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Genetic Diversity Analysis and Validation of Microsatellite Markers Linked with Tolerance to Powdery Mildew Disease in Mungbean [*Vigna radiata* (L.) Wilczek]

B.S. Pavithra[1], Laxmipreeya Behera[1], K.C. Samal[1]

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ABSTRACT

Background: Mungbean [*Vigna radiata* (L.) Wilczek] is a self-pollinated diploid grain legume (2n=2x=22) crop and has a genome size of 560 Mb. The present study was concentrate to portray the nature and extent of genotypic variation exists among mungbean collections for a range of traits of potential agronomic and adaptive interests. Many diseases affect mungbean, causes the major constraint in the increasing production among which Powdery mildew disease caused by *Erisyphe polygoni* is economically significant because it reduces photosynthetic activity and physiological changes which results in 20-40 per cent reduction in yield. Some gene-specific marker were analysed and found associated with powdery mildew resistance in mungbean genotypes.

Methods: The present investigation was carried out to evaluate thirty-one mungbean genotypes (including four checks) collected from the different parts of India. The genotypes were sown in an incomplete augmented bock design along with four checks varieties. *viz*. 'Kamdev', 'OBBGG-52', 'IPM-02-14' and 'IPM-02-3'. All the mungbean genotypes were evaluated for different phenotypic traits and their tolerance to powdery mildew disease at two cropping seasons as well as at two different locations in Odisha. In this investigation, seven molecular markers *viz*., VrCSSTS1, VrCSSTS2, VrCSSSR3, CEDG191, MB-SSR238, CEDG166, CEDG282 were analysed.

Result: SSR marker such as VrCSSSR and VrCSSTS linked with powdery mildew resistance gene were tested in different genotypes with known powdery mildew reaction and the results showed a consistent association of the marker in all the powdery mildew resistant genotypes and absent in all the powdery mildew susceptible genotypes. The results confirmed the validation of these markers with the powdery mildew resistance gene in different genetic backgrounds. Similarly, CEDG191, CEDG166, CEDG282 markers, reported to be linked to powdery mildew resistance, amplified the respective marker fragment of 100 to 300 bp in mungbean genotypes and were polymorphic. The above PCR-based and locus-specific markers could be employed for marker-assisted breeding (MAB) program as well as genotype conservation. These linked markers will boost the efficiency and precision of powdery mildew resistance breeding in mungbean.

Key words: Genetic diversity, Micro-satellite marker, Mungbean, Powdery mildew.

INTRODUCTION

Greengram or Mungbean [*Vigna radiata* (L.) Wilczek] is an important pulse crop. It is a self-pollinated crop (2n=22) and genome size of 560 Mb. It is an important legume (Pulse) crop after Chickpea and Pigeon pea in India and widely cultivated in different parts of India as it is well adapted to multiple cropping systems in the drier and warmer climates. In India during 2019-20, about 31.15 lakh hectares area was covered under greengram. Among all the states Rajasthan had the highest area 18.30 lakh ha where Odisha covers only 1.63 lakh ha. In particular, the state of Odisha has recorded very low productivity (337 kg/ha) mainly due to inherent genotype failures and losses due to pests and diseases. On account of its dense crop canopy, photo insensitivity and short duration, it assumes special significance in crop diversification, intensification and conservation of natural resources and sustainability of production systems. The significant constraints behind the production of this crop arethe non-availability of high yielding and disease-resistant varieties. Among all Powdery mildew disease caused by *Erisyphe polygoni* is economically significant because it reduces photosynthetic activity and physiological changes which results in 20-40 per cent reduction in yield. So now it is a crucial time for achieving a gain in yield and for developing resistant and well adaptable

[1]Department of Agricultural Biotechnology, College of Agriculture, Odisha University of Agriculture and Technology, Bhubaneswar-751 003, Odisha, India.

Corresponding Author: Laxmipreeya Behera, Department of Agricultural Biotechnology, College of Agriculture, Odisha University of Agriculture and Technology, Bhubaneswar-751 003, Odisha, India. Email: klaxmipreeya1@gmail.com

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genotypes through the breeding program. For this purpose scientific collection, characterization and evaluation of mungbean germplasm are highly essential. It also helps in the proper identification of genotypes with the desired characteristics.

Simple sequence repeats (SSRs) or microsatellites (as short tandemly repeated DNA sequences 2-5 bp in length) markers have been developed for major crop plants and this marker system is predicted to lead to even more rapid advances in both marker development and implementation in breeding programs. A potentially powerful technique for DNA fingerprinting and phylogenetic studies (Suman *et al.,* 2019) is SSRs, which have long been known to be polymorphic and wide in plant genomes. SSR marker VrCSSR1 and one STS marker VrCSSTS1 which were reported as co-segregating with RFLP marker VrCS65 are found to be associated with PM resistance. These markers explained about 16.02% and 20.18% of the total phenotypic variance respectively. By composite-interval mapping, Kasettranan *et al*. (2010) reported two QTLs on two linkage groups, qPMR-1 and qPMR-2 which accounted for 20.10% and 57.81% of the total variation for plant response to powdery mildew disease. In this study, marker MB-SSR238, which was reported to be very closely linked to qPMR-2 locus and marker CEDG191, which was reported to be very closely linked to qPMR-1 were also used.

MATERIALS AND METHODS

Experimental details

The present investigation was carried out to evaluate thirty-one mungbean genotypes collected from the different parts of India. The genotypes were sown in an incomplete bock design augmented with four checks varieties in the month of January 2017 at KVK, Sakhigopal, Puri and November, 2018 in the Experimental Field station, EB-1, OUAT, Bhubaneswar. The accessions were accommodated in 3 blocks each having 13 plots and 13 treatments and each plot contains one treatment and seeds of each variety were sown in three lines in each plot which is 2 m in length, along with four checks 'Kamdev', 'OBGG-52', 'IPM-02-3', 'IPM-02-14'. The checks were randomly allocated along with the test genotypes within the blocks and replicated as many times as the number of blocks.

