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Short Communication

Inheritance pattern and validation of RGA marker for powdery mildew resistance in sesame

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Abstract

The inheritance of powdery mildew resistance in sesame was studied in the segregating generations of a cross involving Rama (susceptible) and VRI-1(resistant) genotypes. The F_2 plants segregated in a ratio of 9 (resistant):7 (susceptible) indicating a digenic mode of inheritance with complementary epistasis for powdery mildew resistance in sesame. The RGA marker (h2_13m22a) reported to be linked to the powdery mildew resistance in sesame found to produce the expected allele (280bp) in the resistant parent (VRI-1) and the resistant bulk, while the allele was absent in the susceptible parent and the susceptible bulk. In F_2 generation plants, the marker h2_13m22a co-segregated with the resistant plants indicating its tight linkage with the gene for powdery mildew resistance. The marker would be useful in marker assisted selection of plant in the breeding for powdery mildew resistance in sesame.

Key words: Inheritance, powdery mildew resistance, RGA marker, *Sesamum indicum*

Sesame (*Sesamum indicum* L., 2n=26), commonly known as "Queen of Oilseeds" is a self-pollinated crop and belongs to the family Pedaliaceae. It is a short duration crop grown throughout the year and fits well into various cropping sequences/systems (Imran et al. 2018). World production of sesame seeds was estimated at 6.016 million tons, led by Tanzania, Myanmar and India (FAO, 2020). In India, it is mainly cultivated in the states of Gujarat, Rajasthan, Madhya Pradesh, Tamil Nadu, Odisha, Andhra Pradesh, Uttar Pradesh, Karnataka and Chhattisgarh. Powdery mildew is the most devastating disease of sesame causing considerable yield loss and is almost appear in all sesame growing areas with high rainfall and humidity coupled with low night temperature (Mallaiah et al. 2016). The study of the inheritance pattern of the disease along with mapping the resistance gene(s) and tagging these against the disease can help the breeding programme much easier and reliable. The use of RGA-markers is comparatively recent and efficient for genotyping of disease reactions (Panigrahi et al. 2016). Hence, the present investigation was undertaken to study the inheritance pattern of powdery mildew (PM) resistance and validate available RGA markers linked with powdery mildew resistance in sesame.

To study the genetics of powdery mildew resistance, a cross was made between resistant genotype VRI-1 and susceptible genotype Rama during 2017-18 at OUAT, Bhubaneswar. The F_1 plants along with the parental genotypes were raised during *kharif*, 2018 and obtained the F_2 seeds. The F_2 plants were raised in the field during *rabi*, 2018-19 under artificial inoculation for assured disease expression. The level of resistance/susceptibility of F_2 plants was calculated by the percent of disease index (PDI) and accordingly the plants were grouped as resistant and susceptible one. The chi-square ($\div^2$) test was applied for testing the goodness of fit for the expected segregation ratio in F_2.

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For molecular genotyping of the plants, the genomic DNA of the parental genotypes, F_1 and F_2 plants were isolated from young tender leaves at the seedling stage using the standard CTAB method (Doyle and Doyle 1987). The DNA was quantified using a UV-VIS Nanodrop-2000 spectrophotometer and their quality was checked by using the ratio of absorbance at 260nm and 280nm. One pair of resistance gene analogue (RGA) marker i.e., h2_13m22a-F/ h2_13m22a-R (Table 1) was used in PCR for detection

Table 1. Details of RGA primers

Primer	Nucleotide sequence	Annealing temperature (T_m)	Fragment size(bp)
h2_13m22a-F	TCAAACTCAA GCCACCACAA	47°C	280
h2_13m22a-R	GCTCGAGTCA TGGAGGGTAA		

of polymorphism as suggested earlier (Pujar and Patil, 2016). PCR amplification was performed in a reaction volume 25µl containing 1X reaction buffer, 2.5mM each of dNTPs, 10ng of the primer pair, 50ng of genomic DNA and 1 unit of *Taq* polymerase. DNA amplification was carried out in the Bio-Rad Thermocycler, programmed for 5min at 95°C for initial denaturation, 40 cycles of 1min at 94°C for denaturation, 30 sec. at annealing temperature and 2min at 72°C for synthesis and final extension for 10 min at 72°C followed by storing at 4°C till loading to the agarose gel. The amplified products were separated in 1.8% agarose gel and visualized by 0.5mg/ml of ethidium bromide staining. The gels were scanned by the gel doc system for detection of RGA primer specific alleles. The size of the amplicons was determined by comparing with the lambda DNA ladder (500bp) with known size (bp) fragments.

