Varietal and Species-Interrelationship between Cultivated and Wild Sesamum

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The relative distance between the five cultivated varieties of *Sesamum indicum* viz., *AVT-3, Rama, Krishna, B-67* and *Local* and the wild *S. mulayamum* was studied on the basis of similarities and dissimilarities of parameters as morphology, seed isozyme profiles and total protein profiles prior to undertaking a hybridization program. The dendrogram revealed that the varieties *AVT-3, Rama* and *Local* were distant from *S. mulayamum* but the grouping of the varieties Krishna and B-67 with *S. mulayamum* indicated greater chance of introgression of the desired insect resistance by hybridizing the wild with either *Krishna* or *B-67.*

Key words : Cultivated Varieties, Dendrogram, Relationship, *Sesamum*, Wild Species

Sesame (*Sesamum indicum* L.; family Pedaliaceae) is an important oil crop cultivated in several parts of India. The consumption of sesame oil is more advantageous than conventional mustard and rape oil with respect to polyunsaturated fatty acid composition (Brar and Ahuja, 1979). Cultivated sesame suffers considerable yield loss every year due to attack of the insect pest *Antigastra catanaulis,* although resitance to the latter is noted in its wild relatives : *S laciniatum* Klein and *S. mulayamum* Nair (Mitra and Biswas, 1983). A preliminary report of incorporation of this insect resistant trait from wild to cultivated sesame is available (Biswas and Mitra, 1990).

The objective of the present programme was to introgress *Antigastra* – resistance trait of *S. mulayamum (S. mul)* into a few cultivated high yielding but susceptible varieties of sesame, through hybridization and anther culture of F1 hybrids. A critical study of the genetic relationship between the wild species and the cultivated varieties is a prerequisite before undertaking such a breeding and biotechnological program. Hence, as an immediate objective we report here on the similarities and dissimilarities between the varieties of sesame and *S. mul* primarily on the basis of conventional seed parameters such as morphology, micro-morphology, isozyme profiles and total protein profiles. A dendrogram was computed to determine the relationship in order to make a successful breeding program.

Materials and Methods

Five varieties of *Sesamum indicum,* viz., *AVT-3, Rama, Krishna, B-67* and Local and the wild, *S. mulayamum* were used during the present investigation. The seeds were procured from the Experimental Farm of Bose Institute, Madhyamgram, West Bengal, India. The germplasms were initially collected from the Pulses and Oilseed Research Station, Berhampore, Government of West Bengal, India. Seeds of the wild species were collected from a population growing at the vicinity of the Experimental Farm of Bose Institute.

To study seed morphology, scanning electron microscopy (SEM) was done with dried seeds that were mounted on stub, sputtercoated with gold using the Edward S150 Sputter Coater, Scanned with Philips 500 SEM and photographed at an angle of 45°.

For the study of isozyme (esterase) and total protein, 2 g seeds of each variety/species were extracted separately with 5 ml of 0.05 M Tris-HCl buffer, pH 7.6 and centrifuged at 12,500 rpm for 20 min at 4°C (Das and Mukherjee, 1995). The clear supernatant was used as the source of enzyme/protein. Protein was quantified by the Folin-phenol method (Lowry *et al.*, 1951). Isozyme (esterase) and SDS-PAGE profiles of total protein were determined using native, non-denaturing polyacrylamide gel (8.5%) electrophoresis and denaturing SDS-PAGE (10%) respectively (Basu *et al.*, 1997). Electrophoretic runs were made for 3-4 h at 2 mA per lane basis at 4°C with gels loaded on an equivalent protein amount. The gels were developed following the procedures of Wetter and Dyck (1983) in case of isozyme profile and Hames (1981) in case of polypeptide profiles. Densitometric scans of the gels were carried out using the Biorad Gel Documentation System (Gel Doc 1000, version 1.5).

Pairing affinity values (PA) for different combinations of materials were calculated on the basis of morphological and biochemical parameters (Mitra *et al.*, 1998). In case

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of the electrophoretic band profile, PA values were calculated following the formula of Khan (1992). Three separate similarity matrices were derived from morphology, isozyme profiles and seed protein profiles and three separate dendrograms were computed accordingly using a statistical package SPSS-X release 3.1. For the sake of brevity, the combined similarity matrix and the dendrogram computed on the basis of these matrices is presented.