Phenotyping

Screening of germplasm according to DUS descriptor

The observations were taken as per the DUS Guidelines for the Conduct of Test for Distinctness, Uniformity and Stability on mungbean framed by the "Protection of Plant Varieties and Farmers' Rights Authority (PPV and FRA)" (ACC, 2007). These included both quantitative and visual characters (Table 1).

Disease screening

Mungbean germplasm was evaluated in the field as per the DUS guidelines. The germplasm was screened using a 0-5 scale for Powdery mildew disease as recommended by Reddy *et al*. (1994) under field conditions. The disease scoring was carried out at 35 days and 55 days after sowing under natural conditions. Genotypes for powdery mildew disease were scored and were classified into five different classes. Observations were recorded on numbers of spots on each leaflet in a plant at different scales. Finally, the mean of each observation was calculated and the per cent disease index (PDI) was determined by using the following formula:

$$PDI = \frac{\sum \text{ of numerical values}}{\text{Total number of leaves examined} \times \text{Maximum grade value}} \times 100$$

Statistical analysis

The selected genotype was compared with the rest of the genotypes by DIST Coefficient Matrix. Higher the value of the coefficient, compared variety will be more similar to the selected variety. In addition to this, the value of coefficient also signifies about the extent of dissimilarity between the two varieties. DIST Coefficient Matrix was subjected to generate a dendrogram using software program NTSYS pc Ver 2.2. Exeter Software, N.Y. (Rohlf, 2000).

Genotyping

DNA Isolation and purification

The leaves were first washed, air-dried and then crushed on to a sterilised pestle and mortar in liquid nitrogen to obtain a fine powder. Care was taken to avoid thawing of the material. Extraction of total genomic DNA was carried out as described by Doyle and Doyle, (1987) with minor modifications to suit the materials under consideration. Then the DNA was dissolved in 200 µl of TE (10:1) buffer (pH 8.0) and kept overnight for complete dissolution. RNase solution (10 mg/ml) was added @ 15 µl/100 µl to the tube containing dissolved DNA and incubated at 37°C for 1 hour. An equal volume of phenol: chloroform: isoamyl alcohol (25:24:1) was added and mixed properly for 2 min and spun 5 min and then purification steps were followed to remove the contaminants. Then the DNA was dried under vacuum, dissolved in TE (10:1) buffer at room temperature and then stored at -20°C for further use.

Standardisation of PCR for gene specific primer

Optimisation of primers is essential to make the assay perfect. Poor optimisation leads to lack of reproducibility between replicates as well as inefficient and insensitive assays, hence to pass up this intact problem prior to PCR amplification a gradient was set up such that it spans the calculated annealing temperature of the primers ±2°C. This range can then be further customised to polish up the assay. This step can be combined with various primer concentration combinations to optimise both annealing temperature and primer concentration simultaneously. In this study, all the primers were optimised by the setting of a gradient temperature. Once the experiment has ended, the samples are run and visualised on an agarose gel (1.5%) to assess the results. The optimal annealing temperature and primer

Table 1: Phenotyping profiling of 31 mungbean genotypes based on DUS characters.

Genotypes	Hypocotyl: colouration	Time of flowering	Plant: growth habit	Plant: habit	Stem: colour	Stem: pubescence	Leaflet:Lobes (terminal)	Leaf : shape (terminal)	Leaf colour	Leaf: vein colour	Petiole: colour	Leaf: size	Flower: colour of petal	Pod: Colour	Pod pubescence	Pod: position	Plant height	Pod: colour	Pod: curvature	Pod: Length (mature pod)	Seed: colour	Seed: lusture	Seed: shape	Seed: Size
V1	1	5	7	3	1	9	9	2	2	2	2	3	5	2	9	2	3	1	3	3	3	2	3	3
V2	9	3	7	3	1	9	9	2	2	2	2	3	5	1	9	2	3	1	3	3	1	2	3	3
V3	9	7	5	1	2	9	9	2	2	2	2	3	5	2	9	1	3	1	1	3	2	1	1	5
V4	1	5	5	1	1	1	9	2	2	2	2	3	5	2	9	1	3	1	3	3	1	2	1	3
V5	1	3	5	3	1	9	9	2	2	2	2	3	5	2	9	2	3	1	3	3	3	2	3	3
V6	9	5	5	3	1	1	9	2	2	2	2	3	5	2	9	1	3	2	3	3	3	2	3	3
V7	1	7	5	3	1	9	9	2	2	2	2	3	5	2	9	3	3	2	1	3	2	1	3	3
V8	1	3	5	3	1	9	9	2	2	2	2	3	5	2	9	2	3	1	3	3	3	2	3	3
V9	9	7	5	3	2	9	9	2	2	2	2	3	5	1	9	2	3	1	1	3	2	1	1	3
V10	1	3	5	3	1	9	9	2	2	2	2	3	5	1	9	2	3	2	1	5	4	2	3	3
V11	1	3	5	1	1	1	9	2	2	2	2	3	5	2	9	2	3	2	3	3	1	2	3	3
V12	9	5	5	1	1	9	9	2	2	2	2	5	5	1	9	3	3	2	3	3	2	1	3	3
V13	9	5	7	1	1	9	9	2	2	2	2	3	5	1	9	2	3	2	1	3	3	2	3	3
V14	1	7	7	1	1	9	9	2	2	2	2	3	5	2	9	3	3	2	3	3	4	2	3	3
V15	1	7	7	3	1	9	9	2	2	2	2	3	5	1	9	2	3	2	3	3	4	2	3	3
V16	1	3	7	3	1	1	9	2	2	2	2	3	5	2	9	2	3	2	3	3	2	1	1	3
V17	9	5	7	1	2	9	9	2	2	2	2	3	5	2	9	2	3	2	1	3	4	2	3	3
V18	1	3	7	3	1	9	9	2	2	2	2	5	5	1	9	2	3	2	3	3	3	2	3	3
V19	1	3	5	1	1	9	9	2	2	2	2	3	5	2	9	1	3	2	3	5	2	2	1	3
V20	9	5	5	1	1	9	9	2	2	2	2	3	5	1	9	3	3	2	3	5	2	1	1	3
V21	9	7	7	3	1	9	9	2	2	2	2	3	5	1	9	3	3	2	1	5	4	2	3	3
V22	1	3	5	3	1	9	9	2	2	2	2	3	5	2	9	2	3	2	3	3	2	2	3	3
V23	1	3	7	3	1	9	9	2	2	2	2	3	5	2	9	2	3	2	3	3	4	2	3	3
V24	9	3	7	3	1	9	9	2	2	2	2	3	5	2	9	2	3	2	3	3	3	2	3	3
V25	1	3	5	3	1	9	9	2	2	2	2	3	5	1	9	2	3	2	1	3	2	1	1	5
V26	1	3	7	3	2	9	9	2	2	2	2	3	5	1	9	1	3	1	1	3	2	1	1	5
V27	1	3	5	1	2	1	9	2	2	2	2	5	5	1	9	1	3	1	1	3	2	1	3	3
V28	9	5	5	1	1	9	9	2	2	2	2	3	5	1	9	1	3	1	1	3	3	1	1	5
V29	9	3	5	1	2	9	9	2	2	2	2	5	5	1	9	1	3	2	1	5	2	1	3	5
V30	1	3	7	1	1	9	9	2	2	2	2	3	5	2	9	3	3	1	1	3	2	1	3	3
V31	1	5	7	3	1	9	9	2	2	2	2	3	5	2	9	2	3	2	3	3	3	2	3	3

