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Molecular mapping and tagging of powdery mildew tolerance gene(s) in sesame (*Sesamum indicum*)

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ABSTRACT

Sesame (*Sesamum indicum* L.) is an important oilseed crop of India. Powdery mildew causes significant yield loss. Identifying sources of tolerance / resistance, studying inheritance of powdery mildew and mapping of disease tolerance gene(s) are essential for development of high yielding varieties with tolerance to disease. Thirty accessions were screened for tolerance to powdery mildew, 18 were found susceptible while, 12 tolerant. Among the genotypes, Swethatil was found to be highly susceptible (87.65 % PDI (Percentage Disease Index)) while PKDS 37 was highly tolerant (30.86 % PDI). The mapping population for tagging the gene(s) conferring tolerance to the disease was developed of the cross between a tolerant variety PKDS 37 and the susceptible parent Swethatil. Study of segregating population revealed that tolerance to powdery mildew was governed by two independent recessive genes in complementary epistasis mode. Sixty eight RAPD markers were found polymorphic between the parents and of them, four markers, viz. OPA 7, OPAE 6, OPAE 11 and OPM 12 were clearly distinguished the susceptible from the tolerant bulks by bulked segregant analysis. Assigned to one linkage group two markers OPAE 6 and OPM 12 have been found to be tightly linked to powdery mildew tolerance gene *Pm 1* at a distance of 3.6 cM and 4.8 cM respectively on either side. The markers flanking the trait can be used in marker assisted selection/breeding for resistance to the disease and this region is a potential target to the fine mapping.

Key words: Mapping, Powdery mildew, RAPD, *Sesamum*, Tagging

Sesame (*Sesamum indicum* L.) regarded as the 'Queen of Oilseeds' is the most ancient oilseed crop of the world and is being cultivated in Asia since 5000 years (Joshi 1961 and Weiss 1971). The quality of its oil being of high nutritional and therapeutic value. The antioxidants 'sesamin' and 'sesmolin' enhance the keeping quality of oil by making it resistant to rancidity. Being the much sought after edible oil source world over, it is its diverse utility value that has made today sesame an important commodity in the international trade. India and China together account for over 70% of the global production. Planted over an area of 1.85 mha., India is the largest sesame growing country in the world producing 0.64 m tonnes, productivity wise it is among the lowests with 345 kg/ha (CMIE 2010).Despite its shorter life cycle, suitability to different cropping systems and land types, adaptation to moisture stress and low input management conditions, sesame's contribution to the country's oilseed production is left sadly much to be desired. Inherently low yield potential apart, biotic and abiotic stresses constitute the major yield destabilizing factors of the crop. Powdery mildew caused by many species/strains of the devastative fungal pathogen, *Oidium* sp. is common all over the sesame growing areas, especially in Andhra Pradesh and Tamil Nadu and causes as high as 50% yield losses under favourable conditions. Host plant resistances although, is the preferred strategy to protect the crop from the disease, unfortunately there is hardly any report on sources of true resistance, genetics of resistance and rapid and reliable screening techniques. Whatever reported to be resistant to the disease, had been on the basis of field screening under natural disease incidence. Also such findings had been in the absence of information on race spectrum and area specific race(s). The foregoing thus call for expeditious development and deployment of innovative breeding/selection approaches to find meaningful solution to them. Practically a virgin crop as for use of molecular tools for improvement, sesame is

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Table 1 Genotypes screened for tolerance to powdery mildew

Genotype	Origin	Source	Genotype	Origin	Source
Swethatil	India	ANGRAU	RT 54	India	JNKVV
Rajeswari	India	ANGRAU	Co 1	India	JNKVV
Chandana	India	ANGRAU	Paiyur 1	India	JNKVV
Gouri	India	ANGRAU	SVPR 1	India	JNKVV
Madhavi	India	ANGRAU	VRI 1	India	JNKVV
YLM 11	India	ANGRAU	TMV 3	India	JNKVV
YLM 17	India	ANGRAU	TMV 4	India	JNKVV
JCS 344	India	ANGRAU	TMV 5	India	JNKVV
JCS 399	India	ANGRAU	TMV 6	India	JNKVV
JCS 9426	India	ANGRAU	Uma	India	JNKVV
RT 338	India	JNKVV	MKN 5	India	NBPGR
AT 101	India	JNKVV	MKN 6	India	NBPGR
PKDS 37	India	JNKVV	EC 346125	Exotic	NBPGR
TAC-89-309	India	JNKVV	EC 346125/1	Exotic	NBPGR
OSC/36-2002	India	JNKVV	EC 377025	Exotic	NBPGR

