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Interspecific Crossing Barriers in Sesame (*Sesamum indicum* L.)

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A B S T R A C T

The genus sesamum has 38 species along with cultivated species *S. indicum.* Some of the wild species are known to have useful genes like disease and pest resistance. The interspecific hybridization utilizing cultivated and wild species of sesamum can lead to broadening both nuclear and cytoplasmic genetic base of the cultivated species. It has been difficult to produce interspecific hybrids due to incongruity fertilization barriers. The barriers to hybridization can occur at any stage from pollination to fertilization or even at later stages of development of the hybrid plants. The information on type of barriers which exists between cultivated and wild species is prerequisite to develop methods to overcome fertilization barriers and further to develop successful hybrids in any wide hybridization programmes. The crossability barriers between *S. malabaricum* and *S. mulayanum* with cultivated species (*S.indicum*) were studied using Aniline blue fluorescent microscopy technique. Pistils of different interspecific crosses were fixed to study pollen germination, pollen tube entry in papillae, style and micropylar region at different intervals after pollination. The fixed pistils of cross between *S. malabaricum* with cultivated species recorded 68.5% of pollen germination as compared to 90% in selfed pistils whereas *S.mulayanum* recorded 62.5% pollen germination as against 91% in selfed pistils. The ovules showed an average of 60% and 58% of micropylar entry as compared to 83% and 79% in selfed pistils of *S. malabaricum* and *S. mulayanum*, respectively. These results of fluorescent microscopy study indicated nonexistence of pre-fertilization barriers between wild (*S. mulayanum* and *S. malabaricum)* with cultivated species of sesame.

Introduction

Sesame (*Sesamum indicum* L.) is an ancient oil yielding crop and known as "Queen of Oilseeds" due to high cooking quality and medicinal value of its oil. It is cultivated in the world mainly for its high quality edible oil. Sesame seed contains over 50 per cent and a balanced fatty acid composition with more or less equal percentages of oleic and linoleic acid. India is considered to be the major centre of genetic diversity. India is the largest producer of sesame covering 35 per cent of world's sesame area and 26 per cent of the production and nearly 8 per cent of the total area under oilseeds in India is under sesame with 8.11 lakh tones of production from an area of 17.78 lakh ha (Anon., 2016).

The seed yields of sesame are the lowest of all the major oil seed crops (India 370 kg/ha; Karnataka 415 kg/ha) The limited breeding efforts to develop high yielding cultivars, in addition to the lack of resistance to biotic and abiotic stresses, is the major cause of low productivity in sesame. Major diseases

causing yield losses are *Alternaria* leaf spot, *Cercospora* leaf spot and phyllody and an important pest attacking sesame is shoot webber. The variability and germplasm resources available in *S. indicum* are limited to combat these diseases and pests (Ashri, 1998).

The genus *sesamum* has 38 species along with cultivated species *S. indicum.* Some of the wild species are known to have useful genes like disease and pest resistance (Prabhakaran, 1996). Many workers have reported genetic male sterility in sesame, and CMS system derived from *S. malabaricum*, indicating the possibility of using interspecific crosses to derive and develop different male sterile systems each in a different genetic background.

Although interspecific hybridization utilizing cultivated and wild species of sesamum can lead to broadening both nuclear and cytoplasmic genetic base of the cultivated species. It has been difficult to produce interspecific hybrids due to incongruity barriers. Thus, the frequency of hybrids obtained is extremely low.

The barriers to hybridization can occur at any stage from pollination to fertilization or even at later stages of development of the hybrid plants (Stebbins, 1958 and Levin, 1971). Depending on the type of barriers, different techniques can be used for overcoming them. Knowledge on type of barriers and methods to overcome the same, is therefore, an important step for developing successful hybrid in any species.

Systematic investigations in various crosses on the type of barriers and associated method to overcome them can provide the basis for obtaining sexual hybrids at higher frequency and between desired genotypes. Such systematic studies in crosses between wild and cultivated species of sesamum are limited. Keeping these things in view the present experiment was undertaken to study the fertilization barriers using Aniline blue flouroscent microscopy in interspecific hybrids.

