

Study of parental polymorphism in sesamum (*Sesamum indicum* L.) using SSR markers

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SUMMARY

The present investigation has been undertaken to study the polymorphism between the two diverse sesamum genotypes *i.e.* Swetha til, a high yielding popular white seeded variety but susceptible to powdery mildew and BB3-8 accession of *Sesamum mulayanum* resistant to powdery mildew from RARS, Jagtial. The variation among these parents was characterized using 300 SSR markers, out of which 240 showed clear amplification pattern and 24 markers exhibited polymorphism (10%). The results indicating that there is high conservation of coding sequences among the genotypes within the species.

Key Words : Sesamum, Parental polymorphism, SSR markers

How to cite this article : Sravani, D., Anuradha, G., Rajendar Reddy, M., Vijay, Y., Malathi, S. and Sudhakar Rao, K.V. (2014). Study of parental polymorphism in sesamum (*Sesamum indicum* L.) using SSR markers. *Internat. J. Plant Sci.*, 9 (1): 263-265.

Article chronicle : Received : 02.10.2012; Revised : 05.12.2013; Accepted : 20.12.2013

Sesame (*Sesamum indicum* L.) also known as sesamum, til, gingelly, simsin, gergelim etc. is the most ancient oilseed crop in the world (Joshi, 1961 and Weiss, 1971). Belonging to the family Pedaliaceae it is regarded as 'Queen of Oilseeds', the quality of its oil being of high nutritional and therapeutic value. High stability of its oil with distinct sweet flavor and oil meal with rich protein make it ideal for domestic and confectionary uses, respectively. The

antioxidants 'sesamin' and 'sesmolin' enhance the keeping quality of oil by making it resistant to rancidity, its diverse utility value that has made today sesame an important commodity in international trade.

Despite its shorter life cycle, suitability to different cropping systems and land types adaptation, moisture stress and low input management conditions sesame's contribution to the country's oilseed production remains sadly left much to be desired.

Sesame is susceptible to several pests and diseases of which powdery mildew and phyllody among diseases and capsule borer among insect pests contribute for significant losses. Powdery mildew caused by *Oidium acanthospermi*, showed considerable economic damage upto 45 per cent throughout the sesame growing areas in post rainy/severe winter season. Shambarkar *et al.* (1997) reported that powdery mildew alone could cause yield loss up to 45 per cent. Powdery mildew is common in all the sesame growing areas, especially in Andhra Pradesh and Tamil Nadu. It is a particular problem where climate is relatively cool and moist. Optimum development of powdery mildew occurs at temperatures between 59°F and 71°F, High nitrogen application and high stand density can also favor the disease. Powdery mildew is

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easy to recognize as the fungus is visible as white cottony patches on the leaves. Wind or rain-borne spores spread the disease and new spores can be produced every 7 to 10 days. Yield is reduced both due to reduced seed size and reduced number of capsules per unit area.

It is managed to an extent in the initial stages with sulphur. But often it fails to give total protection against the disease. Therefore, in addition to breeding for yield contributing characters, more attention should be given for breeding resistant varieties and hybrids with higher yield.

Molecular marker technology, among its variety of applications, enables precision in selection/screening at genotype level and in unfolding the hitherto hidden variability of breeding value. However, recent advances in molecular biology have equipped scientists with a wide choice of marker assisted techniques to identify both quantitative and qualitative traits. Of the several PCR based markers employed in genetic mapping studies, microsatellite markers, also known as simple sequence repeats (SSR), are genetically more informative, crop and trait specific, co-dominant and robust. SSRs are highly effective in assessing the genetic diversity existing in the genotypes as well as in genetic mapping studies.

MATERIAL AND METHODS

Two genotypes Swetha til, a high yielding popular variety of Andhra Pradesh susceptible to powdery mildew from RARS, Jagtial and BB3-8 accession of *Sesamum mulayanum* line resistant to powdery mildew were chosen as parents to make cross viz., Swetha til × *Sesamum mulayanum* and further to develop mapping population.

Genomic DNA was extracted from young tender leaves from a random sample of five plants from each parent following the standard cTAB method (Porebski *et al.*, 1997). The DNA quantification was done by using a Nanodrop spectrophotometer (Sigma, USA.) as well as using known amount Lambda DNA as standards. PCR optimization for SSR a markers was done by varying concentration of template DNA, Taq polymerase, dNTPs, primers and MgCl.