combination give the band with the highest intensity (yield) with no non-specific products.

Microsatellite marker analysis

Seven Microsatellite markers tested for PCR amplification according to annealing temperature for 31 genotypes. The experiment was performed in eppendorf Thermal Cycler programmed under standard conditions for all the primer pairs using 1 U of Taq polymerase with 1X polymerase chain reaction buffer (100 mMTris-HCl at pH 9, 500 mMKCl and 15 mM $MgCl_2$), 2.5 mMdNTP, 3 mM $MgCl_2$, 20pM of each primer and 50 ng of DNA template with a final reaction volume of 25 µL. The PCR reactions were denatured at 94°C for 5 minutes followed by 35 cycles of 94°C for 1 minute, required annealing temperature for 1 minute and extension at 72°C for 2 minutes. The final extension was 72°C for 7 minutes. The amplicons were run on an agarose gel (1.8%) with 100 bp DNA ladder, which was then visualised through UV GELDOC unit to assess the results.

RESULTS AND DISCUSSION

Morphological Characterization

Analysis of data of thirty-one genotypes on the basis of 24 visual characters and various categorical descriptors revealed the occurrence of 62 numbers of variants. Eighteen variants (erect plant growth, purple stem colour, absent in leaflet lobes, deltoid leaf shape, lanceolate leaf shape, cuneate leaf shape, green leaf colour, green leaf vein colour, purple leaf vein colour, green petiole colour, purple petiole colour, large leaf size, yellow flower colour of petiole colour, absent pod pubescence, medium and long plant height, long pod length of the mature pod, large seed size) out of possible 62 variants could not be observed in any of the genotypes. Only one limited number of categorical descriptors (yellow seed colour) that have contributed to the distinctiveness of specific mungbean genotypes had been observed. Yield assessment was analyzed for each mungbean genotypes under field conditions and calculated the yield (kg/ha) given in the (Table 4). Majority of genotypes showed good yield *viz*. 'OBGG-52' (870 kg/ha), 'Kamadev' (830 kg/ha), 'KalahandiLocal-1' (846 kg/ha), 'BerhempurLocal-1' (920 kg/ha), 'Banpur Local' (952 kg/ha), 'IPM-02-03' (895 kg/ha) and while rest of the genotypes showed low yield *viz*. 'Digapahandi Local' (275 kg/ha), 'Sambalpur Local' (290 kg/ha), 'Nadika Local' (220 kg/ha), 'NayagarhLocal-6'(380 kg/ha).

Cluster analysis based on morphology

The genetic dissimilarity matrix was analysed using UPGMA clustering algorithms for the construction of dendrogram that depicted the pattern of genetic relationships among mungbean genotypes (Table 3). The analysis revealed four main clusters at 8.75 dissimilarity coefficients with best possible discrimination between groups. The genotype pairs 'Badahana Local' and 'Hinjili Local' showed the maximum dis-similarity (2.67) followed by dis-similarity between 'OBGG-52' and 'Berhampur Local 1' among the thirty-one genotypes (Table 2), whereas, 'Nayagarh Local' and 'Nayagarh Local 6' showed lowest dis-similarity (0.01) followed by dis-similarity between 'Kukudakhandi Local' and 'Banpur Local'(0.20). Fourteen genotypes *viz*., 'Banpur Local', 'Kukudakhandi Local', 'PuriLocal- 4', 'Kalahandi Local 1', 'Kalahandi Local 1', 'Balangir Local', 'Nadika Local', 'NayagarhLocal-6', 'Nayagarh Local', 'Bhapur Local', 'Bolangiri local-1', 'Bhapur Black Local', 'Kamdev' and 'KVK Puri-2' were included in Cluster I. But cluster-II was represented by only one genotype (Sambalpur Local 2'. Ten genotypes *viz*., 'Nuagaon Brown Local', 'Kaniapada Local', 'SundergarhLocal 11-1', 'IPM-02-14', 'Behrampur Local 2' , 'KantapadaLocal', 'Berhampur Local 1', 'IPM-02-3', 'Kendrapada Local 1' and 'Purushottamapur Local' were represented in the Cluster III and cluster IV had six genotypes *viz*., 'Badahana Local', 'Andapur Local', 'Hinjili Local', 'Seragada Local', 'OBGG-52' and 'Digapahandi Local'.