Materials and Methods

Pollination and fixation

Flower buds of female parent were hand emasculated one day before anthesis and the pistils were bagged using butter paper bags to avoid contamination from foreign pollen. Next morning, fresh pollen was collected from the male parent and dusted on the stigma of the female parent and pollinated pistils were labeled. Thirty six hours after pollination (36HAP) and 48 HAP, 50 pistils each of fifteen crosses were fixed in 3:1 absolute alcohol: glacial acetic acid (v/v) solution. After twenty four hours pistils were transferred to 70 per cent alcohol and stored at 10°C. Using aniline blue fluorescence (ABF) method (Dumas and Knox, 1983) pollen germination, pollen tube growth in stigma, style and ovary were observed in each pistil and details were recorded.

Aniline blue fluorescence method

Aniline blue is a dye which is used as a callose staining fluorochrome (Dumas and Knox, 1983). Fixed pistils were transferred to glass vials containing 8N NaOH and kept in a sand bath inside oven. They were macerated at 57°C for 18 minutes. When pistils appeared clear, they were rinsed with tap water thoroughly to remove the traces of NaOH and kept in distilled water until used for staining. They were later stained in 0.005 % decolorized aniline blue in 1/15 M K_3PO_4 (pH 11.00) containing 20% of glycerol. When gently pressed under cover slip, the pistils spread out evenly.

Preparation of stain

Five mg decolourized aniline blue powder was dissolved in 10 ml of 1/15 M K_3PO_4 solution and to this 20ml glycerol was added and dissolved. Later the pH was adjusted to 11.00 using 0.1N NaOH solution.

Observations and photographs were taken under a Zeiss microphot fluorescence microscope model BX-41 illuminated at 425-490nm UV light using transmission filter B. Under these filter combinations callose exhibits a bright fluorescence contrasting against bluish green non-fluorescing pistil tissue and blue to purple pollen grains.

Pollen grain germination was scored as the number of pollen grains that had germinated on the stigma, with or without having penetration into the stigmatic papillae.

$$\text{Pollen grain Germination (\%)} = \frac{\text{No. of pollen grains germinated}}{\text{Total number of pollen grains}} \times 100$$

Pollen tube growth in style were scored and expressed as

$$\text{Pollen tubes in style (\%)} = \frac{\text{No. of pollen tubes in style}}{\text{Total No. of pollen grains germinated}} \times 100$$

It was assumed that fertilization has taken place if pollen tubes entered the micropylar end of the ovule. In the studies of pollen tube growth, number of ovules with visible micropylar end in each pistil and ovules showing pollen tube entry were counted and expressed as

$$\text{Micropylar penetration (\%)} = \frac{\text{Ovules showing pollen tube entry}}{\text{Total No. of ovules with visible micropylar end}} \times 100$$

Results and Discussion

Sesame, is one of the world's most important oil seed crops due to its relative superior oil quantity, having an oil content generally over 50 per cent (Yermanos *et al.,* 1972), a balanced fatty acid composition with more or less equal percentages of oleic and linoleic acids, one of which is approximately 40 per cent (Liu *et al.,* 1992) and resistance of oil to oxidative deterioration because of the presence of endogenous antioxidants, sesamol and sesaminol together with tocopherols (Baydar *et al.,* 1999). Although sesame is one of the oldest cultivated plants in the world, its production and extension has been limited, particularly because of its low yield (Ashri, 1989).

Intercrossing two divergent plant species would be successful if there is a harmony between the pollen of paternal parent and the pistil of maternal parent. A normal relationship implies that a chain of processes can take place unhampered before fertilization. This requires perfect coordination and interaction between genes in the pollen parent and genes in pistil parent (Hogenboom, 1975). Each of the matching genes consists a link in the chain of processes and interactions that are needed for successful hybridization. Incomplete matching may cause a missing link in the chain and may inhibit interbreeding through incompatibility. In case of sesame, there is lack of information in one species about the mechanism prevailing in others. Hogenboom (1975) termed this phenomenon as 'incongruity'.

