Inheritance study and identification of RAPD marker linked to Alternaria blight resistance in sesame (Sesamum indicum L.)

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ABSTRACT

Alternaria blight of sesame (Sesamum indicum L.), has been identified as a predominant biotic pressure of single origin, that limits seed yield both qualitatively and quantitatively to incidence is high in southern parts of India with considerable yield loss. The resistance mechanism in sesame is obscure. The affected between RT-273 a resistance line identified to through field screening with Gulbarga Local Block a popular land race, but susceptible to Alternaria blight and advanced to F2 generation. The inheritance for resistance was under the control of single dominant allele and segregated has 3:1 (chi-square is NS), further 10kb RAPD marker was identified for OPA-6 primer through F2 bulked segregant analysis.

KEY WORDS: Alternaria resistance, dominant gene, F2 bulked segregant analysis, OPA-6 Primer, RAPD marker, Sesame

INTRODUCTION

Sesame (Sesamum indicum L.) is the oldest oil seed crop known to man and fourth important oil seed crop in Indian subcontinent only after groundnut, rape seed, mustard and sunflower. Sesame oil contains two antioxidants viz. sesamin and sesamol which import a high degree of resistance against rancidity (Ashri, 1989). The sesame oil with high-unsaturated fatty acids and unique nutritive composition makes it near a perfect food (Lokesha and Teertha Prasad, 2006). India ranks first both in area (1.63 million hectares) and production (0.518 million tonnes) globally. India also has global market for white seeded types. Despite these privileges, the yields level of sesame in India is very low and highly inconsistent compared to world’s average. Susceptibility of sesame crop to biotic (disease) pressure is one of the major constraints identified. Captivating, it is being grown by resource poor farmers who follow a strategy of sow it, forget it and harvest it. More often the seeds produced in unscientific means eu route international markets hence of a concern (Lokesha et al., 2005).

Alternaria leaf blight, caused by Alternaria alternata and Alternaria sesami (Mohanty and Behera, 1978; Barbera et al., 1966; Chavan, 1978 and Naik et al., 2003), has been identified as a predominant disease of fungal origin (Ellis and Holliday, 1970), incidence has been recorded to be more than 80 per cent in Northern Karnataka
and takes heavy toll resulting in sufficient loss (Naik et al., 2003); Complete loss of crop was reported in Maryland (Thomas, 1959; and Barbara et al., 1996). Intriguingly, the incidence of this disease is wide spread and not only in India but also elsewhere (Russia: Krashina, 1928; Japan: Kawamura, 1931; Costarica: Mendoza, 1940; Uganda: Hansford, 1943; Nicaragua: Litezenberger and Stevenson, 1957). The disease becomes serious during kharif (Dolle, 1981; Reddy, 2001). The fungal spores are not only seed borne and capable of surviving for 56 weeks under normal environmental conditions (Naik et al., 2006) but also survives on a wide range of host plants and affects both seed quality and quantity (Ellis and Holliday, 1970). Undeniably, Alternaria has been one of the predominant mycoflora associated with seeds as well (Ashish Kumar and Tribhuvan Singh, 2001).

Development of resistant varieties against Alternaria blight is one of the major thrust areas identified in sesame improvement (Kariyallappa et al., 2003; Lokesha et al., 2005) because the adoption of technologies by farmers is almost murky. Several approaches, Conventional and biotechnological, are being attempted to develop varieties that are resistant to Alternaria blight (Lokesha et al., 2005).

Resistance breeding begins with searching of germplasm that are resistant to Alternaria blight. Incidentally most cultivated/popular varieties and germplasm are highly susceptible, whilst RT-273 is the lone cultivated Sesamum indicum genotype that has been identified through field screening (Naik et al., 2003). Though a number of wild species viz. Sesamum radiatum, Sesamum prostatum, Sesamum lacinietum, Sesamum mulayanum and Sesamum occidentale var. Malabaricum were found to be resistant, inter-specific hybridization is not that encouraging owing to exitinace of fertilization barriers (Prakash Chavhan, 2001; Tarihal, 2003; Rudresh, 2004).

Molecular markers have been widely used in breeding disease resistant in several crops (ying, et al., 2003). However, there are no documented evidences of use of molecular marker technology for breeding disease resistance in sesame crop vis a vis for Alternaria blight. An AFLP based marker has been successfully used in identification of closed capsule in sesame (Uzun et al., 2003). Identification of a marker for Alternaria resistance would help in screening segregants/germplasm lines/mutants in sesame crop. One of the methods widely used was F2 bulked segregants analysis, thus the present endeavor.

