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Short communication

Molecular analysis of powdery mildew (*Erysiphe polygoni* DC) resistance in black gram (*Vigna mungo* L. Hepper)

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Black gram (*Vigna mungo* L. Hopper)) is a major pulse crop grown in tropical and sub tropical Asian countries. In India it covers an area of 3.17 m. ha, with annual production of 1.33 mt and average productivity of 419 kg/ha. [1]. Over the years the trend in its production and productivity has shown a decline in the country. Amongst different biotic and abiotic factors impact of powdery mildew (*Erysiphe polygoni* DC) disease might be the major constraints in its production causing 20-40 per cent yield losses in black gram [2]. Host plant resistance (HPR) is the most effective means to over come this.

Field screening of resistant and susceptible varieties requires specific environmental conditions for appearance of disease. Therefore, Marker Assisted Selection (MAS) is a helpful tool for identifying such materials even during off-season. The gene(s) for powdery mildew resistance in black gram appears to be an ideal candidate for tagging. The closely linked molecular markers can be used for quick and efficient selection of disease resistant lines in fixed and segregating populations. The present study aims to find out the Random amplified polymorphism DNA (RAPD), Inter simple sequence repeats (ISSR) and Amplified fragment length polymorphism (AFLP) markers showing polymorphism for resistant and susceptible cultivars through Bulked Segregant Analysis (BSA), followed by Co-Segregant Analysis (CSA) of *Vigna mungo* against powdery mildew and tagging of resistant gene(s).

The study was conducted at Research Farm of Indira Gandhi Krishi Vishwavidyalay (IGKV), Raipur and Bhabha Atomic Research Centre (BARC), Mumbai in *rabi* 2002-03 and *kharif* 2003. Powdery mildew susceptible genotype Nayagarh local and resistant genotype LBG 17 were used as parents. The F_1 and onward generations of the cross were raised at gamma field of BARC, as well as at lGKV, Raipur, were screened against powdery mildew resistance. Pedigree of each single plant was maintained. For the molecular study F_8 progenies were used as Recombinant Inbred Lines (RIL).

DNA was extracted from the seedlings of LBG 17 (resistant) and Nayagarh local (susceptible) and F_8 progenies of their cross by the method described by the Dellaporta *et al*. [3]. RAPD, ISSR and AFLP analyses were performed to identify polymorphism in parents, bulks of 10 powdery mildew resistant and susceptible plants in F_8 generation of the mentioned cross.

RAPD PCR amplification was performed with random decamer primers. Amplification was performed in a 25 µl reaction volume, which contains 10 mM Tris HCl (pH 8.3), 50 mM KCl, 2.5 mM $MgCl_2$, 0.01% gelatin, 0.2 µM primers, 50 µg of total genomic DNA and 0.5 units of *Taq* DNA polymerase [4]. Amplification was performed in an eppendorf Master Cycler PCR (Eppendorf Netherler-Hinz GMBH, Hamburg). The amplification conditions were an initial denaturation at 94°C for 4 min and 45 cycles at 1 min denaturation at 94°C, 1 min. annealing at 37°C, 2 min. polymerization at 72°C, and 5 min. final extension at 72°C. The amplified products were loaded on 1.5% agarose gel by using IX TBE buffer at 75 v for 3 hr. The gels were

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stained in 0.5 µg/ml Ethidium bromide solution and visualized under UV light in transilluminator. The size of amplified products were determined with Eco RI + Hind III double digested λ DNA marker.

ISSR amplification reaction was carried out using 25 µl volume containing 50 ng of template DNA, 0.5 units of *Taq* DNA polymerase, 0.2 mM each of dNTPs, 1.0 µmole primer in 1 X TBE (Tris Boric acid EDTA) reaction buffer containing 10 mM Tris HCl (pH 8.3), 50 mM KCl, 2.5 mM $MgCl_2$, 0.01% gelatin. The amplification conditions were as 90°C for 4 minutes, followed by 5 cycles at 94°C for 30 sec., 55°C (–1°C) for 45 sec., 72°C, 2 minutes, and for 94°C, 30 sec., 50 °C for 45 sec., 72°C for 2 minutes and final extension at 35 cycles at 72 °C for 7 minutes.

In AFLP analysis about 150 ng/ µl of genomic DNA from both the parents and the resistant and susceptible bulks comprising were digested with the restriction enzymes *Eco* R I and *MSe* I. The digested DNA fragments were ligated to *Eco* R I and *MSe* I adapters with T_4 DNA ligase enzyme. The restriction-ligation products were used as a primary template DNA for the first Polymerase Chain Reaction (PCR) step (pre-amplification) with *E-A* and *M-C* primers. The temperature profile for pre amplification was 94^oC for 30 seconds 20 cycles followed by 65^oC 1 minute 20 cycles and 72^oC 1 minute 20 cycles. The ligated products were used as primary template DNA for the preamplification with E- A and M- C primers. The initial PCR products were used in the selective PCR amplification with 7 primer combinations (E-A _ and M-C_ primers) having two selective nucleotides at the 3' end of each primer. The temperature profile for selective amplification were as follows, 94^0C, 65^0C each for 30 seconds 1cycle, 72^oC for 1min 1cycle, followed by 94^0C, 65^0C each for 1 minute 12 cycles, 72^oC for 1 min 12 cycles and 94^0C, 65^0C each for 30 seconds 23 cycles, 72^oC for 1min 23 cycles. The denatured selective amplified PCR products were run on 6% denaturing polyacrilamied gel and subjected to silver staining [5].

