28	0.28	0.21	0.55	0.42	0.38	1.00