constrained with many problems, solution to which, requires biotechnology intervention. Keeping in view the seriousness of the disease stress, basic information gap on the level and reliable sources of resistance and genetics of resistance the present investigation was carried out for identifying sources of tolerance, studying the genetics of powdery mildew and mapping of disease tolerance gene(s).

MATERIALS AND METHODS

Thirty genotypes comprising exotic accessions (3), India bred improved varieties (16) and advanced breeding lines (11) constituted the experimental material for screening for resistance to powdery mildew. These genotypes were screened for resistance/tolerance to the disease under field conditions raising the susceptible check, Swethatil as infector rows all around at SRTC, Rajendranagar, Hyderabad (Table 1). Each of the genotypes was sown in two rows of 3 m length with 30 cm × 15 cm spacing. The crop was raised adopting the recommended package of practices (Anon 1985). The screening was done (50 days after sowing) when the disease incidence was maximum on the susceptible check. To make the disease screening still more effective, the entries were challenged artificially by treating with the inoculum of mycelial spores prepared from the diseased susceptible check. Observation on disease reaction was made on five randomly selected plants in each entry. Nine leaves were scored in each plant, three each from the apical, middle and basal regions and all of them were graded. The disease intensity was scored adopting the following 0-9 grade (TNAU 1980). (Table 2). Level of resistance/susceptibility of the entries to the disease was determined by Percentage Disease Index (PDI) following the formula of McKinney (1923).

$$\text{PDI} = \frac{\text{Sum of grades}}{\text{Total number of leaves analyzed} \times \text{Maximum disease grade}} \times 100$$

On the basis of the PDI, the entries were grouped into four categories (Raja Ravindran 1990) (Table 3).

To study the genetics of powdery mildew tolerance and for mapping and tagging of powdery mildew tolerance gene(s), Swethatil, a high yielding variety from RARS, Jagtial and PKDS 37 an accession received from Jawaharlal Nehru Krishi Vishwa Vidyalaya (JNKVV), Jabalpur respectively

Table 2 Grading of powdery mildew disease intensity

Disease grade	Description
0	No lesions or specks
1	Small sized powdery specks infecting less than 1 % leaf area
3	Enlarged irregular powdery growth covering 1-5 % leaf area
5	Powdery growth to form big patches covering 5-25 % leaf area
7	Powdery growth covering 25-50% leaf area followed by yellowing
9	100% leaf area covered with powdery growth, yellowing and dropping of infected leaves

Table 3 Classification of the entries based on Per cent Disease Index (PDI)

PDI	Disease reaction
0	Immune (I)
1- 30	Resistant (R)
31-50	Moderately Resistant (MR)/ Tolerant (T)
>51	Susceptible (S)

susceptible and highly tolerant to powdery mildew were chosen as parents for hybridization. Crosses were affected at the Seed Research and Technology Centre, Rajendranagar. The F_1s were selfed as well as backcrossed with the parents to produce F_2 and BC_1 (B_1 + B_2) respectively. Thus the mapping population comprised F_2 (120 plants), B_1 and B_2 generations (50 plants each). All the six populations, viz. P_1, P_2, F_1, F_2, B_1 (Backcross with PKDS 37) and B_2 (Backcross with Swethatil) were screened for powdery mildew tolerance at the College farm, College of Agriculture, Rajendranagar, Hyderabad.