At 8.07 dis-similarity coefficient, Cluster-I was further sub-divided into two sub-clusters. The sub-cluster I A was represented by two genotypes *viz*., 'Kamdev' and 'KVK Puri-2'. whereas Cluster IB had 12 genotypes *viz*., 'Banpur Local', 'Kukudakhandi Local', 'PuriLocal-4', 'Kalahandi Local 1', 'Kalahandi Local 1', 'Balangir Local', 'Nadika Local', 'NayagarhLocal-6', 'Nayagarh Local', 'Bhapur Local', 'Bolangiri local -1' and 'Bhapur Black Local'.

Variability for disease reaction

Based on disease scale (0-5) given by Reddy *et al*., 1994, observations were carried out mainly on the basis of weekly intervals and disease incidence was recorded from the field given in the Plate 2 and 3. Majority of genotypes (22.58%) showed highly resistant for powdery mildew and 16.13% were resistant to powdery mildew. In comparison, 19.35% of genotypes exhibited were highly susceptible, with moderate resistant and susceptible. Among all genotypes, 3.23% of genotypes were exhibited susceptible to powdery mildew.

Molecular characterisation

The study was aimed to validate the powdery mildew associated marker (Table 4) among mungbean genotypes. Seven primers of specific microsatellite loci were used to amplify the repeated regions in the mungbean samples. For all loci, 25 µl reaction mixture containing genomic DNA concentration of 25-50 ng, primer concentration of 1 pM and $MgCl_2$ concentration of 1.5 mM, dNTPs 2.5 mM, 10X buffer and 1 unit of Taq polymerase were given. In the present study primers were found to be polymorphic. These polymorphic loci amplified were in the mungbean genotypes given in the Fig 2 approximately of size *viz*. VrCSSTS1 (300 bp), VrCSSTS2 (300 bp), VrCSSSR3 (200-300 bp), MB-SSR238 (200-300 bp), CEDG191 (100-150 bp), CEDG166 (220-300 bp) and CEDG282(100 bp) which are associated with powdery mildew disease recorded during field evaluation.

Relationship between genetic and phenotyping profiling

The results of this study support the existence of a relationship between genetic and phenotypic character

Table 2: Details of morphological characterisation of mungbean genotypes and their proportional distribution based on DUS parameters.

Characteristics	States	Note	Number of entries	Percentage of entries
Hypocotyl: AnthocyaninColoration	Absent	1	19	61.29
	Present	9	12	38.70
Time of flowering	Early (<40 days)	3	16	51.61
	Medium (40-50 days)	5	9	29.03
	Late (>50 days	7	6	19.35
Plant: Growth habit	Erect	3	0	0
	Semi-erect	5	17	54.83
	Spreading	7	14	45.16
Plant: Habit	Determinate		13	41.93
	Indeterminate	3	18	58.06
Stem: Color	Green	1	25	80.64
	Green with purple splashes	2	6	19.35
	Purple	3	0	0
Stem: Pubescence	Absent	1	5	16.12
	Present	9	26	83.87
Leaflet: Lobes (Terminal)	Absent	1	0	0
	Present	9	31	100
Leaf: Shape (Terminal)	Deltoid	1	0	0
	Ovate	2	31	100
	Lanceolate	3	0	0
	Cuneate	4	0	0
Leaf: Color	Green	1	0	0
	Dark green	2	31	100
Leaf: Vein color	Green	1	0	0
	Greenish purple	2	31	100
	Purple	3	0	0
Petiole: Color	Green	1	0	0
	Green with splashes	2	31	100
	Purple	3	0	0
Leaf: size	Small	3	27	87.09
(at 5th node from the base)	Medium	5	4	12.90
	Large	7	0	0
Flower: Color of petal (standard)	Yellow	3	0	0
	Light yellow	5	31	100
Pod: Color of premature pod	Green	1	14	45.16
	Green with pigmented suture	2	17	54.83
Pod pubescence	Absent	1	0	0
	Present	9	31	100
Pod: Position	Above Canopy	1	8	25.80
	Intermediate	2	17	54.83
	Not visible	3	6	19.35
Plant height	Short(<50 cm)	3	31	100
	Medium (50-70 cm)	5	0	0
	Long (>70 cm)	7	0	0
Pod: Color	Brown	1	11	35.48
	Black	2	20	64.51
Pod: Curvature of mature pod	Straight	1	13	41.93
	Curved	3	18	58.06
Pod: Length (mature pod)	Short (<8 cm)	3	26	83.87
	Medium (8-10 cm)	5	5	16.12

Table 2: Continue...

Table 2: Continue...

	Long(>10cm)	7	0	0
Seed: color	Yellow	1	3	9.67
	Green	2	13	41.93
	Mottled	3	9	29.03
	Black	4	6	19.35
Seed: lusture	Shiny	1	12	38.70
	Dull	2	19	61.29
Seed: shape	Oval	1	8	25.80
	Drum shaped	3	23	74.19
Seed: Size(weight of 100 seeds)	Small(<3g)	**3**	27	87.09
	Medium(3-5g)	**5**	4	12.90
	Large(>5g)	7	0	0

Table 3: Euclidian distance matrix of 31 mungbean genotypes based on 24 visually assessed characters.

