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IDENTIFICATION OF RAPD AND ISSR MARKERS LINKED FOR POWDERY MILDEW RESISTANCE IN MUNGBEAM (*Vigna radiata* L. WILCZEK.)

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ABSTRACT

The present study was aimed to identify RAPD and ISSR markers associated with powdery mildew resistance in mungbean (*Vigna radiata* L. Wilczek). Mapping population was developed by crossing mungbean genotypes BPMR-48 (resistance) X Kopergaon (susceptible). The DNA from four F_2 resistant and four susceptible plant along with their parents were isolated and purified. The resistant and susceptible bulk DNA of the mapping populations were analyzed with their parents. Out of 120 RAPD and 100 ISSR markers thirty one RAPD and six ISSR primers produced specific bands in resistant parent and which was absent in susceptible parent. These bands were also observed resistant bulk progenies and remained absent in susceptible progenies. To validate these primers, amplification of individual DNA samples of progenies and their parents were amplified and revealed that OPM-6_{810} and ISSR 834_{2626} present in all resistant F_2 plants as will as in resistant parent indicating that the markers OPM-6 810 and ISSR 834_{2626} may be associated with powdery mildew resistance in mungbean, which can be exploited for development powdery mildew disease resistant genotypes through MAS.

Key words : *RAPD and ISSR markers, powdery mildew mungbean.*

Mungbean (*Vigna radiata* (L.) Wilczek) (2n=2x=22) is 3rd important leguminous species, grown as pulse crop principally for its protein rich edible seeds which is originated from Indian sub-continent (1). In India it is grown on an area of 43.05 lakh ha with production of 20.70 lakh tonnes and 481 kg/ha productivity during the year 2016-17 (2). Among the plant protein sources, mungbean seed proteins form an excellent source of high quality and easily digestible good protein in Asian diet (3).

The production of mungbean in India is adversely affected by foliar diseases *viz. Cercospora* leaf spot and powdery mildew. Severe infection of powdery mildew can reduce yield of mungbean by between 20 and 40 per cent (4) 20 to 100 per cent (5). The crop incurs maximum damage when powdery mildew infects plants just before the flowering stage (6). Therefore, the development of varieties by incorporation of powdery mildew resistance genes into commercially cultivated varites is an effective strategy to check the disease. However in classical breeding programme selection of the line are influence by the environment. Thus the present investigation was carried out with an objective to identify the molecular markers associated with the powdery mildew resistance trait.

MATERIALS AND METHODS

The present investigation was carried out at All India Co-ordinated Pulses Improvement Project, MPKV, Rahuri and State Level Biotechnology Center, MPKV, Rahuri during 2009-11.

Development of mapping population : The powdery mildew resistant parent BPMR-48 was crossed with susceptible parent Kopergaon during summer 2009. The cross seed were sown during *Kharif* 2009 and true single F_1 plants was forwarded to F_2. The F_2 seeds were sown during summer 2010. The 200 F_2 individuals were tagged and leaf samples were collected and stored in -80^{0}C for genotyping. The seeds from each F_2 plant were collected separately and forwarded to F_3 generation for evaluation of powdery mildew reaction.

Phenotyping of mapping population : The F_3 seeds (25 numbers) from each F_2 plant were sown in two replication during Kharif 2010 with susceptible check Kopergaon after every 10 rows. The susceptible check was sown ten days earlier to increase spore load. The individuals in F_3 family were scored for powdery mildew reaction using 0-5 scale rating for each plant. Based on mean score, the homogenous resistance and susceptible F_2 plant were identified.

DNA extraction : Genomic DNA was extracted from the leaves of 15-day-old seedlings of seven parental genotypes grown in glasshouse, by modified CTAB (Hexacetyltrimethyl ammonium bromide) protocol of (7) with modifications.

In the present investigation, an attempt was made to identify the RAPD and ISSR marker associated with powdery mildew resistance, using bulked segregant analysis. One of the most time consuming requirements in marker development is the need to screen entire in mapping populations with

