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Molecular Evaluation of Mungbean Recombinent Inbried Lines from Cross, Chinamung and BL849 for Powdery Mildew Disease Resistance through Bulk Segregant Analysis

N. K. Parimala, K. M. Harinikumar and D. L. Savitramma
Department of Plant Biotechnology, College of Agriculture, UAS, GKVK, Bengaluru - 560 065
E-mail: harinikumarkm@gmail.com

Abstract

In mungbean yield reduction is mainly due to wide spread of powdery mildew disease, which is the most devastating in mungbean. It can be effectively controlled through incorporation of disease resistance genes into susceptible species through Marker Assisted Selection (MAS). In present study, 96 Recombinant Inbred Lines (RIL) derived from cross between Chinamung (highly susceptible topowdery mildew disease) and BL849 (highly resistant) lines were analyzed by PCR technique by using Simple Sequence Repeats (SSR) markers. Phenotypic data was collected by screening the 96 F_8 RILs population according to the presence of infections by pathogen. Results revealed that 9 are moderately resistant, 60 are moderately susceptible, 24 are susceptible and 3 are highly susceptible. Parental polymorphism study with 248 SSR primers revealed that 14 showed polymorphic for parents. For Bulked Segregant Analysis (BSA) to identify Polymorphic DNA marker linked to a powdery mildew resistance DNA was pooled from selected segregants and genotyping was followed. The markers association was also studied by Single Marker Analysis (SMA) for 14 polymorphic markers. The SMA revealed that two markers DMBSSR130 and DMBSSR125 showed significant association with powdery mildew resistance with 79 per cent and 73 per cent to total variation, respectively. The DMBSSR130 was confirming the association in both the BSA and SMA but DMBSSR125 showed association in SMA not in BSA.

Keywords: Bulked segregant analysis, Single marker analysis, Powdery mildew, Mungbean and SSR markers

Mungbean (*Vigna radiata* (L). Wilczek) also well known as green gram. It is a *kharif* pulse cultivated since long time in India. To meet the demand of mungbean at global level, there is a need to improve the average production and productivity.

The yield in case of mungbean is greatly affected by numerous biotic and abiotic factors. Among biotic factors, Powdery mildew is one of the major disease that leads to reduction in crop yield. Powdery mildew disease is a foliar nature caused by the fungal pathogen *Erysiphe polygoni* DC. Occurrence of this disease can be widely seen under cool-dry season. This powdery growth which covers the leaf surface affects photosynthesis and leads to yield reduction to an extent of 40 per cent. This disease severe sysmptons can be observed in 35-40 days standing crop (Khare *et al.,* 1998).

Powdery mildew disease can be controlled by using strategies such as cultural practices and chemical application. In case of cultural practices disease can be controlled to the some extent but not up to the level. Chemical control of powdery mildew disease is one of most effective practice. However, in developing countries, poor farmers cannot meet the expense of fungicides to control the disease. Use of chemicals also leads to serious environmental hazards. Continuous use of chemical fungicides also leads to the appearance of resistant races of the pathogen. Practically, the chemical treatment turns out to be ineffective, if not performed at proper plant growth stage. High infestation demands huge quantity of fungicides, which often may not be economic, accessible and also large scale indiscriminate use may consequently have serious ecological repercussions. The fungus also overcomes chemical treatments, which is a continuous threat. Further the fungus as evolved resistance over almost varieties. Therefore the practice of effective approaches to control powdery mildew disease is very important to get good yield, as it is inflicting heavy

yield loss so this study was taken to evaluate available mungbean genotypes (RILs) for identification of resistance genotypes (RILs) to identify resistance cultivars for powdery mildew disease.

Material and Methods

During the 2018 *rabi*, the field experiment was cultivated at the 'K' block of the University of Agricultural Sciences, GKVK, Bengaluru. The mungbean RILs used were developed from a cross, Chinamung × BL-849. All the RILs used were collected from the Department of Genetics and Plant breeding at the University of Agricultural Sciences, Bangalore.

The current F_8 RILs were developed from the cross Chinamung × BL849, the F_1 from this cross was forwarded up to F_5 generation by selfing to attain stability in population further, F_6 and F_7 generations were forwarded to the present generation *i.e.,* F_8 (Divya and Savithramma *et al.*, 2014).