In the present study, all individual plants of the sesame variety "Rama" showed susceptibility to powdery mildew with PDI value 85%, while the resistant parent cv. VRI-1 had a PDI value 5%. All the F_1 plants resulted from the cross (Rama x VRI-1) appeared to be resistant with an average PDI of 10.1%. Out of the total 130 F_2 plants, 71 plants appeared to be resistant while 59 plants appeared to be susceptible ones (Table 2). Thus, the F_2 plants found to segregate in a ratio of 9(resistant):7(susceptible) indicating a digenic mode of inheritance with complementary epistasis i.e., complete dominance at both gene pairs; however, when either gene is homozygous recessive, it hides the effect of the other gene. A similar result reported earlier by Raja Ravindran and Rathinam (1996), who studied F_2 progenies of 24 cross combinations involving Co-1 as a resistant parent and reported resistant and susceptible plants to segregate in the ratio of 9:7 indicating resistance to be governed by two pairs of dominant genes showing complementary gene action.

Resistance gene analogue (RGA) markers are designed from conserved repeat motifs of the nucleotide-binding site- leucine-rich repeats (NBS-LRR) region of plant "R" gene families and therefore, these can effectively trace the disease resistance genes against fungus, bacteria and viruses in a set of genotypes. In the present study the RGA marker (h2_13m22a) produced the expected 280bp amplicon (allele) in the resistant parent as well as resistant bulk, while the susceptible parent and susceptible bulk did not produce any amplification product indicating close linkage of the marker with the disease resistance. The F_1 plants, which were resistant, also showed the expected 280bp allele of the RGA (Fig. 1). The RGA marker found to co-segregate with the powdery mildew resistance in the F_2 plants and almost all the F_2 resistant plants showed the expected amplification product (280bp). The present study demonstrated that the RGA marker (h2_13m22a) is strongly associated with the powdery mildew resistance and hence would

Table 2. Mode of Inheritance of powdery mildew resistance in sesame

Materials	Total plants	PDI (%)	Observed frequencies		Expected frequencies		Ratio (R:S)	Chi-square	Probability value
			R	S	R	S			
Rama(Susceptible parent)	10	82.0	-	10	-	-	-	-	-
VRI-1(Resistant parent)	10	5.0	10	-	-	-	-	-	-
F_1(Rama x VRI-1)	10	6 -14	10	-	-	-	-	-	-
F_2(Rama x VRI-1)	130	3 -99	71	59	73.1	56.9	9 : 7	0.137*	0.7-0.8

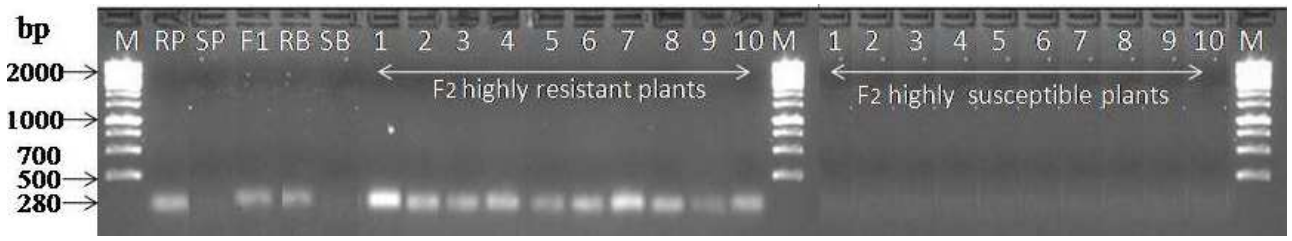

Fig. 1. DNA profile of parents and cross (Rama x VRI-1) in F_1 and F_2. M- 500 bp Mol. Marker, RP-Resistant parent (VRI-1), SP-susceptible parent (Rama), F_1-1st Filial generation of the cross, RB-Resistant bulk, SB-Susceptible bulk, Lane 1-10: Resistant and susceptible plants respectively

be useful in marker-assisted selection for the development of resistant cultivars in sesame.

Authors' contribution

Conceptualization of research (BB & TRD); Designing of the experiments (BB, TRD, MK); Contribution of experimental materials (BB, MK); Execution of field/ lab data collection (MK, MD, SKT); Analysis of data and interpretation (BB, TRD, SKT); Preparation of the manuscript (TRD, BB, MK)

Declaration

The authors declare no conflict of interest.

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