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Study of fertilization barriers in crosses between *Sesamum indicum* and its wild relatives

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Abstract

Crosses were made using the cultivated species *S. indicum* cv. DS-1 and cv. E-8 as the male parents and the wild species *S. occidentale, S. radiatum* and *S. mulayanum* as the female parents. Studies on field pollination and *in vivo* pollen germination, the barriers to hybridization were classified as post-zygotic, in the crosses *S. occidentale* × DS-1, *S. occidentale* × E-8, *S. radiatum* × DS-1 and *S. radiatum* × E-8. Seeds observed were shrivelled and failed to germinate. Embryo abortion at the later stages may be the major cause for hybrid inviability. In the crosses with *S. mulayanum* as the female parent, viable F1's were obtained indicating absence of fertilization barriers. The possible roles of the enzymes, esterases and peroxidases in cross-compatibility were studied but none was found to affect inter-specific compatibility.

Key words: *Sesamum*, Aniline Blue, Cross-(in)compatibility, esterases, peroxidases, zygotic barriers

Introduction

Some of the wild species of sesame (*Sesamum indicum*) like *S. radiatum*, *S. occidentale* and *S. mulayanum* possess resistance to the diseases *viz*, *Alternaria* leaf spot [1] and *Cercospora* leaf spot [2] and insect pest like sesame shoot webber (*Antigastra catalaunalis*) [3]. In addition, these species are tolerant of drought. Therefore introgressive hybridization can be used to incorporate the genes for resistance into the cultivated species. Development of male sterile lines can also be possible by transferring genes from the wild species *S. mulayanum* [4]. However, many efforts to produce viable hybrids from the crosses between *S. radiatum* and *S. occidentale* and the cultivated species *S. indicum* [5,6,7,8] have failed because of cross-incompatibility. Further, efforts in the case of the cross *S. mulayanum* × *S. indicum* have been limited to transfer of genes [9] and understanding species relationship. Very few reports are available on the type of barriers to hybridization. No report is available on histochemical basis of cross-compatibility in *Sesamum*.

This investigation attempted to understand whether the hybridization barrier in crosses between *S. indicum* and the wild species *S. occidentale*, *S. radiatum* and *S. mulayanum* were pre- or post-fertilization. In addition, an attempt was also made to understand the role of peroxidase and esterase enzymes in cross-(in) compatibility.

Materials and methods

Two cultivars, DS-1 and E-8 of the cultivated species *Sesamum indicum* and three wild species *S. occidentale, S. radiatum* and *S. mulayanum* were used as parents in the hybridization programme. Seeds of the cultivated species were obtained from the Department of Genetics and Plant Breeding, College of Agriculture, Dharwad and those of the wild species were obtained from Directorate of Oilseeds Research, Rajendra Nagar, Hyderabad and Tamil Nadu Agricultural University, Coimbatore. Seeds were sown in *kharif*, 1999 at the Botany Garden, College of Agriculture, Dharwad and extensive care was taken to raise the crop successfully.

S. indicum cv. DS-1 and *S. indicum* cv. E-8 were used as the male and the wild species *S. occidentale, S. radiatum* and *S. mulayanum* as the female parents to make six crosses. Flowering started 30 to 35 days after germination in plants of cultivated species where as about 60 to 65 days after germination in wild species. The crosses were done four to five days after first flowering. As controls, self-pollinations within each of the above species were also carried out. Flower buds of the female parents were hand emasculated one day before anthesis in the evening and the pistils were bagged using butter paper bags to avoid stray pollen. Pollinations were carried out between 7.00 A.M. and 10.00 A.M on the next day using a mixture of freshly collected pollen from several plants of the respective male. Each pistil was pollinated with an abundant amount of pollen. From each cross, 50 pistils were collected 36 hours after pollination (HAP), for microscopic observations and a minimum of 100 pistils were left on the plant to study seed development.

To estimate the effect of post-zygotic barriers, the proportion of developing capsules at 10 days after

pollination (DAP) and 30 DAP, the percentage of capsule set at harvest and the number of seeds per pollination was determined in each of the crosses performed. Germination percentage of seeds from each cross was assessed.