DNA extraction from sesame was difficult due to presence of contaminants such as polyphenol and polysaccharides. These compounds have also been reported to cause difficulty in DNA purification in other plant species and inhibit enzyme action. Therefore, five different DNA extraction protocols were tried in the present study to obtain high quality and pure DNA (Table 4) and of them the method developed by Porebski *et al.* (1997) found efficient was used. A set of 160 operon RAPD decamer primers (OPA, OPAE, OPC, OPD, OPH, OPM, OPP, OPR, OPS and OPT kits, Operon technologies, Almaeda, CA designated by an "OP" prefix and then the kit letter and primer number) were screened in the parents, Swethatil and PKDS 37. Following parental polymorphism using appropriate RAPD primers, closely linked markers to resistance were identified by bulked segregant analysis (BSA). BSA is a method used for rapidly identifying markers linked to any specific gene or genomic region. These markers which showed same polymorphism in parents and susceptible and tolerant bulks were used to screen the segregants in the mapping populations (F_2–120 plants, B_1 and B_2–50 plants each). Molecular map was constructed using the MAPMAKER/EXP version 3.0 (Lincoln *et al*. 1992) following Kosambi Mapping Function (Kosambi 1944) and MapDisto software version 1.7b 132 (Lorieux 2006). Linkage group was determined using 'group' command with LOD (Logarithm of odds ratio) score of 3 and recombination fraction of 0.4.

RESULTS AND DISCUSSION

Of the 30 entries evaluated, 18 were susceptible to the disease to different degrees (PDI ranging from 51.0 to 87.5%) while, 12 genotypes recorded tolerance reaction (PDI from 30.86 to 48.15%) (Table 5). Among the susceptible genotypes, Swethatil scoring the highest PDI (87.65%) was identified as the highly susceptible genotype. The genotype PKDS 37 recording the lowest PDI (30.86%) was identified as the most tolerant to the disease. None of the genotypes evaluated showed immune response to powdery mildew. While the findings broadly agree with many earlier reports by pathologists and breeders that no reliable source of resistance/ immunity could be found (Karunanithi and Dinakaran 1996), a few have reported existence of resistant sources (Ganesh *et al.* 1992 and Gopal *et al*. 2005). The contradictory findings could be due to differences in the disease rating methodology, screening method, species/and race spectrum as the reports are from different regions. The differential reaction of genotypes to the pathogen at different regions however, need to be studied by pathologists for racial/species differences.

To study the inheritance pattern of the powdery mildew tolerance, six populations, viz. P_1, P_2, F_1, F_2, B_1 and B_2 were evaluated for powdery mildew tolerance under field

Table 4 Isolation of DNA of Swethatil by different extraction protocols

Isolation Method
Murray and Thompson (1980)
Porebski *et al.* (1997)
Lin *et al.* (2001)
Laurentin and Karlovsky (2006)
Sigma DNA extraction kit

Table 5 Reaction of 30 genotypes to powdery mildew

Genotype	Source	PDI (%)	Reaction
Swethatil	ANGRAU	87.65	Susceptible
Rajeswari	ANGRAU	75.31	Susceptible
Chandana	ANGRAU	80.25	Susceptible
Gouri	ANGRAU	66.67	Susceptible
Madhavi	ANGRAU	70.37	Susceptible
YLM 11	ANGRAU	71.60	Susceptible
YLM 17	ANGRAU	72.84	Susceptible
JCS 399	ANGRAU	51.85	Susceptible
JCS 9426	ANGRAU	67.90	Susceptible
JCS 344	ANGRAU	82.72	Susceptible
Paiyur 1	JNKVV	55.56	Susceptible
SVPR 1	JNKVV	58.02	Susceptible
VRI 1	JNKVV	56.79	Susceptible
TMV 3	JNKVV	65.43	Susceptible
TMV 4	JNKVV	85.19	Susceptible
TMV 5	JNKVV	80.25	Susceptible
TMV 6	JNKVV	77.78	Susceptible
Uma	JNKVV	54.32	Susceptible
RT 338	JNKVV	40.74	Tolerant
AT 101	JNKVV	45.68	Tolerant
PKDS 37	JNKVV	30.86	Tolerant
TAC-89-309	JNKVV	44.40	Tolerant
OSC-36/2002	JNKVV	48.15	Tolerant
RT 54	JNKVV	43.21	Tolerant
EC 346125	NBPGR	32.10	Tolerant
EC 346125/1	NBPGR	35.80	Tolerant
EC 377025	NBPGR	37.03	Tolerant
MKN 6	NBPGR	33.30	Tolerant
Co 1	JNKVV	38.27	Tolerant
MKN 5	NBPGR	34.57	Tolerant