Results

The morphological features of flower and seed of the varieties of *Sesamum* and *S. mulayamum* are summarised in Table 1. In general, all varieties of *S. indicum* possess white flowers though there are tinges of pink in variable degrees in the inner corolla of *Rama, Krishna, Local* and *AVT-3*. The colour of the flower of *S. mulayamum* is, however strikingly violet. Moreover, it has a conspicous methyl violet marking along the longitudinal line of the anther (Table 1). The seed coat colour is variable. The seeds of *AVT-3* were differentiated from all others for its white colour. The seeds of *Krishna* are totally black while those of *Rama* and *Local* are brown. The seeds of *B-67* and *S. mulayamum* are blackish (Table 1). In general, the spermoderm is reticulate, reticulation is brought about by polymorphic, deeply concave cavities which are surrounded by straight anticlinal walls. The spermoderm of *Krishna, B-67* and *S. mulayamum* possessed ridges. The ridges are occasional in *Krishna* and *B-67* but very profuse and conspicuous in *S. mulayamum* (Table 1; Fig. 1 A-I).

With one exception, the esterase profiles of the seeds displayed three bands (band 1, rmf 0.53; band 2, rmf 0.57; and band 3, rmf 0.64). The variety *AVT-3* did not show band number 3 (Fig. 2). Similar to the isozymic profile, total seed protein profiles also exhibited general similarity in banding pattern and is represented by six