Pollen-stigma interaction and pollen tube growth in the style of each of the 50 pollinated pistils was studied using Aniline Blue Fluorescence Method [10]. Pistils excised from pollinated buds and flowers after 36 HAP were fixed in 3 absolute alcohol: 1 glacial acetic acid (v/v) solution. After staining in 0.001% decolourized Aniline Blue stain, pistils were squashed in a drop of glycerol. Microscopic observations were made to assess the per cent pollen germination, pollen tube entry in to papillae, pollen tubes in the style and per cent ovules showing entry of pollen tubes at the micropylar end. Z-test [11] was used to analyse the data from pollen-pistil interaction and the significance was tested at 1% probability level.

Assessment of esterase enzyme activity was made to understand their role in cross-compatibility, using the method of Mattsson *et al.* [12]. Pollinated pistils (10 to 12 in each cross) at 36 HAP were placed in a humid chamber. 5 mg α-naphthyl acetate was dissolved in a few drops of acetone and mixed with 10 ml of 0.15 M phosphate buffer. To this, 1g of sucrose and 25 mg of Fast Blue B salt were added and mixed thoroughly in a screw cap bottle. A few drops of reaction solution were taken on a clean dry microslide and the stigma was placed in the cavity such that stigmatic surface completely dipped in the solution. Later the preparations were incubated in a humid chamber at room temperature for 10 to 20 minutes till the reddish colour was obtained. After that pistils were removed from the reaction solution and rinsed thoroughly with phosphate butter (0.15 M) and mounted in a drop of glycerine. The pollen grains, that stained dark red, were classified as having high enzyme activity and which stained light red were classified as having low enzyme activity.

The peroxidase enzyme activity was studied using the method of Gomori [13]. Pollinated pistils (10 to 12 in each cross) 36 HAP, were kept fully immersed in the reaction solution prepared by dissolving 100 mg of benzidine in 100 ml of 0.2 M acetate buffer (pH 5.6), 100ml of 1% hydrogen peroxide (v/v) and 20 ml of 5% ammonium chloride (w/v) and mixing thoroughly in a watch glass. These pistils in solution were incubated at room temperature for 2-3 minutes. The pollen grains, which stained light brown, were considered to be having low and those, which stained intense brown, were considered to be having high enzyme activity.

Results and discussion

On self-pollination of *S. occidentale*, *S. radiatum* and *S. mulayanum* a number of capsules with fully developed seed could be harvested (data not presented here). However, the interspecific crosses between *S. indicum* and *S. occidentale* and *S. radiatum* yielded underdeveloped capsules (Fig. 1a) with shrivelled seeds (Fig. 1b). Number of seeds per capsule was 8.6 in the cross *S. occidentale* × DS-1 and 14.6 in the cross *S. radiatum* × E-8. A large number of capsule drop was observed at 30 DAP possibly because of post-fertilization barriers. The seeds obtained from these inter-specific crosses failed to germinate (Table 1). Presence of post-fertilization barriers was also found previously in the cross-involving *S. radiatum* (female) and *S. indicum* (male) [5, 6] and *S. schinzianium* and *S. indicum* [7]. Failure of the cross may be due to early abortion of embryos as in the cross *S. indicum* × *S. alatum* [14]. Contrary to this, normal seeds were obtained from the crosses *S. mulayanum* × DS-1 and *S. mulayanum* × E-8, suggesting these crosses are free from fertilization barriers.

Germination of pollen grains was scored as the number of pollen grains that had germinated on the stigma, with or without having penetrated the stigma papillae (Table 2). Self-pollination within *S. occidentale*, *S. radiatum* and *S. mulayanum* resulted in germination of a large number of pollen grains and penetration by numerous pollen tubes of the papillae and then growing of long pollen tubes in to the styles. However, in the

Table 1. Results of field pollination using *S. indicum* as male parent

Cross	No. of pollinations	No. of capsules set		No. of capsules obtained at harvest (%)	Total No. of seeds	No. of seeds per capsule	Seeds germinated (%)
		10 DAP	30 DAP				
S. mulayanum × DS-1	110	96	88	71 (64.50)	1488	20.96	24
S. mulayanum × E-8	161	136	103	89 (55.28)	2209	24.82	40
S. occidentale × DS-1	465	296	118	81 (17.42)	697	08.60	00
S. occidentale × E-8	410	278	127	85 (20.73)	758	08.92	00
S. radiatum × DS-1	436	278	101	85 (20.18)	811	09.23	00
S. radiatum × E-8	466	253	136	82 (17.60)	891	10.87	00

Table 2. Pollen grain germination, pollen tube growth in papillae and style and micropylar penetration in crosses using *S. indicum* as male parent