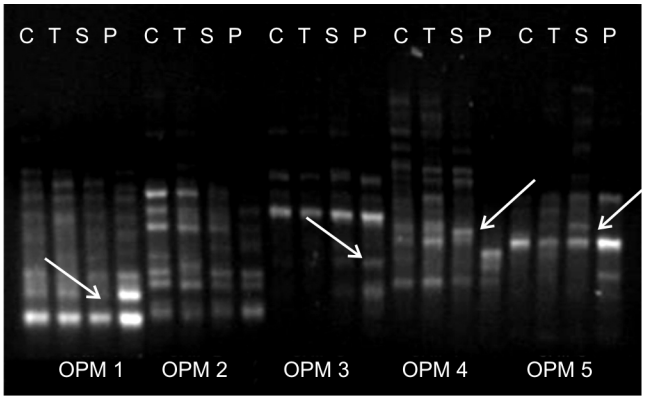
PDI, Per cent Disease Index

Table 6 Mode of inheritance of powdery mildew tolerance in various segregating generations of the cross Swethatil × PKDS 37

Material	Total plants	PDI (%)	Observed frequencies		Expected frequencies		Ratio T:S	χ^2	Probability
			Tolerant (T)	Susceptible (S)	Tolerant (T)	Susceptible (S)			
Swethatil (P1)	10	87.65		10					
PKDS 37 (P2)	10	30.86	10						
Swethatil × PKDS 37	10	60.49		10					
F2	120		52	68	52.5	67.5	7:9	0.007	0.5-0.7
F1 × PKDS 37 (B1)	50		35	15	37.5	12.5	3:1	1.16	0.1-0.2
F1 × Swethatil (B2)	50		2	48		50			

conditions using the standard disease screening methodology as detailed under Materials and Methods. The PDI of the individual plants in the F_2 ranged from 80.24 to 34.56%, while it was in the range of 82.71 to 30.86% and 82.71 to 48.14% in B_1 and B_2 respectively. Based on PDI, the F_1 of this cross showed susceptible reaction (60.49% PDI) indicating susceptibility to be dominant over resistance, whereas the F_2 population segregated in the proportion of 68 susceptible to 52 tolerant plants. Chi-square analysis showed the observed ratio to confirm the expected ratio of 7:9 ratio (tolerant: susceptible) (Table 6). It indicates that the tolerance was governed by two independent recessive genes with complementary epistasis. The results corroborate with the findings from the ratios in the study of the backcross populations, wherein, i e the backcross of F_1 with susceptible parent (Swethatil) without segregating all the plants were susceptible as expected, while the backcross population of F_1 with the tolerant parent (PKDS 37) segregated with a good fit to 3 tolerant : 1 susceptible ratio, i e 35 tolerant:15 susceptible. These findings are quite contrary to those reported by earlier workers. Krishnaswami *et al.* (1983) and Raja Ravindran and Amritha Devarathinam (1996) have reported powdery mildew tolerance to be controlled by two pairs of dominant genes showing complementary gene action.

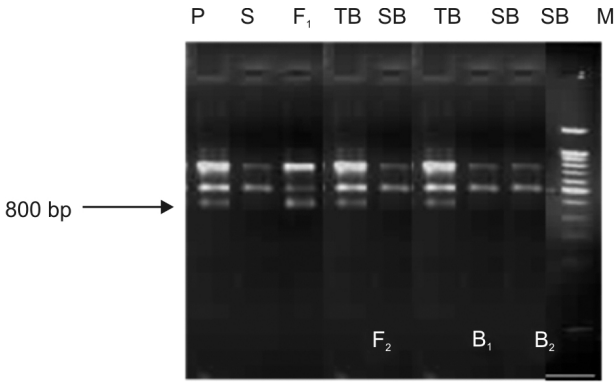
For mapping and tagging of powdery mildew tolerance gene(s), a set of 160 operon RAPD 10-mer primers (OPA, OPAE, OPC, OPD, OPH, OPM, OPP, OPR, OPS and OPT kits) were screened between the susceptible parent Swethatil and the tolerant parent PKDS 37. Of the 330 repeatable amplified fragments produced by these 160 primers, 68 showed polymorphism between the parents (Fig 1). Each polymorphic primer was tested at least twice to determine if both the polymorphism and banding pattern were reproducible. Polymorphic RAPD primers, closely linked to tolerance were identified by BSA, which provides a rapid, technically simple alternative for identifying markers linked to specific genes/traits. The 68 markers identified to be polymorphic between parents were used for bulked segregant analysis of the tolerant and susceptible bulks from F_2 and B_1 population and only susceptible bulk from B_2 population. Out of the 68 markers, four, viz. OPA 7, OPAE 6, OPAE 11 and OPM 12 showed polymorphism between the susceptible



