Indian J. Genet., 78(3): 386-388 (2018)
DOI: doi.org/10.31742/IJGPB.78.3.17

Short Communication

Validation of molecular marker associated with powdery mildew resistance in mungbean

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(Received: April 2018; Revised: July 2018; Accepted: August 2018)

Abstract

Powdery mildew caused by *Erysiphe polygoni*, is one of the major diseases of mungbean (*Vigna radiata* L. Wilczek), causes yield loss up to 20-40%. The present investigation was undertaken to study the SSR markers associated with powdery mildew resistance in mungbean. The resistant line, TARM1 was crossed with highly susceptible, but popular variety DGGV2 and the F_1s were selfed to obtain F_2. F_2 population was evaluated for response to powdery mildew under field conditions. Of the 64 SSR markers studied, only four were found polymorphic between two parents (TARM1 and DGGV2). Single marker analysis indicated that one SSR marker (MB-SSR238) showed association with powdery mildew resistance in mungbean, explaining the phenotypic variance of 11.64%.

Key words: Powdery mildew, single marker analysis, SSR markers, mungbean

Mungbean (*Vigna radiata* L. Wilczek) is one of the important food legumes and also a rich source of dietary protein. It is most widely grown crop due to its early maturity, drought tolerance, ability to fix atmospheric nitrogen and also well suited for mixed/inter cropping system. Among the foliar diseases, powdery mildew caused by *Erysiphe polygoni*, an obligate parasite, causes 20-40 % yield loss in the absence of prevention (Fernandez and Shanmugasundaram 1988) and 100 % when it occurs at the seedling stage (Reddy et al .1994).

The desirable means of managing the disease is the development of resistant varieties. The selection for powdery mildew resistance is difficult when there is no favorable weather condition. Hence, the use of molecular markers linked to gene/QTLs governing powdery mildew resistance is most helpful while breeding mungbean varieties resistant to powdery mildew. Quantitative trait loci governing powdery mildew resistance have been reported by several workers (Chaitieng et al. 2002; Zhang et al. 2008; Kasettranan et al. 2010; Chankaew et al. 2013; Savithramma and Ramakrishnan, 2016). Hence a study was undertaken to validate the previously reported molecular markers linked to powdery mildew resistance in new genetic background.

The present study was carried out at the Department of Biotechnology, University of Agricultural Sciences, Dharwad (UAS). A cross was made between the previously identified resistant genotype TARM1 and DGGV2 (highly susceptible) and the F_1 was confirmed using polymorphic marker. F_1 was selfed to produce F_2. One hundred and thirty six F_2 seeds obtained from the selfed F_1 were sown along with their parents in Main Agricultural Research Station, College of Agriculture, Dharwad, during *rabi*, 2016. Susceptible variety, DGGV2 was sown nearby and around the plot as an infector row. In addition, the resistant line TARM1 was also sown for comparison. Individual plants were scored for powdery mildew response under disease pressure by artificial inoculation by following the method described by Wongpiyasatid et al. (1999). The disease was scored from the first incidence of the disease up to maturity using the scoring system described by Mayee and Datar (1986).

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Published by the Indian Society of Genetics & Plant Breeding, A-Block, F2, First Floor, NASC Complex, IARI P.O., Pusa Campus, New Delhi 110 012; Online management by indianjournals.com; www.isgpb.org

Genomic DNA was isolated from 12 days old, 136 F_2 individuals and two parents following CTAB method described by Agbagwa et al. (2012) with certain modification. A set of 64 SSR primers (Wang et al. 2004; Hisano et al. 2007; Zhang et al. 2008; Somta et al. 2009; Wang et al. 2009; Kasettranan et al. 2010; Palaniappan et al. 2012) were used to identify polymorphism between powdery mildew resistant and susceptible parents. Each F_2 individuals were screened with the polymorphic primers. The amplified products were resolved in either 4 % agarose or in 4% MetaPhor agarose and agarose in the proportion of 3:1. The amplified products were scored as "A" for the female parent (homozygous susceptible), "B" for the male parent (homozygous resistant) and "H" for the heterozygotes. Single marker analysis was performed to find the contribution of the markers towards the powdery mildew resistance by one way ANOVA using MS-EXCEL programme. The coefficient of determination of R^2 which explains the percent of phenotypic variance explained by the polymorphic marker was noted.