Cross	Average no. of pollen grains	Germination of pollen grains		Pollen tubes entering papillae		Pollen tubes in style		Average no. of ovules with visible micropylar end	Ovules showing micropylar penetration	
		Mean	%	Mean	%	Mean	%		Mean	%
S. mulayanum (selfed)	137	110	81	99.22	90	64	58	39	36	91
S. mulayanum × DS-1	121	87	72**	58.43	67**	38	43**	35	24	68**
S. mulayanum × E-8	144	104	72**	74.60	72**	37	35**	36	28	78**
S. occidentale (selfed)	136	110	81	97.03	89	54	49	40	36	90
S. occidentale × DS-1	122	73	60**	49.20	67**	35	48	39	29	74**
S. occidentale × E-8	122	72	59**	49.10	68**	33	46	33	21	64**
S. radiatum (selfed)	146	121	83	104.26	86	58	48	35	31	89
S. radiatum × DS-1	116	73	63**	53.54	73**	34	46	39	29	76**
S. radiatum × E-8	124	73	59**	42.28	66**	33	45	33	27	81**

**-Significantly different from respective controls at 1% level of probability

interspecific crosses significantly lower number and percentage of pollen grains germinated. Most of the germinated pollen grains had entered through the stigma papillae indicating compatibility at stigmatic surface (Fig. 2a & 2b). Lelivelt (1993) has noticed similar result in the intergeneric crosses between *B. napus* and × *Brassicoraphanus* or *S. alba* 2x [15]. However, the results obtained in the present investigation are in contrast to the results of Parani *et al.* (1996) in the cross *S. alatum* × *S. indicum* [16]. They found less number of germinated pollen grains entering stigmatic papillae.

The selfed pistils of *S. occidentale*, *S. radiatum* and *S. mulayanum* had numerous pollen tubes in their stylar region. In all the cases, enough number of pollen tubes reached the lower part of the style (Fig. 2c). In the inter-specific crosses with *S. occidentale* and *S. radiatum* the number of pollen tubes present in the stylar canal of the pistils was not significantly different from that of the controls. Similar results were observed in the interspecific cross *S. alatum* × *S. indicum* [16] by Parani *et al.* (1996). Thus there was no evidence of any barrier in the style.

Pollen tube entry into the micropylar end of the ovules could be investigated only in those ovules where the micropyle end was undamaged and clearly visible. The number of ovules with micropylar penetration is expressed therefore, as a percentage of the total number of ovules with visible undamaged micropyle. When selfed, in *S. occidentale, S. radiatum* and *S. mulayanum* 90 %, 89 % and 91%, respectively of the ovules showed pollen tube entry through the micropyle (Table 2). In the interspecific crosses, significantly lower proportions (64% to 81%) of the pollen tubes entered into the micropyle (Fig. 2d). Lelivelt (1993) found similar results from the intergeneric crosses within *Cruciferae* [15]. Further the pistils of all the interspecific crosses had uniform deposition of callose along the pollen tube walls and also small callose plugs spaced at regular intervals in the pollen tubes of the stylar canal indicating the formation of callose plugs even in the crosses where fertilization has occurred. Our observations on callose response were parallel to the results obtained by Lush and Clarke (1997) [17] and different from the results obtained by Williams *et al.* (1982) [18] who observed callose plugs placed at irregular intervals in the pollen tubes of the stylar canal.

The present one is the first study of the pollen-stigma interaction in sesame with respect to enzymatic activities. No differences in esterase and peroxidase activities were found between the inter-specific crosses and the selfs.

Thus, the work presented here has shown that interspecific crosses performed are free from pre-fertilization barriers. However, the operation of post-fertilization barriers appears to be the major cause for failure of hybrid seed development in the crosses namely *S. occidentale* × DS-1, *S. occidentale* × E-8, *S. radiatum* × DS-1 and *S. radiatum* × E-8. The application of ovary-ovule culture or embryo rescue techniques could help in obtaining viable interspecific hybrids in the crosses showing post-fertilization barriers.