S - Swethatil; P - PKDS 37

Total primers	5
Polymorphic primers (Between S and P)	OPM 1, OPM 3, OPM 4 and OPM 5

Fig 1 Gel picture showing polymorphism between parents (Swethatil and PKDS 37)



S – Swethatil
F_1 – Swethatil × PKDS 37
B_1 – F_1 × PKDS 37
SB – Susceptible bulk
P – PKDS 37
B_2 – F_1 × Swethatil
TB – Tolerant bulk

Fig 2 Bulked Segregant Analysis showing RAPD marker (OPM 12) linked to powdery mildew tolerance

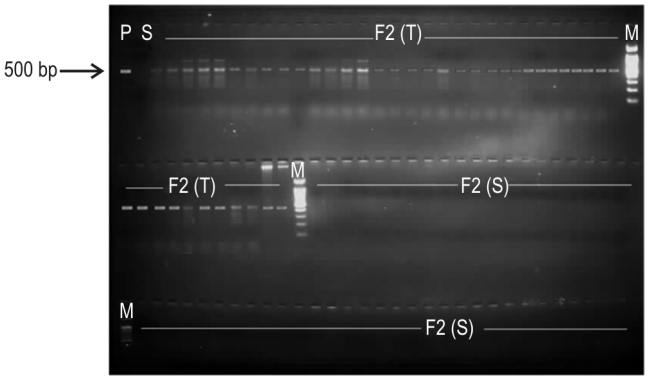


Fig 3 RAPD amplification pattern with OPAE 6 marker in F_2 population (Swethatil × PKDS 37)

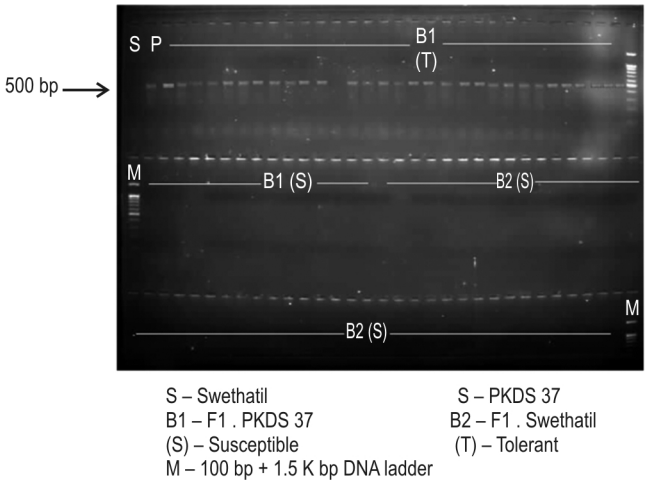


Fig 5 RAPD amplification pattern with OPAE 6 marker in backcross populations

and tolerant bulks. OPA 7 (5' – GAAACGGGT -3') amplified a fragment of about 1200 bp length in the tolerant parent (PKDS 37) and the tolerant bulks. OPAE 11 (5'-AAGACCGGGA -3') amplified a fragment of 1100 bp length in the susceptible bulks and Swethatil. A DNA fragment of 500 bp was amplified by the primer OPAE 6 (5'-GGGGAAGACA -3') in the tolerant bulks and PKDS 37 while, OPM 12 (5'-GGGACGGGT -3') primer amplified a fragment of 800 bp length in PKDS 37 and tolerant bulks (Fig 2).