The amplification reaction with SSR primers was carried out in a final volume of 10 µl .PCR reaction mix containing the following.

Sterile distil water	: 5.0 µl
10X buffer	: 1.0 µl
dNTPs (2.0 mM)	: 1.0 µl
Forward primer (10 mM)	: 0.4 µl
Reverse primer (10 mM)	: 0.4 µl
Taq DNA Polymerase (5U/µl)	: 0.1 µl
Template (50ng/ µl)	: 2.1 µl
Total Reaction volume	: 10.0µl.

PCR condition for SSR analysis included an initial pre denaturation step of 5 min at 94°C and following 35 cycles of amplification:

Table A : Basic steps in Polymerase chain reaction (PCR)

Step	Temperature	Time
Denaturation	94°C for	0.45 min
Primer annealing	50°C-60°C	0.45 min
Extension	72°C	1min

Final extension was carried out at 72°C for 10 min. The amplified fragments were stored at 4°C for short periods and at -20°C for long duration. amplified PCR product from each reaction was separated on 3.0% agarose gel containing ethidium bromide in 1 x TAE buffer at 130 V, finally visualized and photographed using gel documentation (using Bio-Rad Molecular Imager Gel Doc XR System). In the present investigation, 300 SSR markers were used.

RESULTS AND DISCUSSION

Polymorphism among two diverse parental lines of sesamum (Swetha til, *Sesamum mulayanum*) was studied using 300 SSR markers. out of which 240 showed clear amplification pattern and 24 markers exhibited polymorphism (10%) Fig. 1, Table 1. Each polymorphic primer was tested at least twice to determine if both the polymorphism and banding pattern were reproducible. Though the parental genotypes were distinct for various important traits, polymorphism at

Table 1 : List of polymorphic SSR markers

Sr. No.	Polymorphic primers	Allele size
1.	SM 10-102	198
2.	SM 10-103	248
3.	SM 10-106	198
4.	SM 10-115	198
5.	SM 10-116.	146
6.	SM 10-144	163
7.	SM 10- 160	193
8.	SM 10- 176	237
9.	SM 10- 178	175
10.	SM 10- 182	212
11.	SM 10- 183	188
12.	SEM 38	400-450
13.	SEM 76	170
14.	SEM 270	187-224
15.	SEM 249	290-310
16.	SEM 396	175-220
17.	SEM 436	175
18.	SI 2	190
19.	SI 9	150
20.	SI 14	243
21.	SI 15	236
22.	SI 34	250
23.	SI 42	189
24.	SP15	180

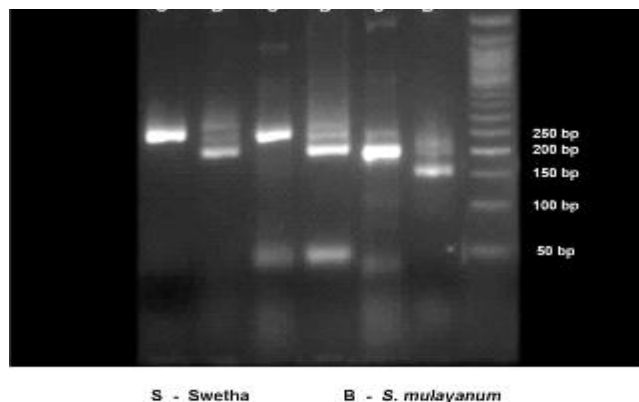


Fig. 1 : Parental polymorphism with SM10-103, SM10-104 and SM10-105

molecular level was quite low, *i.e.*, (10%).

Marri *et al.* (2005) were used two hundred and ten microsatellite markers in rice to screen the parents (*O. rufipogon* and *O. sativa*) for identifying polymorphic markers. Eighty markers (38%) detected polymorphism. The lower percentage polymorphism may be due to a higher degree of genetic similarity between *O. rufipogon* and *O. sativa* used in this study.

Although parents involved in the present study belongs to cultivated and wild species, many primers showed

monomorphic indicating close of the two species. However, some of polymorphic fragments have also been identified which are useful in studies of tagging, mapping and genetic linkage studies.

The above study indicates that SSR markers can be used to study parental polymorphism and can also be utilized for genetic diversity analysis, association mapping and mapping studies in sesame.

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