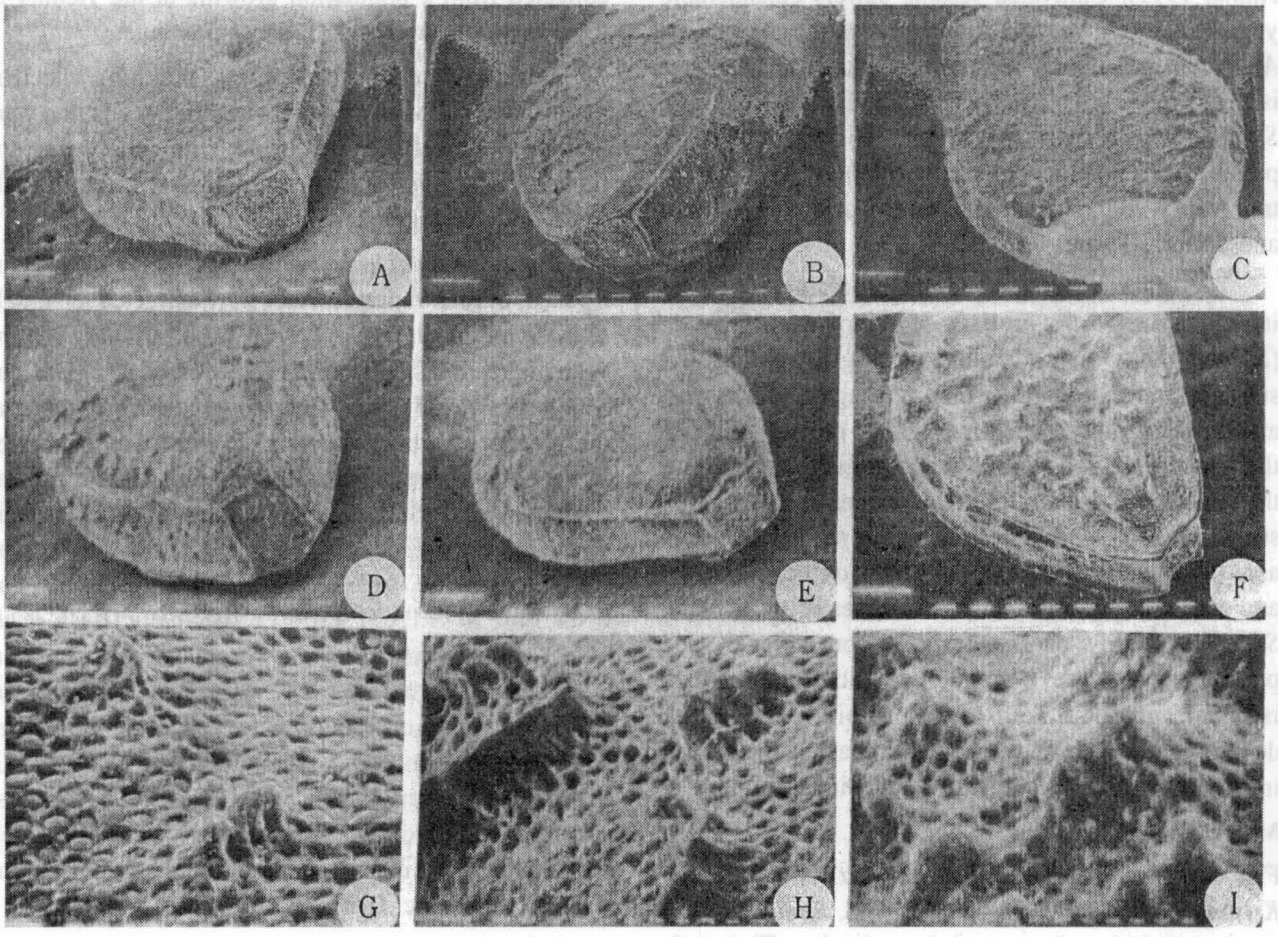

Fig. 1. SEM, seed surface morphology of Sesamum spp. Entire seeds (x50) of Sesamum indicum; (A-F) A, AVT-3, B, Rama, C, Krishna, D, B-67, E, Local; F : Sesamum mulayamum G-I : Seed surface showing ridges (x400), G, Krishna, H, B-67, I, Sesamum mulayamum

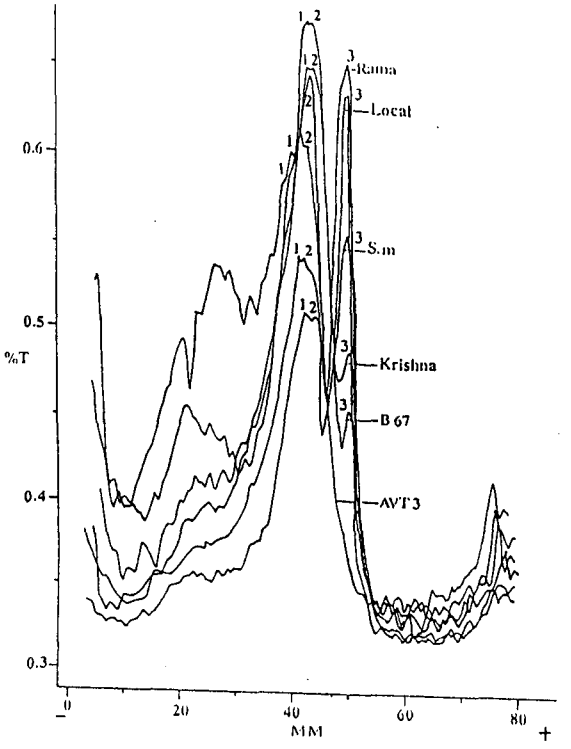

Fig. 2. Densitometric scan of esterase profiles of AVT-3, Rama, Krishna, B-67, Local (all Sesamum indicum) and S.m. (Sesamum mulayamum). Digits indicate band numbers. Migration direction : – → +