The parents used to develop these RILs have distant characteristics. Chinamung is high yielding but highly susceptible to powdery mildew disease used as female parent, BL849 is the low yielding yet highly resistant (HR) to powdery mildew disease with a score of zero in the scale of 1-5 used as male patent. Chinamung, BL849, KKM3, and Pusabaisakhi were used in each block as checks which are help in spreading the disease to RILs.

Genomic DNA was extracted by using modified CTAB method (Doyle and Doyle, 1990) from both the parents and their F_8 RILs. The final DNA concentration was adjusted to 50 ng/µl. Parental polymorphism survey involving 248 SSR markers was carried out. The SSR markers were taken from research papers related to powdery mildew of mungbean includes Chankaew *et al.,* 2013, Zhang *et al.,* 2008. PCR was performed in a 9 µl volume containing 50 ng of template DNA, 0.3 units of Taq DNA polymerase (Bangalore Genei Ltd., Bengaluru, India), 2.5 mM of dNTPs, and 0.2 µM primers in a 1×PCR Taq buffer containing $MgCl_2$. The amplifica-tion was carried out by Eppendorf Mastercycler Germany. PCR conditions included 94°C of 5 min for initial denaturation followed by 35 cycleseach consisting of a denaturation step for 1 min at 94°C, an annealing step for 45 sec at 40 °C, an extension step for 1 min at 72 °C and the final extension for 10 min at 72 °C. Amplified products were separated by 3.0 per cent Metaphor agarose gel electrophoresis at 70 V. The gels were stained with ethidium bromide and visualized on a digital gel documentation and image analysis system (Alpha Innotech, Multimage TM Light, Cabinet Filter Positions - JH Bio Innovation Pvt., Ltd., Bengaluru, India).

Results and Discussion

In order to study the variation among the RILs for Powdery mildew disease, screening for the disease done at weekly intervals along with, parents and checks. This revealed reference for high resistant with scoring 0 from five point scale, among 96 RILs 9 were moderately resistant 'MR' with infection percentage 5.1-30 per cent, 60 were moderately susceptible 'MS' with infection of 30.1-65 per cent, 24 were susceptible 'S' with 65.1-90 per cent disease infection and 3 were highly susceptible 'HS' with 90- 100 per cent infection (Table 1).

Molecular evaluation was carried out to study the Bulk Segregant Analysis (BSA) and Single Marker Analysis (SMA) by using SSR markers linked to powdery mildew resistance. Initially parental polymorphism was studied with 248 primers, out of 248 only 14 are polymorphic for the parents. Among the 14 polymorphic primers only one primer DMBSSR130 showed polymorphism between bulks similar to the parents. So the DMBSSR130 was used to study BSA. Bulk segregating analysis (BSA) did for only one primer *i.e.,* DMBSSR130, as it produced specific polymorphic bands between parents rather than other 13 polymorphic primers (other 13 polymorphic markers did not show much difference between bulks). BSA was carried out using gel electrophoresis technique by serially loading parents (female parent and male parent), bulks (susceptible and resistant bulk),

TABLE 1

Distribution of 96 F_8 RILs of the cross Chinamung x BL849 according to the screened Powdery mildew disease reaction under fields conditions based on five point scale by Reddy *et al.* (1994)