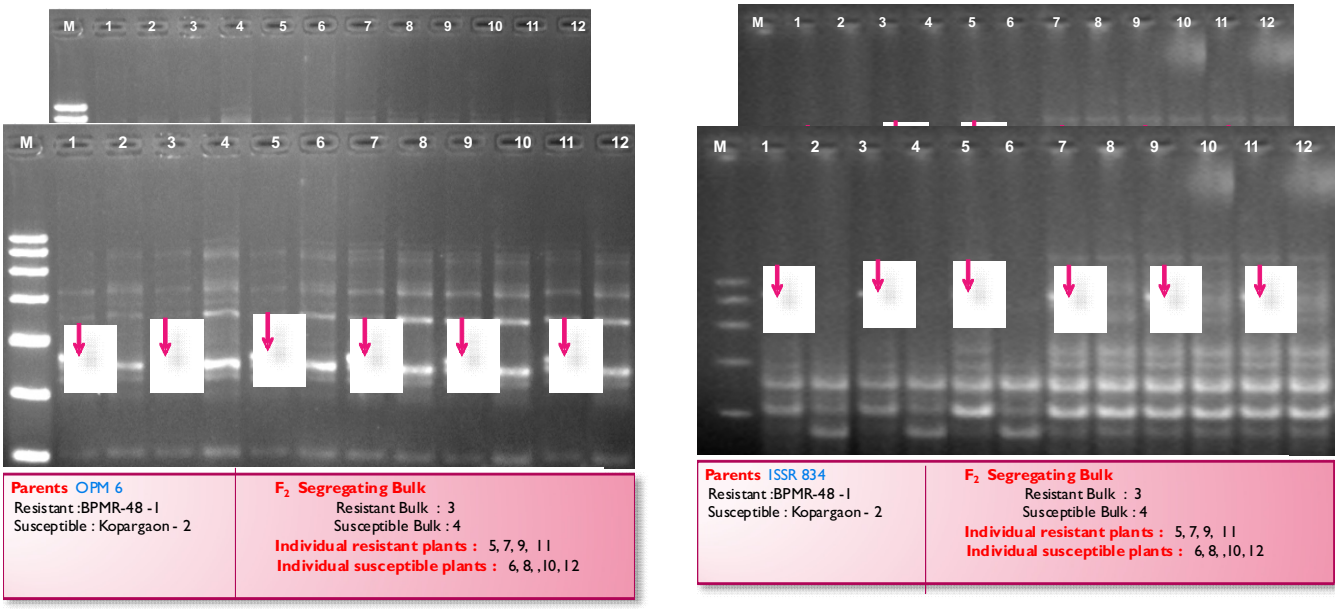

Fig.-1 : Cosegregation of RAPD (OPM-6_{810}) and SSR-834_{2626} markers with powdery mildew resistance in F_2 individuals of BPMR-48/Kopargaon of mungbean.

every primer has been reduced by bulked segregant analysis. The screening of contrasting bulks made from individuals of same phenotype of segregating population, suggests that testing the entire population is required only when polymorphism between the bulks are detected. The result in a considerable saving of time particularly when used with PCR based technique such as RAPD (8).

RESULTS AND DISCUSSION

Tagging of powdery mildew resistant genes with RAPD and ISSR markers : Bulked segregant analysis was employed to identify RAPD and ISSR markers linked to powdery mildew resistant gene of BPMR-48. A total 120 and 100 primers of RAPD and ISSR respectively were surveyed for identification of polymorphic markers between the DNA bulk of resistant and susceptible F_2 individuals and their parents. Thirty one RAPD and six ISSR primers produced specific band for resistant parent which were absent in susceptible parent. Out of 31 RAPD and six ISSR random primers one primers viz., OPM-6(RAPD) and ISSR 834 produced specific fragments in resistant parent and resistant bulk respectively, which were absent in the susceptible parent and bulk. Amplification of individuals DNA samples out of the bulk with putative markers, OPM-6 $_{810}$ and ISSR-834_{2626} only revealed polymorphism in all 4 each of F_2 resistant and susceptible plant (Fig.-1). The primers indicating that the markers OPM-6_{810} and ISSR-834_{2626} were associated with powdery mildew resistance in BPMR-48. The association of OPM -6 $_{810}$ and ISSR-834_{2626} RAPD and ISSR markers respectively with powdery mildew resistance can be employed for selection as well as screening of powdery mildew resistant genotypes. Bulked segregant analysis has been used to detect markers linked to many disease resistant genes including *Uromyces appendiculatis* resistance in common bean (9), leaf rust resistance barely (6) and angular leaf spot in common bean (10). The RAPD markers prepared by the polymerase chain reaction are used widely for mapping genes, because RAPD markers are more rapidly and easily detectable that RFLP markers (8). The RAPD marker has also been used effectively for tagging disease resistant (11) and virus resistant genes (12). However problem associated with the reproducibility of these RAPD and ISSR markers in different laboratories have been reported. SCAR (Sequence Characterized Amplified Region) represents an alternative to increase the reproducibility of these markers (13). Thus, on the basis of present finding the OPM-6 and ISSR-834 should be converted into SCAR for increasing reproducibility and efficiency of these markers for selection of powdery mildew resistant genotypes.

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