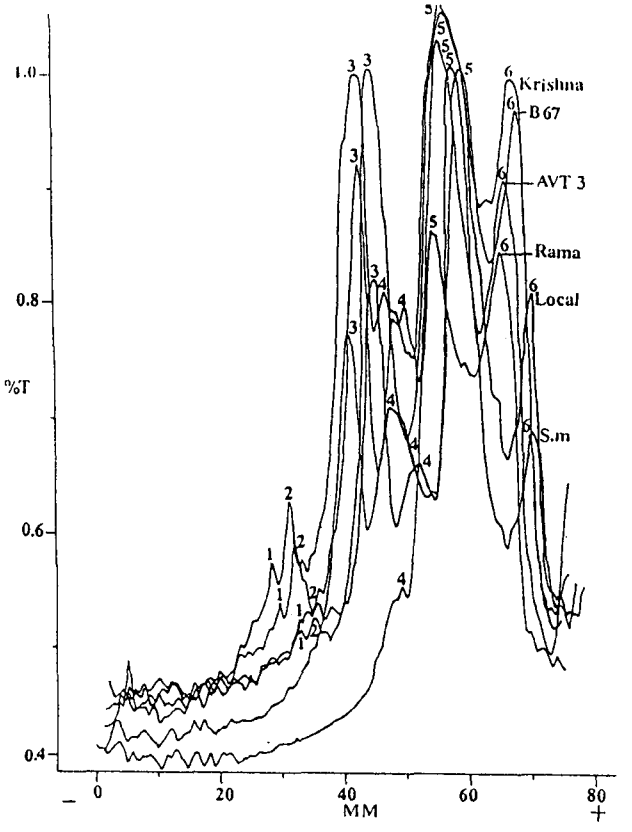

Fig. 3. Densitometric scan of total seed protein profiles of AVT-3, Rama, Krishna, B-67, Local and Sesamum mulayamum. Digits indicate band numbers. Migration direction: – → +

Table 1. Morphological parameters of *Sesamum*

Parameter	*S. indicum*					*S. mulayanum*
	AVT-3	*Rama*	*Krishna*	*B-67*	*Local*	
Flower colour						
Outer corolla	White	White	White	White	White	Violet
Inner corolla	Light Pinkish	Pinkish	Pinkish	White	Light pinkish	Violet
Colour marking along longitudinal line of anther	Pinkish	No marking	Pinkish	No marking	No Marking	Methyl violet
Seed coat						
Colour	White	Brown	Black	Blackish	Brown	Blackish
Spermoderm	Smooth	Smooth	Ridged	Ridged	Smooth	Ridged

bands (band 1, rmf 0.34; band 2, rmf 0.38; band 3, rmf 0.57; band 4, rmf 0.65; band 5, rmf 0.71; and band 6, rmf 0.93). The variety *AVT-3* is distinguishable form the others by the absence of bands 1-3 and in the variety *Rama* two slow migrating bands (1-2) could not be seen. All the six bands are present in *Krishna B-67, Local* and *S. mulayamum* (Fig. 3). Pairing affinity values ranged between 0.36-0.78 (Table 2). High pairing affinity values are shown by *Rama/local* (0.78); *Krishna/ B-67* ((0.78); and *Local/B-67* (0.78) followed by *Local/ Krishna* (0.71). *S.mulayamum* showed comparatively high pairing affinity values with *Krishna, B-67* and *Local* (0.64). However, the value is lowest between *S. mulayamum* and *AVT-3(0.36).* The combined dendrogram revealed two major groups : one comprising *Rama* and *Local,* similar at a distance of 2.2; the other comprising *Krishna* and *B-67,* similar at a distance of 1.8 and together with *S. mulayamum* similar at a distance of 3.1. Variety *AVT-3* at a distance of 4.6 is different (Fig. 4). The distances are Eucleidian but the pattern is similar to that computed following Mahalnobis distances, the dendrogram of which is not shown.

Discussion

Understanding the genetic relationship between the varieties and *S. mulayamum* is a rational approach prior to undertaking a breeding programme. The study of morphological, biochemical and molecular parameters was chosen to evaluate the closest neighbour(s) of interest. From the morphological observations of flower colour, the wild species *S. mulayamum* seemed to be totally different from the five cultivated varieties of *S. indicum* as the former was conspicuously violet while the latter were either white or white-based. The nature of spermoderm gave further differentiating observations as the varieties *AVT-3, Rama* and *Local* were devoid of ridge like structures which were characteristics of seeds of *Krishna* and *B-67* and particularly *S. mulayamum* the wild one. Thus, seed appears to be one of the distinctive features of the spermophytes. Variation in seed size, shape, colour and surface features are of prime importance in identification of seeds and distinction of varieties and species (Das and Mukherjee 1995; Mitra *et al.,* 1998).