Scale	Per cent leaf infection	Disease Reaction	Number of RILs	RILs
0	0	Highly Resistant (R_0)	0	0
1	1-5	Resistant (R_1)	0	0
2	5.1-30	Moderately Resistant (MR)	9	C_1-94-1, C_1-117-3, C_1-118-1. C_1-121-1, C_1-124-4, C_1-190-1, C_1-245-3, C_1-246-1 and C_1-248-1
3	30.1-65	Moderately susceptible (MS)	60	C_1-7-3, C_1-8-1, C_1-9-2, C_1-11-1, C_1-12-3, C_1-16-3, C_1-18-2, C_1-21-1, C_1-28-1, C_1-30-1, C_1-31-3, C_1-36-3, C_1-36-3, C_1-36-3, C_1-49-1, C_1-53-2, C_1-55-3, C_1-65-1, C_1-67-1, C_1-76-1, C_1-77-1, C_1-78-1, C_1-80-1, C_1-81-4, C_1-86-3, C_1-85-5, C_1-91-1, C_1-101-3, C_1-109-2, C_1-114-2. C_1-115-2, C_1-119-2, C_1-120-5, C_1-122-3, C_1-123-2, C_1-126-1, C_1-128-3, C_1-135-3, C_1-149-4, C_1-155-3, C_1-156-1, C_1-159-1, C_1-164-1, C_1-166-2, C_1-167-3, C_1-169-4, C_1-170-2, C_1-171-1, C_1-172-1, C_1-184-1, C_1-186-2, C_1-192-1, C_1-192-1, C_1-192-1, C_1-198-1, C_1-231-1, C_1-233-1, C_1-242-1, C_1-250-2 and C_1-266-1.
4	65.1-90	Susceptible (S)	24	C_1-1-3, C_1-32-4, C_1-46-1, C_1-74-1, C_1-104-3, C_1-107-1, C_1-113-2, C_1-125-2, C_1-160-1, C_1-163-1, C_1-174-1, C_1-194-3, C_1-201-1, C_1-206-2, C_1-207-1, C_1-208-3, C_1-209-2, C_1-218-1, C_1-221-1, C_1-222-1, C_1-249-1, C_1-254-2, C_1-262-1 and C_1-284-1.
5	90.1-100	Highly susceptible (HS)	3	C_1-116-2, C_1-252-1 and C_1-265-2

then ten susceptible and ten resistant individuals which were used in preparing bulks in 3 per cent metaphor agarose gel (Table 2) (Fig. 1).

Similar kind of work was carried out by Priya *et al.,* 2013 in blackgram by screening 80 polymorphic primers for BSA. But out of 80 primers, only two primers showed specific proper bands with difference of some base pairs between parents and also with the bulks. The report was given in 2009 by Somta *et al.,* that is only few SSRs were developed in mung bean by Data base mining and their cross species amplification in 19 Asian *vigna* species. This is also one of the important reason that the SSR markers developed for other crops can produce polymorphism between parents (with less chances) and are not able to distinguish the bulks.

TABLE 2

List of Polymorphic SSR markers between the parents Chinamung and BL849

Sl.No.	Susceptible RILs list	Sl.No.	Resistant RILs list
1	C_1 - 116 - 2	1	C_1 - 94 - 1
2	C_1 - 252 - 1	2	C_1 - 117 - 3
3	C_1 - 265 - 2	3	C_1 - 118 - 1
4	C_1 - 1 - 3	4	C_1 - 121 - 1
5	C_1 - 32 - 4	5	C_1 - 124 - 4
6	C_1 - 46 - 1	6	C_1 - 190 - 1
7	C_1 - 74 - 1	7	C_1 - 245 - 3
8	C_1 - 104 - 3	8	C_1 - 246 - 1
9	C_1 - 107 - 1	9	C_1 - 248 - 1
10	C_1 - 113 - 2	10	C_1 - 7 - 3

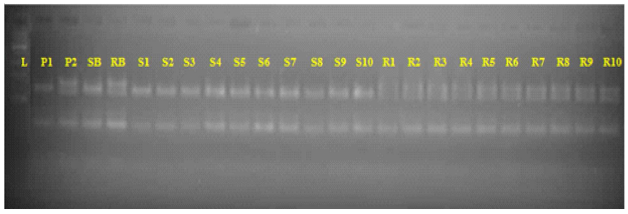

Fig. 1: Bulk Segregant Analysis with DMBSSR130 show polymorphism between the parents, bulks (susceptible and resistant) and the RILs selected for preparing bulks derived from the cross of Chinamung and BL-849 by using 3 per cent metaphor agarose gel

Legend : L=100 bp ladder, P_1= female parent, P_2= male parent, S_1 - S_{10} are susceptible RILs and R_1-R_{10} are resistant RILs listed on Table 2

In this study, SMA was performed with all the polymorphic markers for identifying linkage between selected polymorphic marker and powdery mildew disease resistance trait to confirm the result with BSA. Among the 14 polymorphic markers, the two markers (DMBSSR130 and DMBSSR125) were showed good association in SMA.

Marker DMBSSR130 shows positive result in both BSA and SMA study but marker DMBSSR125 shows association only in SMA not in BSA.