The four markers, which showed polymorphism in the parents as well as the susceptible and tolerant bulks (i e putative linkage), were used to screen individually 120 plants of the F_2 population. Individual F_2 genotypes were scored in terms of molecular markers on the basis of presence (1) and absence (0) of the amplified DNA band. Individual B_1 and B_2 plants were screened with polymorphic markers identified in BSA. Of four molecular markers analyzed, only two, viz. OPAE 6 (5'- GGGGAAGACA -3') and OPM 12 (5'-GGGACGGGT -3') gave specific DNA bands of 500 bp length and 800 bp length in all the tolerant plants but remained absent in the susceptible plants in all the three generations. These results showed the markers OPAE 6 and OPM 12 to be linked to powdery mildew tolerant genes (Fig 3, 4 and 5)

Molecular linkage map was constructed with 2 RAPD markers OPAE 6 and OPM 12 using the MAPMAKER/EXP following Kosambi Mapping Function and MapDisto software. Both the markers were mapped to one and the

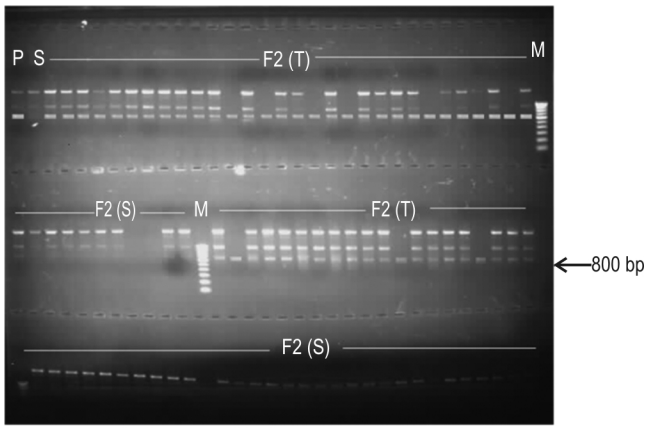


Fig 4 RAPD amplification pattern with OPM 12 marker in F_2 population (Swethatil × PKDS 37)

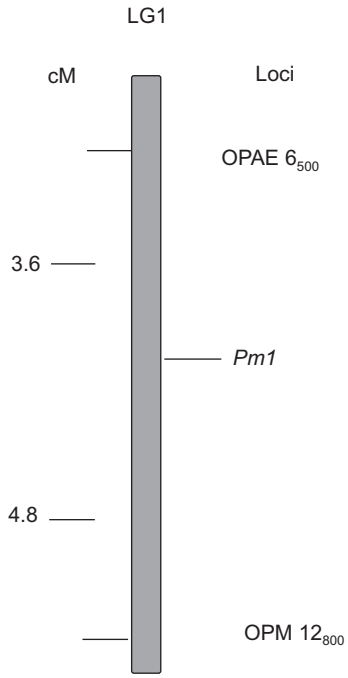


Fig 6 Molecular mapping of powdery mildew tolerance gene

same linkage group at a distance of 8.4 cM between them. The tolerance gene designated as *Pm1* was located at a distance of 3.6 cM from the marker OPAE 6 on oneside and of 4.8 cM from the marker OPM 12 on the other side (Fig 6). The gene *Pm 1* was found to be a major gene explained by quite high phenotypic variance (45.95%).Though the markers are not close enough they can serve as flanking markers enabling the selection effective and reliable. In the absence of reports on either genetic or molecular linkage map, it is not possible to assign the trait/marker to any specific chromosome of sesamum genome. The two markers found linked to the disease tolerance in the present study are not close enough to the markers to be sure about the selection in a breeding population. Fine mapping of the genomic region would help identify still tightly linked markers to the tolerance gene(s) besides reducing the linkage drag. Pending fine mapping and narrowing to candidate genes, the two markers (OPAE 6 and OPM 12) despite their distance to the trait can be utilized for screening large number of varieties and advanced breeding lines to confirm the effectiveness of the linkage between them and the disease tolerance. On confirmation, these markers can be sequenced and converted into SCAR marker.

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