	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12	V13	V14	V15	V16	V17	V18	V19	V20	V21	V22	V23	V24	V25	V26	V27	V28	V29	V30	V31
V1	0.00																														
V2	1.74	0.00																													
V3	1.96	1.31	0.00																												
V4	1.84	2.47	2.44	0.00																											
V5	0.58	1.74	2.04	1.84	0.00																										
V6	2.36	1.81	1.91	1.79	2.36	0.00																									
V7	0.82	1.97	1.85	1.90	1.00	2.43	0.00																								
V8	0.58	1.74	2.04	1.84	0.01	2.36	1.00	0.00																							
V9	1.87	1.14	0.65	2.46	1.96	1.85	1.73	1.96	0.00																						
V10	0.89	1.89	2.09	2.00	0.68	2.44	1.06	0.68	1.99	0.00																					
V11	1.84	2.40	2.59	0.65	1.74	1.79	1.95	1.74	2.59	1.89	0.00																				
V12	1.84	0.91	1.06	2.45	1.84	1.81	1.84	1.84	0.98	1.94	2.42	0.00																			
V13	1.76	0.84	0.96	2.47	1.85	1.80	1.83	1.85	0.91	1.84	2.46	0.79	0.00																		
V14	0.68	2.00	1.95	1.94	1.06	2.46	0.84	1.06	1.93	1.19	1.98	1.85	1.77	0.00																	
V15	0.54	1.94	1.97	1.96	0.98	2.41	0.79	0.98	1.86	1.08	2.02	1.89	1.79	0.50	0.00																
V16	1.77	2.38	2.61	0.82	1.77	1.81	1.97	1.77	2.54	1.89	0.76	2.50	2.47	1.98	1.94	0.00															
V17	1.77	1.00	0.98	2.52	1.86	1.81	1.86	1.86	0.98	1.85	2.50	0.91	0.35	1.76	1.80	2.50	0.00														
V18	0.65	1.74	2.14	1.95	0.65	2.43	1.15	0.65	2.04	0.84	1.84	1.81	1.83	1.06	0.94	1.77	1.86	0.00													
V19	0.98	1.87	1.99	1.76	0.79	2.46	1.24	0.79	2.01	0.87	1.76	1.89	1.90	1.22	1.26	1.80	1.94	0.98	0.00												
V20	1.88	1.00	0.98	2.42	1.88	1.86	1.88	1.88	0.89	1.89	2.45	0.71	0.89	1.89	1.94	2.47	1.00	1.95	1.76	0.00											
V21	1.83	1.21	1.15	2.64	2.00	1.89	1.80	2.00	0.91	1.88	2.67	1.10	0.76	1.79	1.74	2.61	0.79	1.98	2.09	1.02	0.00										
V22	0.65	1.72	2.04	1.81	0.29	2.36	0.96	0.29	1.96	0.74	1.70	1.81	1.85	1.10	1.02	1.74	1.88	0.65	0.74	1.86	2.02	0.00									
V23	0.50	1.77	2.12	1.95	0.50	2.40	1.12	0.50	2.04	0.74	1.84	1.90	1.80	0.94	0.84	1.74	1.79	0.50	0.94	1.95	1.94	0.58	0.00								
V24	1.70	0.50	1.31	2.50	1.70	1.74	1.95	1.70	1.17	1.80	2.42	0.91	0.74	1.89	1.85	2.36	0.76	1.70	1.85	1.00	1.06	1.70	1.65	0.00							
V25	0.82	1.77	2.00	1.88	0.58	2.42	0.87	0.58	1.89	0.61	1.77	1.84	1.80	1.21	1.10	1.79	1.86	0.76	0.89	1.88	1.98	0.50	0.76	1.77	0.00						
V26	0.94	1.83	1.93	1.91	0.94	2.52	1.21	0.94	1.93	1.00	1.94	2.02	1.88	1.35	1.22	1.78	1.91	0.94	1.00	1.98	2.07	0.94	0.94	1.85	0.79	0.00					
V27	1.93	2.48	2.56	0.91	1.84	1.88	1.99	1.84	2.56	1.87	0.76	2.43	2.46	2.08	2.08	1.00	2.48	1.84	1.87	2.53	2.67	1.84	1.93	2.50	1.77	1.87	0.00				
V28	1.90	1.12	0.54	2.43	1.90	1.86	1.90	1.90	0.79	1.91	2.52	0.96	0.79	1.96	1.96	2.52	0.87	1.99	1.87	0.87	1.14	1.93	1.97	1.08	1.86	1.80	2.47	0.00			
V29	2.02	1.10	1.12	2.56	1.94	1.96	2.06	1.94	1.26	1.86	2.49	0.94	1.00	2.15	2.15	2.57	1.06	1.91	1.86	1.02	1.32	1.91	2.00	1.10	1.85	1.88	2.39	0.89	0.00		
V30	0.79	1.78	2.01	1.89	0.79	2.51	1.02	0.79	1.99	0.91	1.78	1.85	1.74	1.04	1.15	1.80	1.78	0.84	0.96	1.89	1.99	0.79	0.79	1.78	0.68	0.87	1.80	1.89	1.90	0.00	
V31	0.20	1.76	1.97	1.85	0.61	2.35	0.79	0.61	1.88	0.87	1.83	1.83	1.74	0.65	0.50	1.76	1.76	0.61	0.96	1.87	1.81	0.61	0.46	1.68	0.79	0.96	1.94	1.91	2.01	0.82	0.00

among the mungbean varieties. This finding is of specific interest in diversity analysis and disease variability reaction with validation of microsatellites marker for mildew resistant make inferences about the genetics and phenotypic correlations among the genotypes. The phenotypic profiling (Table 1) showing that some genotypes are superior as compared to other and the disease variability reaction also showed that these superior genotypes are showing highly resistant, resistant and moderately resistant with better yield. From the molecular profiling analysis (Table 6) of microsatellites marker also revealing similar result and validating that these markers are linked to the powdery mildew disease.