All 96 RILs were genotyped employing DMBSSR 130 primer. Out of which, 44 RILs matched with the female susceptible parent and 52 RILs matched with the male resistant parent (Fig. 2) and when the same

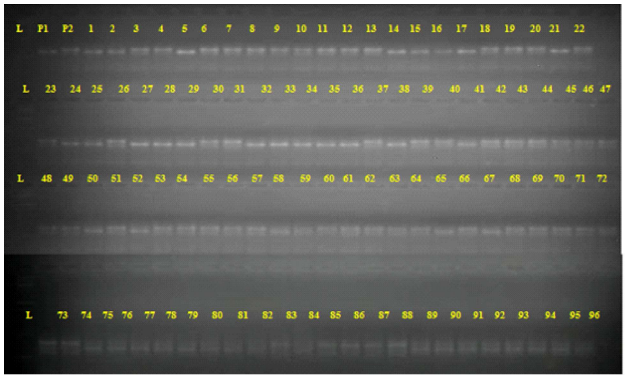

Fig. 2 : Genotyping of 96 RILs derived from cross Chinamung x BL-849 along with parents using polymorphic marker DMBSSR SSR 130 by using 3 per cent metaphor agarose gel

Legend : L=100 bp ladder, P_1 = female parent Chinamung, P_2 = male parent BL849, 1 to 96 are RIL lines.

RILS were subjected to genotyping with DMBSSR 125, 42 RILs resembled the susceptible female parent, 49 RILs showed resemblance with resistant male parent (Fig. 3).

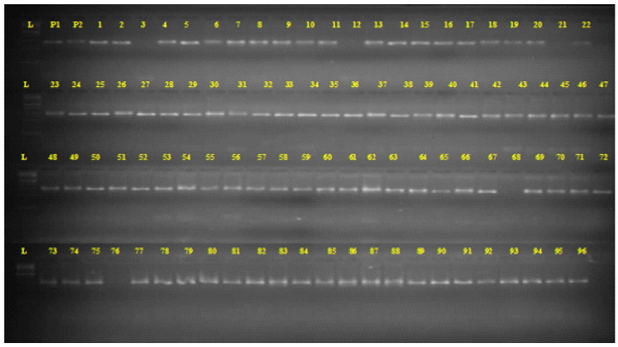

Fig. 3 : Genotyping of 96 RILs with parents derived from cross Chinamung x BL- 849 along with parents using poly morphic marker DMBSSR125 SSR which was not showing difference between bulks by using 3 *per cent* meta phor agarose gel

Legend : L=100 bp ladder, P_1 = female parent Chinamung, P_2 = male parent BL849, 1 to 96 are RIL lines.

SMA was performed to determine the association between SSR markers and powdery mildew resistance. Simple linear regression analysis was carried out to study the linkage between the markers (DMBSSR130 and DMBSSR125) by taking phenotypic data and genotyping scoring data. This analysis explains that the markers (DMBSSR130 and DMBSSR125) were significantly linked with powdery mildew disease resistance. The results indicated that the two markers co-segregate with powdery mildew resistance gene in mungbean. Similar results were explained by Bainade *et al.* (2014) in his work.

One way ANOVA was carried out to study the association of marker and powdery mildew disease resistance trait. The one way ANOVA study on DMBSSR130 and DMBSSR125 markers scoring data and correspondence phenotypic disease scoring data revealed that respective and markers showed 79 per cent and 73 per cent of Phenotypic variance association with Powdery mildew disease resistance. Both the markers (DMBSSR130 and DMBSSR125) were significantly associated with trait at 0.05 per cent level of significance (Table 3).

TABLE 3

Association of molecular markers with powdery mildew resistance by single marker analysis

SSR Marker	MSS	F-value	P-value	R^2 (%)
DMBSSR-130	201.28 ***	380.58 ***	<0.001	79
DMBSSR-125	179.72 ***	294.98 ***	<0.001	73

In a study validation of SSR markers for powdery mildew disease resistance in mungbean carried out by Pooja *et al*. 2018, revealed that single marker analysis for four polymorphic markers CEDG121, CEDG245, MBSSR238 and GMES5773, only MBSSR238 showed significant association with powdery mildew resistance at 0.01 per cent level significance indicated marker was linked to the trait of interest.

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