Seed isozymes and protein profiles proved to be effective tools in testing varieties and species and their genetic relationships (Das and Mukherjee, 1995; 1997). The study indicated that variety *AVT-3* was different from others as it lacked a major fast migrating esterase and three slow migrating protein bands. The variety *Rama* also lacked two slow migrating bands in protein profile.

The dendrogram computed on the basis of combined pairing affinity values probably reflects a true picture of the relative distance between the five cultivated varieties and the wild species, *S. mulayamum.* The variety *AVT-3* was most distant from the others ruling out its consideration for a breeding programme. The grouping of *Rama* and *Local* in a cluster distant from *S. mulayanum*

Table 2. Combined pairing affinity values based on morphology, isozyme and protein profiles

	AVT-3	*Rama*	*Krishna*	*B-67*	*Local*	*S. mulayanum*
AVT-3	1.00					
Rama	0.64	1.00				
Krishna	0.50	0.64	1.00			
B-67	0.43	0.64	0.78	1.00		
Local	0.57	0.78	0.71	0.78	1.00	
S. mulayanum	0.36	0.50	0.64	0.64	0.64	1.00

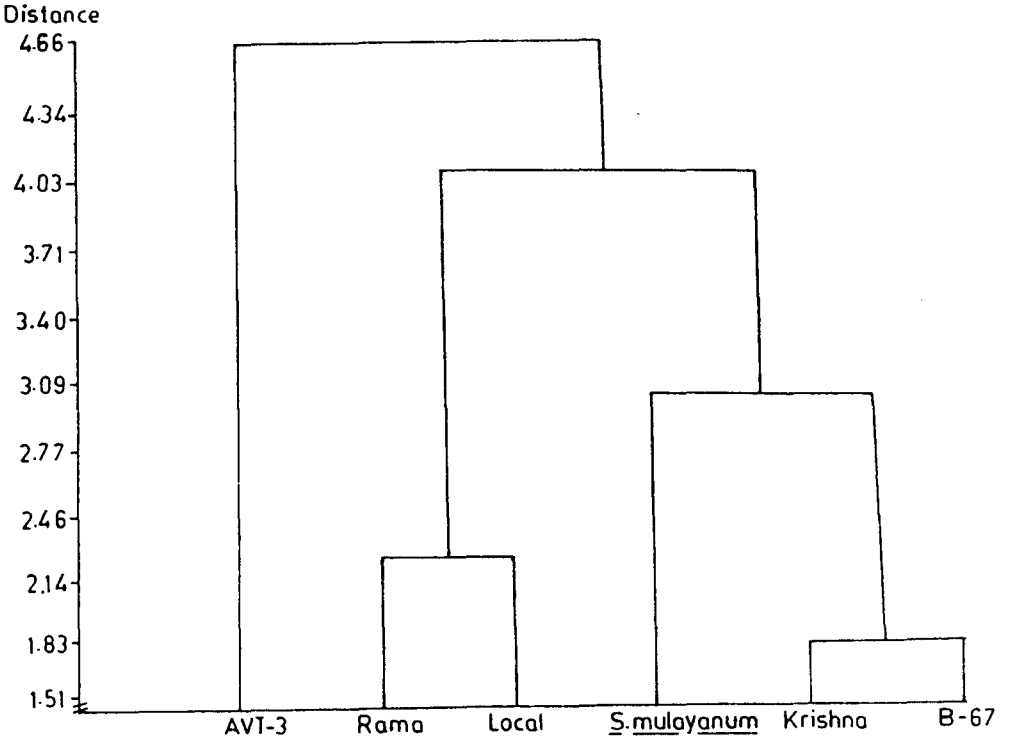

Fig. 4. Dendrogram computed on the compiled pairing affinity values derived from morphology, esterase and protein profiles showing the relative distance between five varieties of Sesamum indicum and the wild Sesamum mulayamum

also indicates their unsuitability for hybridization with *S. mulayamum*. The grouping of *S. mulayamum* with the two varieties *Krishna* and *B-67* strongly points out to the most successful chance of hybridization. These findings confirm the earlier report of hybridization of *S. mulayamum* with the high yielding variety, *B-67* (Biswas and Mitra, 1990).

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