MATERIALS AND METHODS

Parental material consisted of two cultivated Sesamum indicum genotypes viz. RT-273 – a brown seeded variety of Rajasthan origin identified as resistant to Alternaria blight disease in three years of field experimentation conducted at Raichur center (Naik et al., 2003) and Gulbarga local Block (GLB) – a popular white seeded landrace of Gulbarga region of Northern Karnataka but highly susceptible to Alternaria blight. Both direct and reciprocal crosses were affected between RT-273 and GLB by following manual emasculation. F1 plants were raised under controlled condition and advanced to F2. One fully expanded leaf was collected, treated with
liquid nitrogen and preserved and used for molecular marker analysis.

DNA, in all the leaf samples, was extracted following the method outlined by Porebski et al. (1997) (cTAB method) followed by quantification with OD 260 nm. Finally DNA was diluted to a final concentration of 12.5 ng l-1 and 2 l of this DNA was used for the PCR amplification. PCR products were separated on 1.5 per cent Agarose gel (genei, Bangalore and India) gel containing Ethidium bromide (0.5 /ml). The size of the fragments was determined by using (0.1-1Kb and 0.5-5 Kb) commercially available DNA ladder and the gel was visualized under ultraviolet light and documented using gel documentation system (Alpha Innotech Corporation, Bangalore and India). To test the quality of DNA samples, DNA samples were run on 0.8 per cent Agarose gel (genei, Bangalore and India) in 1X TBE buffer and stained with Ethidium bromide and compared with standard undigested DNA sample.

Molecular marker analysis was carried out to using both the parents, RT-273 and GLB were screened with OPA-6 primer (sequence 5’–3’= GGTCCCTGAC) it’s a arbitrary sequence (Operon Technologies Inc., Bangalore and India).isolated DNA collected from both the parents and F2 segregating plants were subjected to PCR analysis using above mentioned primer only. PCR products were separated on a 1.4 per cent Agarose gel containing 0.5µg/ml of Ethidium bromide using 1x TBE buffer. The gels were run for two and half hours at 55V-110V.THE GEL WAS visualized under a gel documentation unit (Alpha Innotech Corporation, Bangalore and India).

**BULKED SGREGANTS ANALYSIS (BSA)**

As a part of confirmation test, the diluted DNA was standardized to a uniform concentration of 12 ng/µl from resistant and susceptible F2 plants were mixed separately to constitute two “resistant bulks” 15S(R) and 11R3(R) and two “susceptible bulks” 12S(S) and 136R2(S) were made respectively as per the protocol outlined by Michemore et al., (1991). Finally the field screening of all 40 labeled F2 plants (RT-273 X GLB = 18; GLB X RT-273 = 22) under controlled condition was collected and corroborated using RAPD molecular marker data.

**RESULTS AND DISCUSSION**

The PCR amplified product using OPA-6 primer generated a polymorphic band in the region of 10kb (plate.1), which clearly differentiated the parents RT-273 (resistant) with GLB (susceptible), and the same polymorphism was also recorded in F2 segregating population. Those that possessed bands were resistant even under field condition. It is interesting that the susceptible plants did show disease symptom in 10 days seedling stage; and died completely at later stages. The field screening of F2 segregants clearly indicated a 3:1 segregation for resistant vs. susceptible (Table.1). The results is in conformity with Ying et al. (2003) where four RAPD markers linked to genetic male sterility in Chinese Cabbage were found to inherit in a simple mendelian manner. RAPD marker studies in sesame are scanty. Bhat et al., (1997) used varying concentration of template DNA ranging from 0-80ng and found that 40ng gave maximum number of reproducible bands in genetically divergent
sesame types. In the present study 25ng amplification gave maximum and high reproducible bands. PCR amplification was achieved in 44 cycles, 12ng DNA per 20μl reaction mixture; 25mM Mg⁺⁺ was suitable for best results.

Lokesha (2006) used 17 random primers of OPA, OPB, OPC and OPD (Operon Primer series) kits in sesame (Sesames indicum L.). OPA-6 primer generated a polymorphic marker of 10 kb band found associated with Alternaria resistance. However, OPA-13 primer though generated polymorphic bands in F₂ segregants failed to differentiate parents. Based on the field screening, it was found that a single dominant gene controlled the resistance. The result of bulked segregants analysis and whole F₂ population screening by primer OPA-6 indicated the association of the marker (OPA-6) with the Alternaria resistance in sesame. Alternaria disease resistance is monogenecally controlled. The resistance and susceptible genotypes different with respect to the domain of protein binding site and it is hypothesized that the resistant should be linked to dominant receptor in a sesame crop as dominant character.

In the present study a total of ten bands, which were consistent, unambiguous and repeatable, produced from the primer OPA-6 were used for analyzing the sesame genotypes of both straight and reciprocal cross for resistant to Alternaria blight. In case of straight cross, out ten bands four bands were monomorphic (Plate.1) and six bands were polymorphic compared with parents are GLB, RT-273 and F₂ segregants using 10Kb ladder. In case of reciprocal crosses, out of ten bands four bands were monomorphic and six bands were polymorphic.

