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Research Article

Pollen pistil interaction in the interspecific cross of *Sesamum indicum* and *S. radiatum*

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Abstract

The study deals with the pollen morphology, *in vivo* pollen germination and pollen pistil interaction in two species of Sesame *viz*., *S. indicum* (CO 1, VRI 3, TMV 7) and *S. radiatum*. As wild species are potential sources of biotic and abiotic stress-resistant genes, strong crossability with cultivated varieties is critical for gene transfer through traditional breeding programmes. The pollen morphology studied under a Scanning electron microscope revealed both species to be stephanocolpate and possessed the shape of prolate. The number of colpi counted assisted in differentiating the pollen of *S. indicum* from *S. radiatum* and represented the evolution of the former. The pollen pistil interaction by aniline blue technique between direct and reciprocal crosses of two species of *Sesamum* reported the pollen germination per cent of *S. radiatum* × *S. indicum* (CO1) (85.00) at one hour after pollination, *S. indicum* (CO1) × *S. radiatum* (80.00) at one hour after pollination and *S. radiatum* × *S. indicum* (VRI 3) (80.35) at two hours after pollination were on par with each other and expressed higher pollen germination per cent, while *S. indicum* (CO1) × *S. radiatum* (22.70), *S. indicum* (VRI 3) × *S. radiatum* (20.30) and *S. indicum* (TMV 7) × *S. radiatum* (20.15) at eight hours after pollination indicated lower pollen germination per cent. In both the direct and reciprocal crosses, the pollen tubes did not reach the ovary even after eight hours of pollination depicting the presence of pre fertilization barrier and hence capsules were not developed.

Keywords : *S*esame, interspecific cross, pollen pistil interactions, fertilization barriers

INTRODUCTION

Sesame (*Sesamum indicum*) is an indispensable oilseed crop as it can be grown throughout the year in the tropics and subtropics as a rainfed crop. It grows well in many cropping systems but is preferably raised under moisture stress with very limited management strategies by small and marginal farmers (Pham *et al*.,2010). Globally, sesame is cultivated on a substantial acreage with an unappreciable productivity. India takes up the second position in the area under sesame cultivation (16.2 lakh ha.) and third position in production (0.657 million tons). In India, Uttar Pradesh ranks first (0.315 million ha.) followed by Madhya Pradesh (0.278 million ha.) and Rajasthan (0.251 million ha.), while in production, West Bengal leads all other states with a total production of 0.134 million tones. In Tamil Nadu, sesame is cultivated over 53,010 ha. area with 36,470 tonnes of total production (INDIASTAT, 2020). Sesame oil is widely recognized for its high concentration of beneficial mono and polyunsaturated fatty acids (Tripathi *et al*., 2013). Sesame seeds are composed of sesamol, a unique anti-oxidant with higher poly-unsaturated fatty acids, because of which it is called “Queen of oilseed crops” (Ashri and Amram,1988). Although sesame is widely utilised for various purposes, its productivity has been declining in comparison to other oilseed crops (Disowja *et al*., 2020)

S. indicum variety CO 1 (2n = 26) has been commercially cultivated since 1983 for its black warty seed, with an oil content of 51 per cent and seed yield potential of 600 to 900 kg/ha. Since 2017, the variety VRI 3 (2n = 26) has been grown extensively for its white seed, 50.1 per cent oil content and seed yield potential of 1105 kg/ha. *S. indicum* variety TMV 7 (2n = 26) has been brought into cultivation since 2009 for its lustrous brown seed with an oil content of 50 per cent and seed yield potential of 820 kg/ha. The wild species of sesame are good source of genes for biotic and abiotic stress tolerance.,. *S. radiatum* (2n = 64) confers resistance to drought (Prabakaran,1996), shoot webber (Srinivasulu,1991), powdery mildew (Thangavelu,1994), Phytophthora blight, Fusarium wilt, leaf blight and seedling blight (Lee *et al*., 1991).

Interspecific hybridization facilitates the transfer of desirable genes from wild species to cultivated species. The study of pollen pistil interaction of interspecific cross of sesame involvs the assessment of *in vivo* pollen germination per cent, growth of pollen tubes in the pistil and fertilization barriers which would ultimately result in identification of the presence or absence of fertilization barriers, which could help in devicing suitable strategies to produce interspecific hybrids. The present study was undertaken to throw light on the pollen pistil interaction on interspecific cross of *S. indicum* (CO 1, VRI 3 and TMV 7) and *S. radiatum* to identify the fertilization barriers operating in the cross.

MATERIALS AND METHODS

The seeds of *S. indicum* varieties CO 1, VRI 3 and TMV 7 and *S. radiatum* were collected from the Department of Oilseeds, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore and were raised in individual rings at Wild Species Garden, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore during the first week of February 2021. Recommended package of practices was followed for raising the crop.

