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IDENTIFICATION OF *SESAMUM ALATUM* X *SESAMUM INDICUM* HYBRID USING PROTEIN, ISOZYME AND RAPD MARKERS

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ABSTRACT

With the objective of transferring phyllody disease resistance, hybridization has been done between *Sesamum alatum* and *S. indicum*. Identical chromosome number of the species, ambiguous morphological characters and lack of segregation in F_2 necessitated to look for molecular markers to establish the hybridity. SDS-PAGE of seed protein revealed transfer of five male-specific proteins to the putative hybrid. Further, inheritance of the est D locus of esterase, *prx* A and *prx* E loci of peroxidase and RAPDs of decamer random primers have not only establishing the hybridity but were also found to be useful as markers to distinguish the hybrids from the selfed progenies.

Key words : *Sesamum alatum, Sesamum indicum,* molecular markers

Sesamum (*Sesamum indicum* L.) is an ancient oil yielding crop, cultivated in South East Asia and African countries. Phyllody is a serious disease in *Sesamum* causing yield loss up to 15% every year. Recently, *S. alatum* Thonn., a wild relative of *S. indicum* has been identified as a resistance source for the disease. But, the two species were not compatible owing to prezygotic and postzygotic barriers. However, overcoming the fertilization barriers, *S. alatum* x *S. indicum* hybrid was successfully developed [1] and the morphological and cytological marker characters of the parents and the hybrid were studied (Table 1). The ambiguity in F_1 morphological characters and absence of segregation in F_2 necessitated to prove the hybridity using molecular markers. Protein [2, 3] and isozyme [4-6] markers have been extensively used to identify the hybrids. Since, these markers are basically variants of certain gene products they are available only in limited number and are not stable all the times. Recently, a class of DNA based markers called RAPDs (Random Amplified Polymorphic

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DNAs) were reported [7] which reflect variation in DNA base sequences of the genome as amplification products of random primers. RAPD makers are available in large number and are stable. The present investigation was taken up to assess the variation in protein, isozyme and RAPD markers between *S. alatum* and *S. indicum* and their utility in hybrid identification.

Table 1. Morphological and cytological characters of *S. alatum*, *S. indicum* and the putative hybrid, *S. alatum* x *S. indicum*

Character	*S. alatum*	*S. indicum*	Hybrid	Remarks
Chromosome number	2n = 26	2n = 26	2n = 26	Hybrid identification requires karotype analysis
Leaf shape	Trilobed at seedling stage become pentalobed later	entire	Trilobed	Hybrid identification is not possible at early stage and no segregation was observed in F_2
Flower colour	maroon	white	violet	violet colour sometimes gets transformed to maroon by pest or disease attack. No segregation in F_2
Seed	winged	wingless	wingless	No segregation in F_2

MATERIALS AND METHODS

SDS-PAGE of seed protein

Seeds of the parents and hybrids were used for extraction of total soluble protein. One gram of seed was ground to fine powder and packed in a filter paper (Watman No. 1). The packet was tied to a thread and suspended in diethyl ether in an air-tight container to remove fat and oil. The container was kept in a shaker (300 rpm) at room temperature for 48 h changing the diethyl either once after 24h. The sample was air dried at room temperature to remove the remaining diethyl ether and then ground with 3ml of sodium phosphate buffer (100mM, pH 7.0). The suspension was centrifuged at 12,000 × g for 6min. Protein concentration of the supernatant was estimated [8] and electrophoresis of equal amount of protein was carried out on 12.5 per cent acrylamide gel [9].

ISOZYME ANALYSIS

Leaves from the parents and hybrid seedlings were collected at three leaf stage and used for preparation of crude enzyme extracts. One gram of leaf tissue was ground in a pre-chilled mortar and suspended in 3ml of sodium phosphate buffer (100 mM, pH 7.0). The suspension was centrifuged at 22,000xg at 4°C for 25 min. The supernatant was used for isozyme analysis. Electrophoresis was carried out on 7% and 8% non-denaturing gel for esterase and peroxidase respectively. The gels were stained according to the standard protocol for isozymes of esterase [10] and peroxidase [11].

DNA isolation and RAPD analysis

DNA from the mucilage rich leaves were isolated following the method described by Kuske and Kirkpatrick [12] using the PS buffer [13]. Concentration of the DNA was estimated spectrophotometrically. The PCR reaction condition reported by Williams *et al* [7] for generating RAPD markers were standardized for Perkin Elmer DNA Thermal Cycler 480 with genomic DNA of *Sesamum*. Each 25µl of PCR reaction mixture contained 10mM Tris-HCl (pH 8.3), 50mM KCl, 2mM $MgCl_2$, 0.001% gelatin, 100µM dATP, dGTP, dCTP and dTTP,15ng of primer, 30-40ng of genomic DNA and 0.5µ of Taq DNA polymerase. The amplification condition included a total of 45 cycles with 1 min (except for the first cycle with 3 min) template denaturation at 94°C, followed by 1 min of primer annealing at 40°C and then 2 min of primer extension at 72°C. The reaction mixture was further incubated at 72°C for 10min and stored at 4°C before the amplification products were analysed by agarose gel (1.3%) electrophoresis. The primers used for amplification were OPA2, 4, 5, 8, 9, 12, 13, 16, 17, 20 and OPF4, 5, 6, 8, 10, 13, 14, 15, 16, 20 (Operon Tech., USA)