Michelmore et al., (1991) use bulked segregate analysis relying on a population that resulted from a cross that segregated for a disease (Downey mildew) resistance. Since then BSA has been extensively used in mapping of molecular marker. Each of these bulks contains individuals that are identifiable for a particular trait such as resistance or susceptibility to a particular disease resistance. RAPD can also be used to identify marker for which no isogenic line exist. Gall midge resistance in rice has been mapped using RAPD marker through BSA (Katiyar et al., 1996). The mapping of genes is being attempted for Alternaria blight resistance in addition to transfer of resistant gene to popular cultivars. In present study resistant and susceptible F₂ plants and was mixed separately to constitute two “resistant bulks” 15S(R) and 11R2(R) and two “susceptible bulks” 12S(S) and 136R2(S) were made respectively. The F₂ bulks were compared with parents and F₁ Genotype was clearly indicated that marker resistant to Alternaria disease (Plate.2).

CONCLUSION

The F₂ bulk segregant population generated from both straight and reciprocal crosses of RT-273X Gulbarga local black. For the standardization DNA extraction protocol, used a parent which was resistance to alternaria blight RT-273 genotype identified as resistant through field experimentation and susceptible (Gulbarga local black-a popular land race) were initially tried for the optimizing the reaction parameter, concentration for the PCR
reaction mixture and standardization of protocol for the isolation of DNA before F₂ bulk segregants were used.

Totally 300 F₂ bulk segregants of sesame genotypes, among 91 genotypes were straight cross and 209 genotypes were reciprocal cross, out of 91 genotypes 20 sesame genotypes(selected based on phenotypic scoring) used to genetic analysis from straight cross and out of 209 genotypes 22 sesame genotypes were selected from reciprocal cross, which includes P₁ P₂ and straight cross of 17S1, 17S2, 4S, 67S1, 67S2, 67S3, 12S, 51S1, 51S2, 20S, 76S, 36S, 23S1, 23S2, 23S3, 15S1, 15S2, 15S3, 15S4 and reciprocal cross which includes 136R1, 136R2, 25R, 11R1, 11R2, 11R3, 120R, 117R, 107R1, 107R2, 107R3, 43R, 34R1, 34R2, 8R1, 8R2, 202R1, 202R2, 202R3, 168R, 157R, 197R. A total of ten bands, which were consistent, unambiguous and repeatable, produced from the primer OPA-06 were used for analyzing the sesame genotypes of both straight and reciprocal cross for resistant to Alternaria blight. In case of straight cross, out ten bands four bands were monomorphic and six bands were polymorphic compared with parents P₁ and P₂ (GLB and RT-273) and 10Kb ladder, in reciprocal cross, out of ten bands four bands were monomorphic and six bands were polymorphic. This is not observed in any other genotypes. The results indicated that a RAPD marker of 10 kb band was found association between resistance and OPA-06 primer. F₂ bulk segregant analysis clearly indicated that the resistance was in the control of a single dominant gene because the susceptible v/s resistant could segregate as 3:1 based on banding pattern. The field observation further confirmed the dominance nature of the resistant gene, so the primer OPA-06 can distinguish resistant and susceptible genotypes. Thus resistant loci can be marked using DNA markers.
Plate 1. OPA-06 RAPD marker gel profile of F2 bulked segregants for straight and reciprocal crosses of sesame genotypes
Plate 2. OPA-6 RAPD marker gel profile of bulked segregant analysis

1. 10kb ladder
2. RT-273 (Resistant parent)
3. GLB (Susceptible parent)
4. 136R2(S) F2 Segregate susceptible plant
5. 12S(S) F2 Segregate susceptible plant
6. 15S(R) F2 Segregate resistant plant
7. 11R(R) F2 Segregate resistant plant
8. 15S(R)+11R(R) F2 Bulked resistant plants
9. 12S(S)+136R2(S) F2 Bulked susceptible plants

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REFERENCES


Naik, M.K., Lokesha, R., K. Ajithkumar, Suvarna, K.V.Bhat, R.K. Mestha and G. Sunkad. 2003, Screening of sesame genotypes against Alternaria blight. Abstracted in Symposium on Recent Developments in the Diagnosis and Management of Plant Disease for Meeting Global Challenges, held by IPS (Southern Chapter), University of Agricultural Sciences, and Dharwad. pp:45.

Naik, M. K., Savitha, A.S., Lokesha, R., Prasad, P.S. and Raju, K. 2007, Preparation OF Alternaria sesami causing blight of sesame in seeds and plants debris. Indian Phytopathology, 60(1):72-75


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