On the basis of seed yield per plant, number of pods per plant, number of branches per plant, number of seeds per pod and 100 seed weight some varieties are considered as superior genotypes for the further breeding program which has shown similar results with Gokulakrishnan *et al.,* (2012); Behera *et al.* (2020). Thirty-one genotypes of mungbean were screened under field conditions and the incidence of disease was present in almost all the genotypes from 35 DAS to 55 DAS (Days After Sowing). Among them, six genotypes showed a moderately resistant and moderately susceptible reaction; five genotypes showed resistance reaction, seven genotypes showed highly resistance reaction, six genotypes showed highly susceptible reaction and only one showed a susceptible reaction. These results are in line with Khare and Lankpale (1998) who carried out mungbean trials during Rabi season over three years against powdery mildew and only seven entries were found to be resistant to powdery mildew over the years. A previous study by Thakur and Agarwal (1995) suggested that environmental factors have a large impact on the extent and severity of powdery mildew. They reported

a high incidence of powdery mildew during *Kharif* than *Rabi* (Mishra *et al.,* 2019). In the present study, high incidence of powdery mildew on mungbean varieties was observed during *Kharif* season under field condition. This indicated that powdery mildew development is largely depended on favourable environmental condition and cool, dry season favours their development and infection.

Among the different DNA markers, microsatellite or simple sequence repeats (SSRs) are the marker of choice for various genetic studies because, which detect sequence variation in the hyper-variable region of tandem repeats of 2-4bp, are a powerful tool for genome analysis because of their co-dominant nature, loci specificity and high reproducibility (Tautz and Renz, 1984). Microsatellite markers have been used for genome mapping and genetic diversity studies in many crop plants (Ford *et al*., 2002; Blair *et al*., 2006; Sangiri *et al*., 2007; Gupta *et al*., 2013; Singh *et al*., 2014 ; Behera *et al.,* 2020). In addition to being

Table 4: Yield performance and incidence of powdery mildew in Thirtyone mungbean genotypes (Reddy *et al*., 1994).

S. no	Landrace / Variety Name	Accession No./ Place of collection/ Collected by	Yield kg/ha	Grade	PDI	Reaction to powdery mildew
V1	Banpur Local	CPR BAM GP 283	952	0	0	Highly resistant (HR)
V2	Nuagaon Brown Local	CPR BAM GP 321	328	5	75.05	Highly susceptible (HS)
V3	Sundergarh Local 11-1	KvkSundergarh-11-1	540	5	79.12	Highly susceptible (HS)
V4	Andapur Local	CPR BAM GP 281	890	0	0	Highly resistant (HR)
V5	Nayagarh Local-6	KVK Nayagarh	380	5	80.24	Highly susceptible (HS)
V6	Digapahandi Local	CPR BAM GP 344	275	5	78.23	Highly susceptible (HS)
V7	Puri Local-4	KVK Puri	670	2	13.08	Moderately resistant (MR)
V8	Nayagarh Local	CPR BAM GP 289	794	2	21.25	Moderately resistant (MR)
V9	Berhampur Local 2	CPR BAM GP 273	563	2	18.69	Moderately susceptible (MS)
V10	Bolangiri local -1	KVK Bolangiri	379	4	65.02	Susceptible (S)
V11	Hinjili Local	CPR BAM GP 290	511	3	26.64	Moderately resistant (MR)
V12	Kantapada Local	CPR BAM GP 258	467	3	34.98	Moderately susceptible (MS)
V13	Kendrapada Local 1	KVK Kendrapada	880	0	0	Highly resistant (HR)
V14	Kalahandi Local	KVK Kalahandi	816	2	11.20	Moderately resistant (MR)
V15	Kalahandi Local 1	CPR BAM GP 302	846	0	0	Highly resistant (HR)
V16	Seragada Local	CPR BAM GP 339	689	1	5.28	Resistant (R)
V17	Purushottamapur Local	CPR BAM GP 280	800	1	4.86	Resistant (R)
V18	Balangir Local	CPR BAM GP 299	550	3	45.92	Moderately susceptible (MS)
V19	Sambalpur Local 2	KVK Sambalpur	290	5	78.45	Highly susceptible (HS)
V20	Berhampur Local 1	CPR BAM GP 272	920	0	0	Highly resistant (HR)
V21	Badahana Local	CPR BAM GP 262	694	1	4.78	Resistant (R)
V22	Bhapur Local	CPR BAM GP 331	863	0	0	Highly resistant (HR)
V23	Nadika Local	CPR BAM GP 293	220	5	81.48	Highly susceptible (HS)
V24	Kaniapada Local	CPR BAM GP 259	358	2	42.64	Moderately resistant (MR)
V25	Bhapur Black Local	CPR BAM GP 334	750	1	6.15	Resistant (R)
V26	Kamdev (check)	Variety released from OUAT, Bhubaneswar	830	1	5.28	Resistant (R)
V27	OBGG-52 (check)	Variety released from OUAT, Bhubaneswar	870	0	0	Highly resistant (HR)
V28	IPM-02-14 (check)	Variety released from IIPR, Kanpur	489	2	47.32	Moderately resistant (MR)
V29	IPM-02-3 (check)	Variety released from IIPR, Kanpur	895	2	39.27	Moderately susceptible (MS)
V30	Puri Local-2	KVK Puri	650	2	11.10	Moderately resistant (MR)
V31	Kukudakhandi Local	CPR BAM GP 337	720	2	21.53	Moderately resistant (MR)

Table 5: List of Microsatellite markers used in the present study for validation (Zhang *et al.,* 2008 and Kasettranan *et al.,* 2010).