RESULTS AND DISCUSSION

The diagramatic representation of the protein profile of the parents and hybrid is shown in Fig. 1. Careful examination of the profiles showed eight protein bands (band number 1, 2, 4, 6, 10, 21 and 22) clearly distinguishing the male parent, *S. indicum* from the female parent, *S. alatum*. Among the eight male-specific protein bands, five were found in the hybrid (band number 1, 2, 4, 10 and 13) indicating gene transfer from the male parent to the hybrid.

The zymogram for esterase and peroxidase are given in Fig. 2 and 3 respectively. Both esterase E.C.3.1.1. and peroxidase E.C1.11.1.7 are monomorphic enzymes and therefore individual isozyme are coded by separate alleles. Esterase and peroxidase isozymes which are products of alleles of the same locus (allozymes) usually displayed only a little difference in mobility such that isozymes of each distinct zone in the

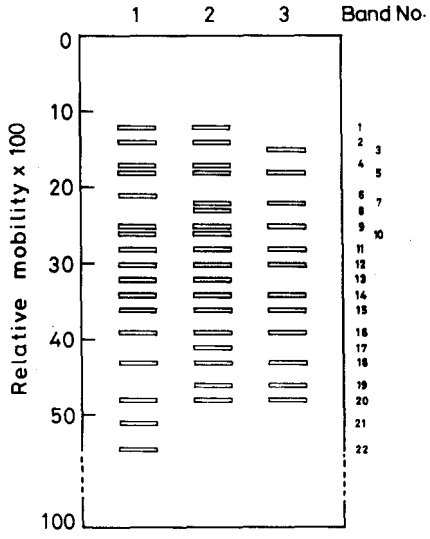

Fig. 1. Seed protein profile of (1) *S. indicum*, (2) *S. alatum* × *S. indicum* hybrid and (3) *S. alatum*

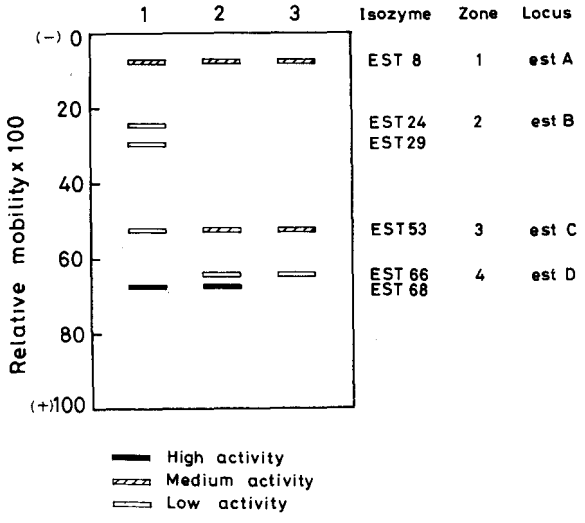

Fig. 2. Zymogram of esterase isozymes of (1) *S. indicum*, (2) *S. alatum* × *S. indicum* hybrid and (3) *S. alatum*

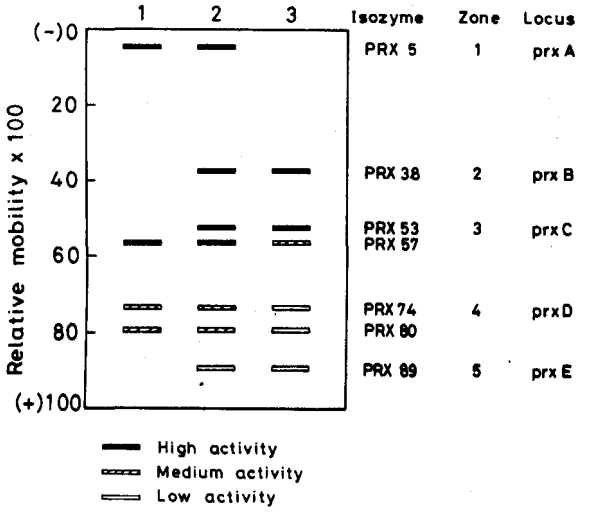

Fig. 3. Zymogram of peroxidase isozymes of (1) *S. indicum*, (2) *S. alatum* × *S. indicum* hybrid and (3) *S. alatum*