Out of 64 SSR primer pairs used, four (CEDG121, CEDG245, MBSSR238 and GMES5773) were polymorphic between the parental lines. F_1 plants were confirmed using polymorphic marker (MB-SSR238) (Fig. 1). F_2 seeds from single plants were used to raise F_2 and were used for both phenotyping and genotyping (Fig. 2).

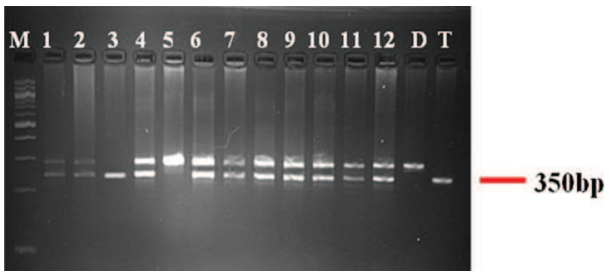

Fig. 1. Confirmation of F_1s using MB-SSR238 for the cross between DGGV2 (female) × TAMR1 (male). M: 100 bp ladder, D: DGGV2 (susceptible parent), T: TARM1 (resistant parent), 1-12: DNA samples from putative F_1 plants

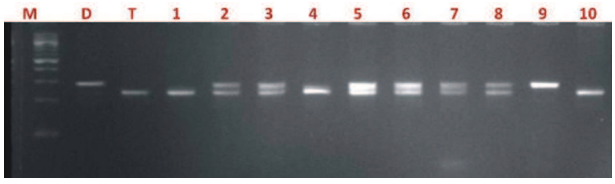

Fig. 2. Amplification profile of F_2 with SSR primer MB-SSR238 [M: 100 bp ladder, D: DGGV2; T: TARM1, 1 to 10: Samples of F_2 (DGGV2 x TARM1) on 4 % agarose]

Single marker analysis performed to validate the previously identified molecular markers (Zhang et al. 2008; Kasettranan et al. 2010; Chankaew et al. 2013) (CEDG121, CEDG245, MB-SSR238 and GMES5773) which were polymorphic for powdery mildew indicated the significant association of the trait with only MB-SSR238 (Table 1) at both 0.05% and 0.01% level of

Table 1. Association of molecular markers with powdery mildew resistance in mungbean

S.No.	Markers	Single marker analysis		
		F value (cal)	P value	% R^2
1	CEDG121	0.645	0.526	0.97
2	CEDG245	0.614	0.542	0.86
3	MBSSR238	8.854	0.0024	11.64**
4	GMES5773	1.280	0.281	1.83

significance with a phenotypic variance of 11.67 %. Previously Kasettranan et al. (2010) also reported that the MB-SSR238 is linked to QTL (qPMR2) governing powdery mildew resistance on LGII which accounted for 57.81% by composite interval mapping. The difference in the PVE of present study with Kasettranan et al. (2010) may be due to different races or strains of *Erysiphae polygoni* prevailing in the test site. Gene for resistance in TARM1 appears to be influenced by the environment. Reddy (2007, 2009) independently showed that a highly resistant mungbean cultivar expressed different disease response to two race of an *E. polygoni*. Chankaew et al. (2013) did mapping of QTL governing powdery mildew resistance using four different resistant sources (V4718, RUM5, ATF3640 and VC6468-11-1A). The study revealed that the major QTL controlling powdery mildew resistance on LG9 in V4718, RUM5 and ATF 3640 are the same locus or linked. While qPMR-2 in LG9 of VC6468-11-1A was at a different location. The difference might be due to the distorted segregation of the markers around qPMR-2 (Kasettranan et al. 2010). As the TARM1 showed the score of PDI 4.7% , it is necessary to have a variety with higher level of resistance, pyramiding resistant genes/ QTLs from different resistant sources into DGGV2 is highly desirable and molecular markers tightly linked to these resistant genes/QTLs are required to achieve the goal in shortest possible time.

Authors' contribution

Conceptualization of research (SB); Designing of the experiments (SB); Contribution of experimental

materials (SB); Execution of field/lab experiments and data collection (PSK, APS); Analysis of data and interpretation (PSK, SB); Preparation of manuscript (PSK, SB).

Declaration

The authors declare no conflict of interest.

Acknowledgements

We would like to thank AICRP on MULLaRP, Main Agricultural Research Station, University of Agricultural Sciences, Dharwad for sparing the materials and field assistance. Also we thank University of Agricultural Sciences for financial assistance to Pooja S.K in the form of fellowship.

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