Oligo name	Forward primer (5′-3′)	Reverse primer (5′-3′)	Annealing temp.
VrCSSTS1	ATTACTTGAGGTGGGGATAAT	AATAGACCACTTTTTCCGT	52.5°C
VrCSSTS2	ATTTGATGACGATGTATTTAA	TAAAGATAATGCTGAGGG	53.0°C
VrCSSSR3	GCAGACACAACCATAAATCC	GGTCTTTGACGGCAATCTC	59.0°C
CEDG191	CAATAAGCAATCTGTGGAGAG	CTGCAGGAAACTTGGAATTGC	60.0°C
MB-SSR238	AGCTATTGGTGCATAGGTTC	GATATGATGAGTATGGTGTAG	54.0°C
CEDG166	GGTACAACATTCTTCTATTTG	GGCTTATGAGTTTATCTTATC	48.0°C
CEDG0282	CAGCAACAAGACATGGAGTG	GGTGACCACTTAGACAGAC	56.0°C

Table 6: Association of amplified DNA banding and disease reaction pattern.

S.no.	Genotypes	Disease reaction	VrCSS TS1	VrCSS TS2	VrCSSS R3	CEDG1 91	MB-SSR238	CEDG16 6	CEDG282
V1	Banpur Local	HR	1	0	1	1	1	1	1
V2	Nuagaon Brown Local	HS	0	0	0	0	0	0	0
V3	Sundergarh Local11-1	HS	1	0	0	0	0	1	0
V4	Andapur Local	HR	1	1	0	1	1	1	1
V5	Nayagarh Local-6	HS	0	0	0	0	0	1	0
V6	Digapahandi Local	HS	0	0	0	0	0	0	0
V7	Puri Local-4	MR	1	0	0	1	1	1	1
V8	Nayagarh Local	MR	0	1	0	1	1	1	1
V9	Behrampur Local 2	MS	1	1	0	0	0	1	1
V10	Bolangiri local -1	S	0	0	0	1	0	1	1
V11	Hinjili Local	MR	1	0	0	1	1	0	0
V12	Kantapada Local	MS	1	0	0	1	0	0	1
V13	Kendrapada Local 1	HR	0	1	0	1	1	1	1
V14	Kalahandi Local	MR	1	0	0	1	1	1	1
V15	Kalahandi Local 1	HR	0	1	1	1	1	1	1
V16	Seragada Local	R	0	1	0	1	1	1	1
V17	PurushottamapurLocal	R	1	1	0	0	1	1	1
V18	Balangir Local	MS	1	0	0	1	0	0	0
V19	SambalpurLocal 2	HS	0	0	0	0	0	0	0
V20	Berhampur Local 1	HR	1	0	1	1	1	1	1
V21	Badahana Local	R	1	0	0	1	1	1	0
V22	Bhapur Local	HR	1	0	1	1	1	1	1
V23	Nadika Local	HS	0	0	0	0	0	1	0
V24	Kaniapada Local	MR	0	0	0	1	1	0	1
V25	Bhapur Black Local	R	0	0	1	1	1	1	1
V26	Kamdev (check)	R	0	1	0	1	1	1	1
V27	OBGG-52 (check)	HR	1	1	1	1	1	0	1
V28	IPM-02-14 (check)	MR	1	0	0	1	1	0	0
V29	IPM-02-3 (check)	MS	0	0	0	0	1	1	1

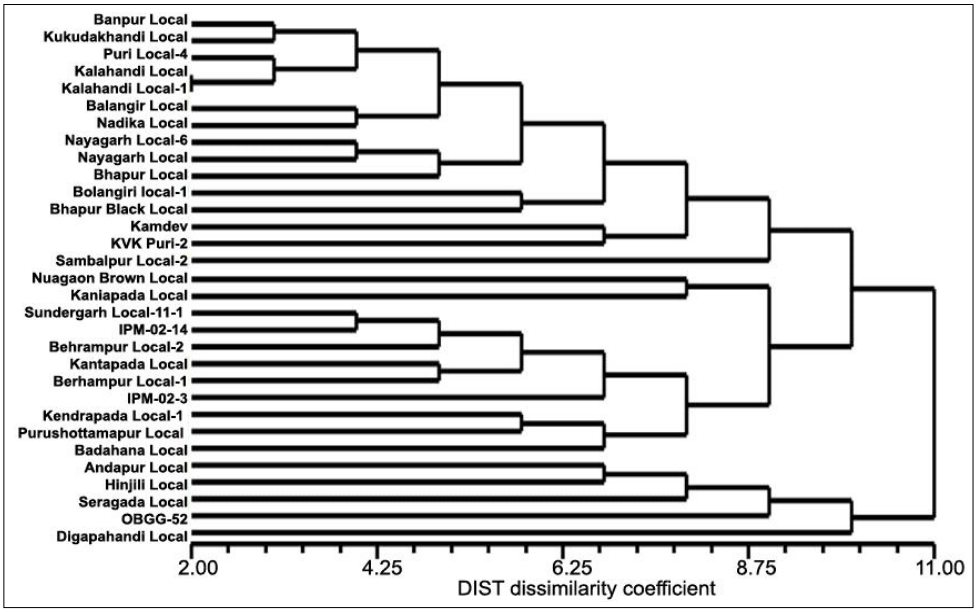

Fig 1: Dendrogram depicting the classification of 31 mungbean genotypes constructed through software programme NTSYS pc Ver 2.1 methods based on DUS characteristics. The scale at the bottom is DIST dissimilarity coefficient.