gel could be presumed to be under the control of individual locus/gene. In our study, four loci viz. est A, est B, est C and est D for esterase and five loci viz. *prx* A, *prx* B, *prx* C, *prx* D and *prx* E for peroxidase were observed in Sesamum. Among them, polymorphism in *est* D, *prx* A and *prx* E were found to be useful in hybrid identification. Alleles coding for the isozymes EST66 and EST68 (individual isozymes named after the standard acronym of the corresponding enzyme in capital letters followed by a numeral representing relative mobility of the particular isozyme) of *est* D were mutually exclusive in the parents under homozygous condition. Heterozygosity of this locus in the hybrid was evident from the double banded (EST66 and EST68) phenotype observed in the corresponding zone. In case of peroxidase, PRX5 of homozygous *prx* A was present only in *S. indicum* while PRX89 of homozygous *prx*E was resent only in *S. alatum*. Hemizygous condition for the two loci lead to the appearance of PRX5 and PRX89 together in the hybrid progeny enabling easy hybrid identification.

However, applicability of this technique in hybrid identification depends on clarity and reproducibility with which the isozymes are resolved in the gel. Great care should be taken right from collection of the samples to staining the gels to avoid degradation of the enzyme which adversely affects both clarity and reproducibility. Tissue-specific and developmental stage-specific expression of certain isozymes further makes the analysis more cumbersome. But, RAPD markers generated

by amplification of DNA by polymerase chain reaction are not associated with such lacunae and are highly stable and reproducible under the given set of controlled experimental condition (chemical and physical). These markers are being widely used to identify artificial as well as natural hybrids [14, 15]

In the present study, genomic DNA of *S. indicum*, *S. alatum* and the putative hybrid *S. alatum* × *S. indicum* were amplified with twenty decamer primers of arbitrary sequences. The RAPD profiles of the parents and hybrid for nine primers are shown in Fig. 4. A total of 127 DNA fragments were amplified by the twenty primers. Among them there were 56 fragments polymorphic between the parents, and almost all the fragments observed in the hybrid were shared either by *S. indicum* or by *S.alatum* which established the hybridity. However, not all the polymorphic fragments could be used in hybrid identification as many of them were female-specific (e.g. primer OPA2, OPA8). Altogether, 25 male-specific fragments were identified and were all found to be present in the hybrid progeny also (shown as bold lines in Fig. 4). These fragments could be used as potential markers to differentiate the hybrids from the selfed progenies. Further, three primers OPA5, OPA8, OPF1 which amplified male-specific fragment(s) were selected and used to amplify the DNA from twenty randomly selected plants of both the parental species and the hybrid. The identified male-specific fragments produced by these primers were present in all the male plants and the hybrids but absent in all the female plants (data not shown) showing the reliability and reproducibility of these fragments as markers for hybrid identification.

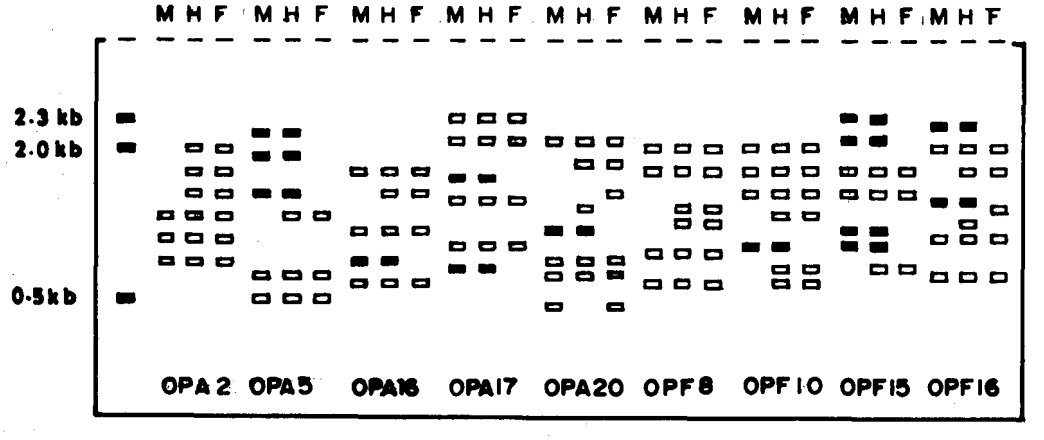

Fig. 4. RAPD profile of *S. indicum* (M, male parent), *S. alatum* (F, female parent) and *S. alatum* × *S. indicum* (H, hybrid) resolved on 1.3% agarose gel. Bold lines indicate male specific fragments.

From the transfer of male specific protein bands observed in SDS- PAGE and inheritance pattern of the isozyme loci and RAPD fragments, the present study establishes the hybridity of *S. alatum* x *S. indicum*. It is also inferred that when large scale screening of the hybrids is required, RAPD analysis would be easy, economical and reliable. And, the protocol described in this report could serve as a reference material for molecular marker based studies like marker assisted selection in *Sesamum*.

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