Aniline blue fluorescence technique is a procedure to study the *in vivo* pollen tube growth. The process of pollen germination on the stigma, growth of pollen tube in the style and penetration of pollen tube into the ovule followed by double fertilization rather requires detailed precise adjustment between

the pollen and the pistil (de Nettaneourt, 1977). Kallo and Chowdhary (1992) reported that such adjustment was probably an integral component of evolution of each species.

So pollen germination on stigmatic surface is the first step to indicate favorable pollen-pistil interaction for successful fertilization.

Six interspecific crosses were studied to assess the fertilization barriers under *in vivo* condition using aniline blue fluorescence method at a interval *24* hours after pollination. In all the interspecific crosses the pollen tubes successfully reached micropylar end of ovule and thereby effected fertilization at 24 hours after pollination.

S. malabaricum as a female parent

Selfing

All the 25 pistils observed had strong fluorescence in the stigmatic papillae showing good pollen germination (89.8% @ 24 HAP) and average number of pollen tubes entering the stylar region were 83.3%. The ovules of all the pistils showed good micropylar penetration (83.4% @ 24 HAP).

With DS-5

Sixty seven per cent of pollen germination with 71.4 per cent pollen tube growth in stylar region at 24HAP. Mean number of ovules showing pollen tube entry was 60.8 per cent at 24 HAP.

With DSS-9

Seventy per cent of pollen germination with 73.3 per cent pollen tube growth in stylar region at 24HAP. Mean number of ovules showing pollen tube entry was 53.2 per cent at 24 HAP.

With E-8

Good percentage of pollen germination (68.8 at 24 HAP) and pollen tube entry into stylar region (76.5%) was observed in all the 25 pistils studied. Per cent ovules showing micropylar penetration was 67.7 per cent at 24HAP.

Table.1 Pollen germination, pollen tube entry in style and micropylar region at 24 hours after pollination (HAP) in interspecific crosses of sesame

CROSS	Average No. of Pollen grains	Average No. of Pollen Germinated		Average No. of Pollen Tubes Entering Style		Average No. of Ovules	Av. No. of ovules showing micropylar entry	
		MEAN	%	MEAN	%		MEAN	%
S.malabaricum selfing	202.3	181.7	89.8	151.3	83.3	46.5	38.8	83.4
S.malabaricum x DS-5	161.4	108.3	67.1	77.3	71.4	44.1	26.8	60.8
S.malabaricum x DSS-9	153.9	107.2	69.7	78.6	73.3	41.9	22.3	53.2
S.malabaricum x E-8	168.2	115.7	68.8	88.5	76.5	43.3	29.3	67.7
S. mulayanum selfing	138.7	126	90.8	101.2	80.3	40	31.7	79.3
S.mulayanum x DS-5	111.2	78.4	70.5	35	44.6	41.2	23.3	56.6
S. mulayanum x DSS-9	131	77.2	58.9	33.3	43.1	37.7	20.8	55.2
S. mulayanum x E-8	138.6	80.5	58.1	38.1	47.3	39.8	25.3	63.6