able to be readily assayed, they are highly informative, transferable, widely distributed in most plant species and highly polymorphic between genotypes. The present molecular data are showing amplification of VrCSSSR3 at 200-300 bp regions, VrCSSTS1 and VrCSSTS2 at 300bp region, which is in congruent with the result reported by Zhang *et al*. (2008) who developed new SSR and STS markers co-segregated with RFLP marker VrCS65 by BAC cloning. In his study, SSR marker VrCSSR1 and one STS marker VrCSSTS1 which were reported as co-segregating with RFLP marker VrCS65 are found to be associated with PM resistance. These markers explained about 16.02% and 20.18% of total phenotypic variance respectively. CEDG191amplified at100-150bp region, CEDG166 amplified at 220-300bp region andCEDG282 at100bp region are considering associated with powdery mildew disease recorded during field evaluation. Multiple-interval mapping by Chankaew *et al*. (2013) also shows the similar types of result with little divergence. He reported that two markers from LG 6 (CEDG121 and CEDG169) were associated with qPMRUM2 locus and two markers from LG9 (VRSSR010 and DMBSSR130) were associated with qPMV4718-3 locus and he reported that Z test indicated that marker DMBSSR130 and CEDG121 showed significant association with powdery mildew resistance. The MB-SSR238 marker exhibits amplification band within 200-300 bp regions showing the association with powdery mildew. The composite-interval mapping by Kasettranan *et al*. (2010) reported two QTLs on two linkage groups, qPMR-1 and qPMR-2 which accounted for 20.10% and 57.81% of the total variation for plant response to powdery mildew disease. In this study, marker MB-SSR238, which was reported to be very closely linked to qPMR-2 locus and marker CEDG191, which was reported to be very closely linked to qPMR-1 were also used.

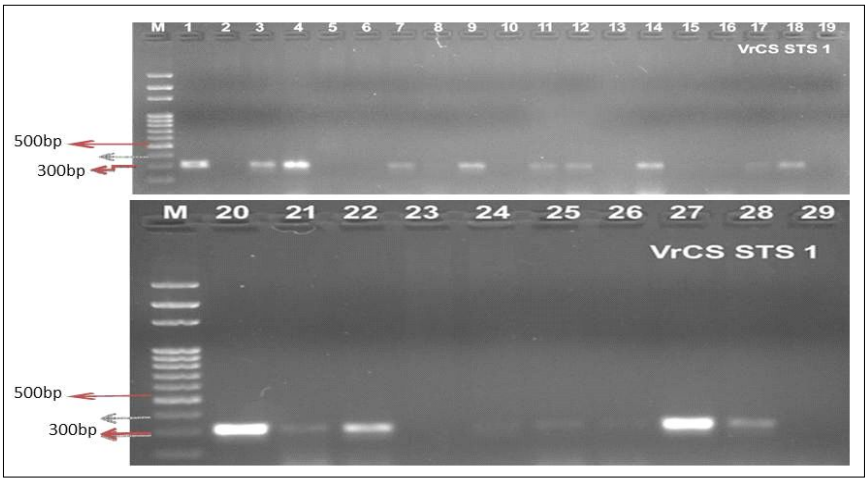

Fig 2a: Amplification profile of 29 mungbean genotypes employing VrCS STS1 gene specific marker, M: DNA Ruler, No on the margin represent the molecular weight of the ruler DNA in bp. Number on the top of the lane correspond to the genotypes of mungbean in Table 6.

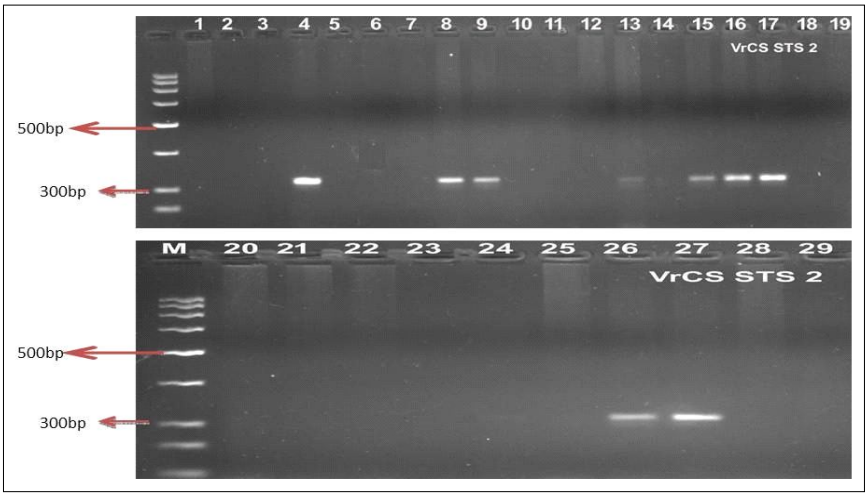

Fig 2b: Amplification profile of 29 mungbean genotypes employing VrCS STS2 gene specific marker, M: DNA Ruler, No on the margin represent the molecular weight of the ruler DNA in bp. Number on the top of the lane correspond to the genotypes of mungbean in Table 6.

CONCLUSION

Based on the present study, significant differences among mungbean genotypes were observed for most of the yield attributing traits. The resistant sources obtained in the present study can be utilised in the breeding program for evolving varieties resistant to powdery mildew. The powdery mildew linked validated markers (VrCSSTS1, VrCSSTS2, CEDG191, CEDG166, CEDG238 and MB-SSR238) could be utilised for the breeding programme for the development of powdery mildew resistant mungbean genotypes. The genotypes identified for different characters serve as a source for breeders to select them and use them for constructing mapping population. The germplasm identified with different characters serves as genepools which provide sufficient scope for a breeder to select within the genotypes. The promising germplasm lines could be used in various breeding programs.

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Conflict of interest

All authors declare that they have no conflicts of interest.

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