RAPD, ISSR and AFLP analyses were carried out to identify the molecular markers closely linked with powdery mildew resistance in black gram. Eighty-three RAPD primers were screened to observe polymorphism between resistant and susceptible parents which produced 397 discrete bands, when resolved on agarose gel electrophoresis. These primers produced different amplification patterns and the amplified fragments ranged from 400 bp to 2500 bp in size. Out of 397 bands, 53 showed polymorphism, and results of only 24 primers were found reproducible. In bulked segregant analysis, twenty four RAPD primers out of eighty-three were detected for resistant and susceptible parents. Amplification pattern of 18 RAPD primers revealed polymorphic bands in resistant lines whereas, six RAPD primers exhibited polymorphism for susceptible strains only. Primer OPN 14 produced bands of 800 bp, 600bp, 300bp and below whereas, primers OPN 19 produced bands of higher molecular weight of 1500 bp, 1200 bp, 700 bp and below. Similarly, primer OPI 19 produced the bands of size 800 bp, 600 bp, 500 bp and below (Fig. 4) and primer OPN 7 produced a 2 kbp band specific to susceptible parent Nayagarh local (Fig. 3) and susceptible bulk advanced for co-segregation analysis. Although results were satisfactory but since RAPD has low reproducibility, dominant nature, amplification of multiple loci and contrasting results regarding polymorphism detected in different populations, hence not always efficacious in MAS. Hence, the similar set of material was tested and amplified with four ISSR primers. ISSR 809 produced bands size 400 bp, 300 bp, 200 bp, 100 bp and below while ISSR 811 produced bands of 1400 bp, 400 bp, 300 bp, 200 bp and below (Fig. 2). Primer ISSR 808 produced among various bands, with a 1300 bp band specific to the resistant parent and the resistant bulk (Fig. 1). The same primer produced a 1200 bp band specific to the susceptible parent, which could not be evidenced in susceptible bulk. This primer was advanced for co-segregation analysis in which a 1300 bp band specific to the resistant parent was observed in 9 out of 10 individual resistant plants, depicting a

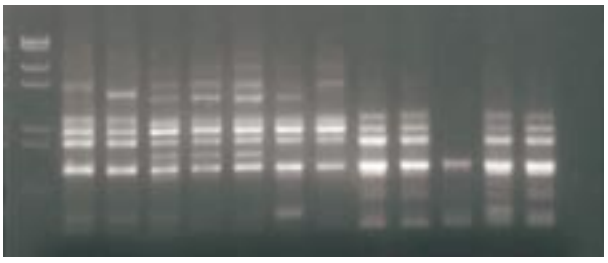

Fig. 1. Results of Primer ISSR 808 (1-5 N)

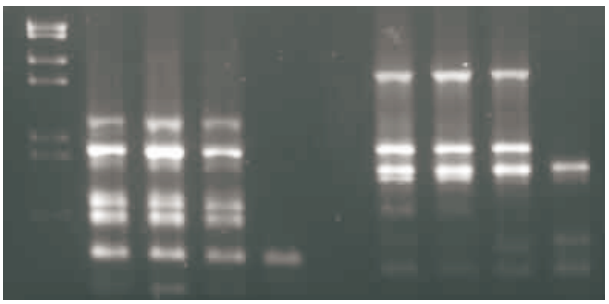

Fig. 2. Results of Primer ISSR 809-811

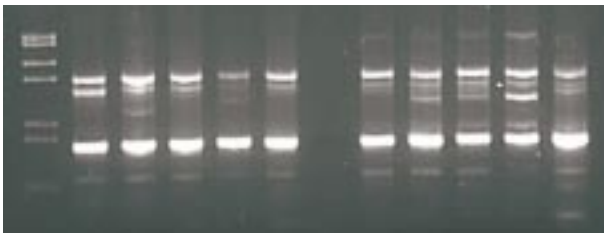

Fig. 3. Gel of primer OPN 7

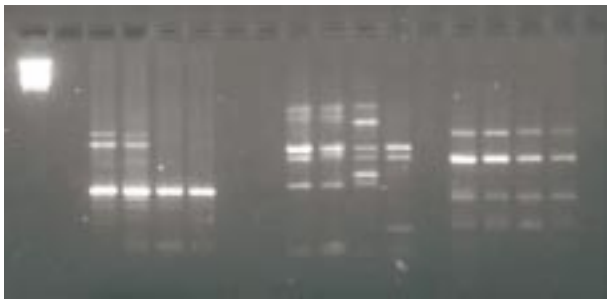

Fig. 4. Gel of Primers OPN 14, 19 and OPI 19

close linkage between the marker genes. Similar work on different crops are reported by different workers [6-8].

AFLP based bulk segregation analysis carried out with seven primer combinations generated a total of 258 bands, of which 38 were polymorphic. For each of the seven primer combinations the resistant and susceptible parents had a unique profile. Out of seven primer pairs used, five pairs showed polymorphism between the resistant and susceptible parents while similar banding pattern observed in the resistant and susceptible bulks.

Primer combinations of E - AAG / M- CTC produced two polymorphic bands of 300 bp and 800 bp in resistant parent LBG 17 as well as in the resistant bulk. Other four primer combinations for E- ACC / M- CAT, E- ACC / M- CAG, E- ACG / M- CAT and E- AGG / M- CTC also revealed a great deal of polymorphism between the parents and respective bulks. Two primer combinations of E- AAC / M- CAT and E- AAC / M- CAA could not reveal a great deal of polymorphism between the parental genotypes.

Therefore, the RAPD primers OPN 14, OPN 19, OPI 19 and OPN 7 can be used as markers for powdery mildew susceptible population. ISSR primers ISSR 808, specific to the resistant parent (1300 bp) and the resistant bulk, whereas same primer produced a band specific to the susceptible parent (1200 bp). AFLP primer combinations of E- AAG / M -CTC can be used as marker for resistant parent LBG 17 as well as in the resistant bulk since they produced bands of 300 bp and 800 bp.

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