Fig.1A Cultivated species pollen germination on wild species stigmatic surface

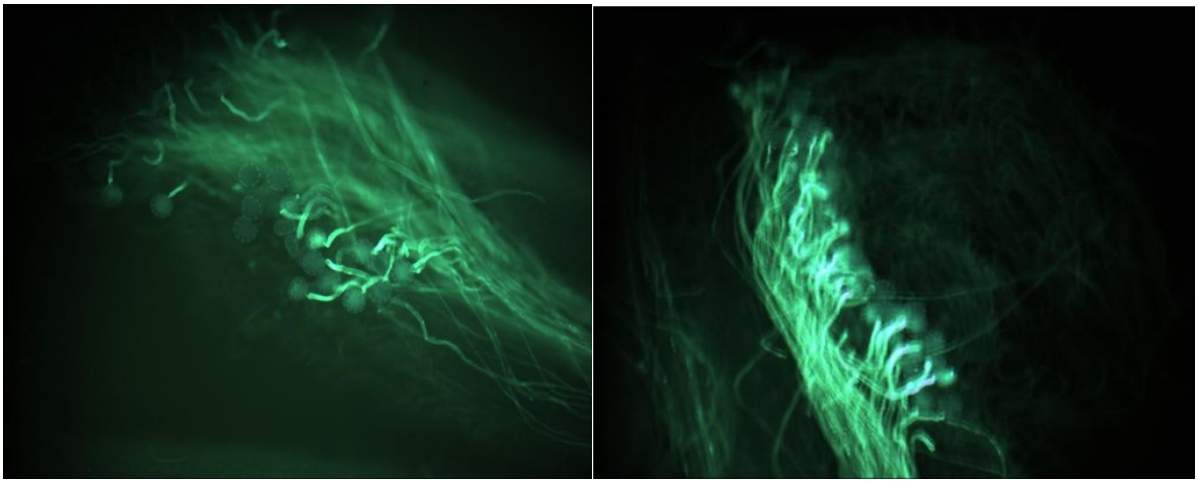


Fig.1B Cultivated species pollen tube growth at style region of wild species

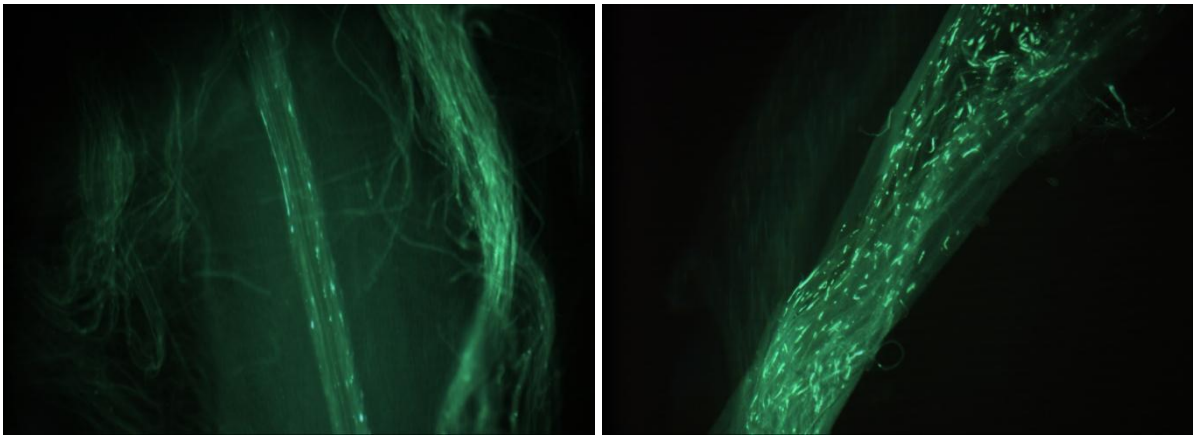
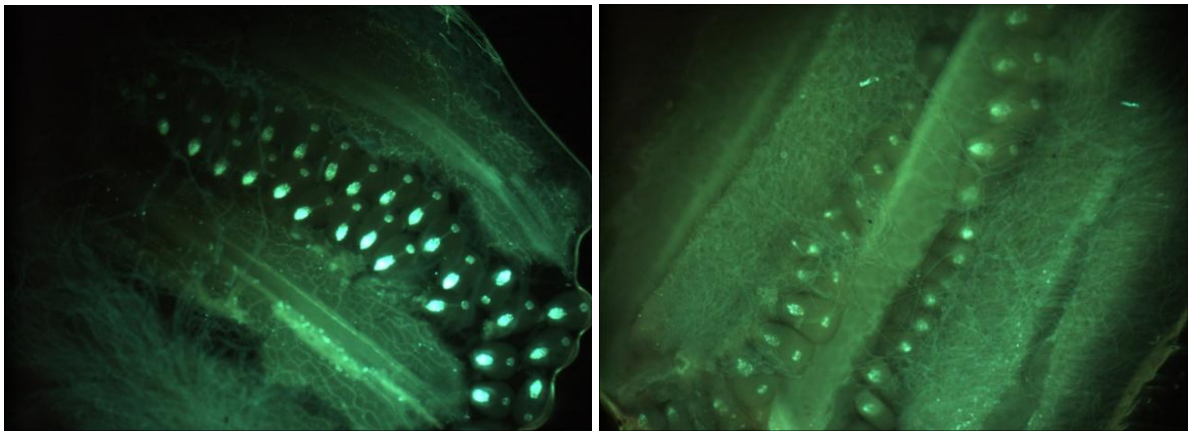