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Fig. 1. Capsules and seeds of parents and hybrids (a) Capsules: (1) *S. occidentale* x *S. occidentale* (2) *S. occidentale* x DS-1 and (3) *S. occidentale* x E-8; (b) Seeds: (1) *S. occidentale* (2) E-8 and (3) *S. occidentale* x E-8

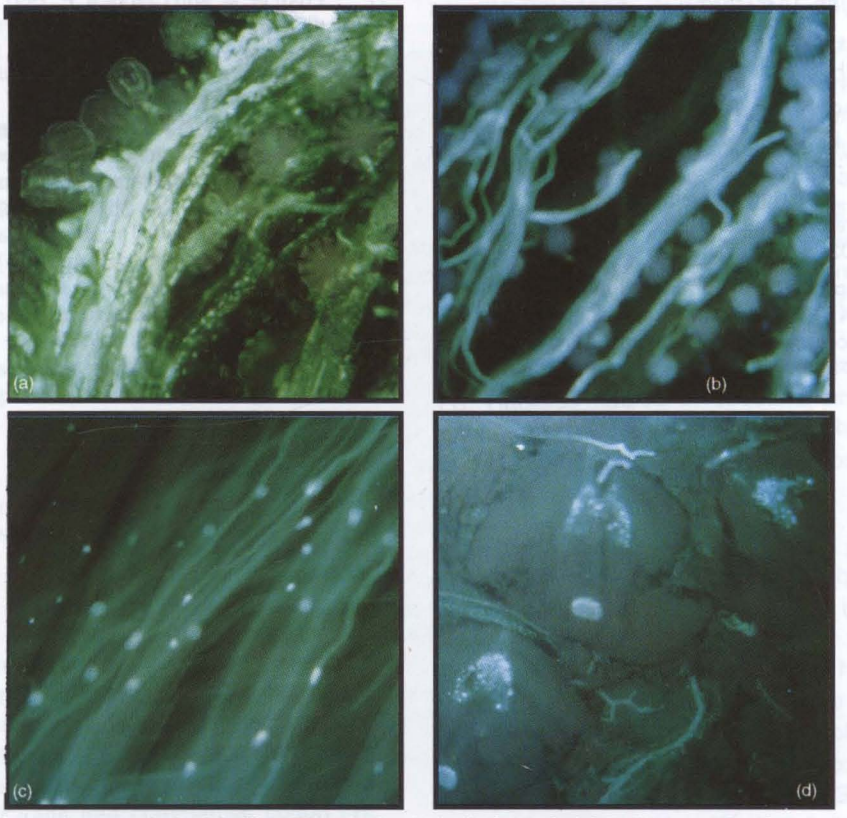

Fig. 2. Fluorescence photomicrographs* of Aniline Blue stained pistils (100 x): (a) Pollen germination and pollen tube entry into papillae in the cross *S. mulayanum* x DS-1; (b) Pollen tube entry into papillae in the cross *S. radiatum* x DS-1; (c) Pollen tubes with callose plugs in the style of the cross *S. radiatum* x DS-1 and (d) The cross *S. radiatum* x E-8 showing micropylar penetration of ovules. *Microscope used-Zeiss microphot fluorescence microscope model Axioskop; Illumination-425 to 490 nm uv light; Transmission filter-G 365

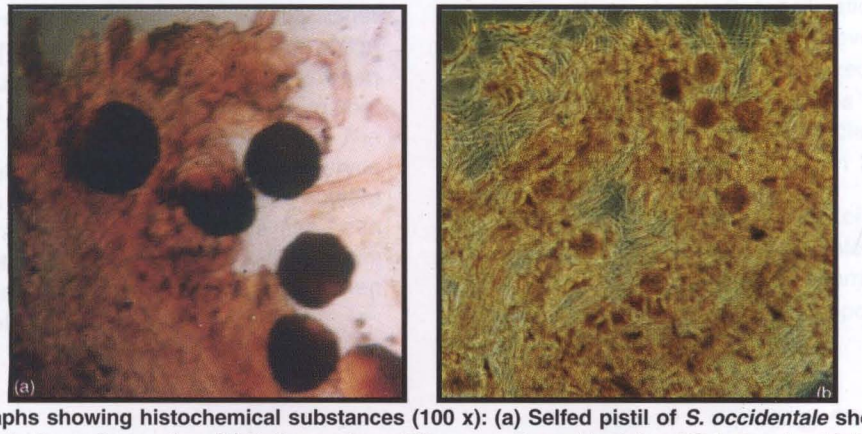

Fig. 3. Photomicrographs showing histochemical substances (100 x): (a) Selfed pistil of *S. occidentale* showing high esterase enzyme activity in the pollen grains; (b) Pollen grains of the cross *S. occidentale* x DS-1 showing high peroxidase enzyme acitvity

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