Fig.1C Cultivated species pollen tube entry into micropylar region of wild species and seed development



S. mulayanum as a female parent

Selfing

All the 25 pistils observed had strong fluorescence in the stigmatic papillae showing good pollen germination (90.8% @ 24 HAP) and average number of pollen tubes entering the stylar region was 80.3%. The ovules of all the pistils showed good micropylar penetration (79.3% @ 24 HAP).

With DS-5

Seventy one per cent of pollen germination with 44.6 per cent pollen tube growth in stylar region at 24HAP. Mean number of ovules showing pollen tube entry was 56.6 per cent at 24 HAP.

With DSS-9

Fifty nine per cent of pollen germination with 37.7 per cent pollen tube growth in stylar region at 24HAP. Mean number of ovules showing pollen tube entry was 55.2 per cent at 24 HAP.

With E-8

Fifty eight percentage of pollen germination and 47.3 per cent of pollen tube entry into stylar region was observed in all the 25 pistils studied. Per cent ovules showing micropylar penetration was 63.6 per cent at 24HAP.

This indicates the absence of pre-fertilization barriers in all ten interspecific crosses studied. In the present study, the percentage of pollen germination in all the crosses was significantly lower than the selfing of corresponding female parent at same time interval after pollination. This is similar to the results obtained by Lelivelt (1993) in intergeneric crosses between *B. napus* and *S. alba* (2x) and by Ramesh *et al.,* (2003) in interspecific crosses of sesame. This shows that for pollen germination on stigma, proper recognition substances are necessary which might be lacking to promote pollen germination in interspecific crosses.

The percentage of pollen tubes entering the styles was also significantly lower than the control in all the interspecific crosses. However, reports of Lelivelt (1993) and Ramesh *et al.,* (2003) indicated non-significant differences between control and interspecific crosses in *B. napus* x *S. alba* and *S. occidentale* x *S. indicum*, respectively.

Whereas, Similarly, Parani *et al.,* (1996) reported significantly higher pollen germination on stigma and pollen tube entry in style compared to control.

The pollen tubes in all crosses showed uniform deposition of callose along the pollen tube walls and also small callose plugs spaced at regular intervals in pollen tubes of stylar canal.

These observations are in accordance with the results of Lush and Clarke (1997) and Ramesh *et al.,* (2003) in compatible interspecific crosses of Nicotiana and sesame, respectively, but in contrast to the observations of Williams *et al.,* (1982) who noticed striking differences with respect to number, shape and size of callose plugs in *Rhododendron*. All the pistils observed in each interspecific cross showed good but significantly lower micropylar penetration compared to control at 24 HAP.

Thus with these results it could be summarized that in all the crosses of this study, there was pollen germination and fertilization of the cultivated male parent pollen on the wild female parent indicating that there were no pre-fertilization barriers. However, among all Interspecific crosses

studied, it was observed that, the average number of pollen germination, average number of pollen tubes entering style and average number of ovules with micropylar penetration were significantly low compared to the respective selfed pistils in both *S. mulayanum* and *S.malabaricum.*

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