For conducting pollen study, anthers were collected from ten randomly newly opened flowers in the morning (7.00 am to 8 am) and were viewed under light microscope. For study under Scaning Electron Microscope (SEM), pollen grains were suspended and mounted on double-sided conductive carbon tape fixed to the stub, sputter coated with gold alloy for 15 seconds in an EMITECH SC7620 sputter coater (Quorum Technologies Ltd., Laughton, East Sussex, England), and positioned on the sample chamber of a SEM (Quanta 250; FEI Company, Eindhoven, Netherlands). After attaining a higher vacuum, the filament was turned on and different parameters such as electron beam, intensity, spot size, voltage, emission current and images were acquired.

To study the fertiisation barrier operating *invivo*, flowers in female plants were hand emasculated the previous day evening between 4 and 5 pm by pulling out the epipetalous corolla and enclosing with butter paper cover to reduce the risk of contamination from foreign pollen and tagged. Cross-pollination was accomplished by dusting the appropriate pollen grains precisely onto the stigmas of emasculated flowers during on the next day morning between 7.30 to 9 am. All pollinated flowers were immediately covered with wax paper bags following pollination (Thangavelu and Nalathambi, 1982).

The pistils from the tagged cross and self-pollinated flowers were collected at 1, 2, 4 and 8 Hours After Pollination (HAP) and fixed in acetic acid: alcohol mixture (1:3 v/v) for 12 hours. The maximum time interval for the collection of pollinated pistils was restricted to 8 HAP because under natural conditions most of the crossed flowers would wither within 10 HAP. For every collection time, each of the ten crossed and self-pollinated pistils from five tagged plants of sesame was used for microscopic observations. The pistils can be softened in 0.8 N NaOH overnight, stained with 0.1% (w/v) aniline blue in 0.1 M K_3PO_4 for four hours and mounted on microscope slides in 50% glycerol (Sitch, 1990). The slides were observed under a Fluorescent microscope (Nikon Eclipse Ni-U, Japan) using a Nikon filter (330–380 nm excitation filter, 410 nm barrier filter). Images were recorded with the help of a Nikon DS-Fi3 camera using NIS Elements F v.4.60.00 image processing platform.

Pollen germination per cent was recorded by the per cent ratio of the number of pollen grains germinated to the total number of pollen grains. Pollen tube growth was observed based on the rate at which it reached varying positions in stigma, namely stigmatic surface, mid-style region and ovary.

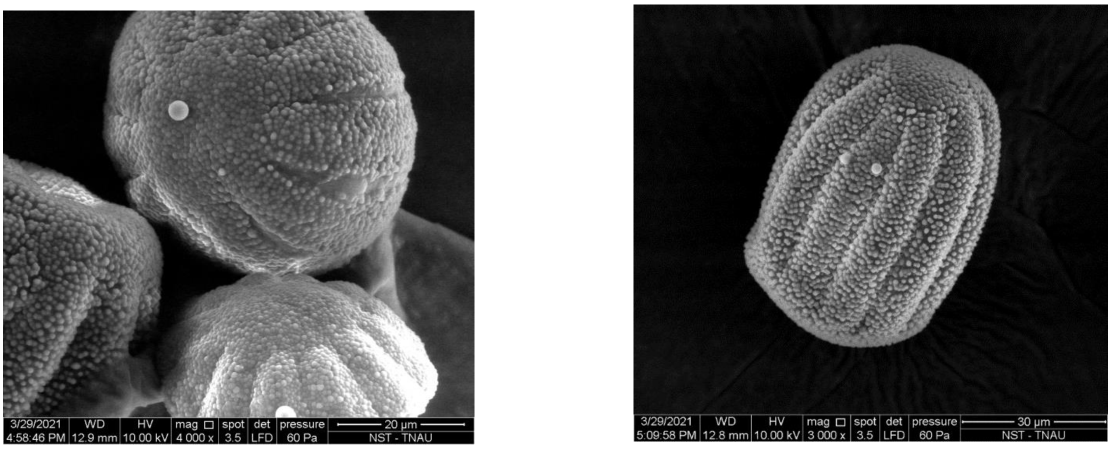
The observations were analyzed by two factorial designs with three replications using the statistical tool package AGRES. The mean pollen germination per cent of selfed and crossed pistils of wild and cultivated genotypes was obtained and the least square difference (LSD) was used to compare the significant differences among them.

RESULTS AND DISCUSSION

The number of colpi in *S. indicum* was found to be 12 (**Table 1 and Fig.1**) and in *S. radiatum* it was eight (**Table 1 and Fig. 2**). Colpi are elongated apertures that are evenly spaced on the equatorial plane or across the general surface of a pollen grain. The number of colpi counted in both species was more than three, hence were concluded as stephanocolpate. The ratio between the polar and equatorial axis of *S. indicum* and *S. radiatum* was 1.39 and 1.76, respectively (**Table 1**). The shape of pollen grains was studied based on the ratio of the polar and equatorial axis of pollen, as proposed by

Table 1. Pollen morphology of *Sesamum Spp*

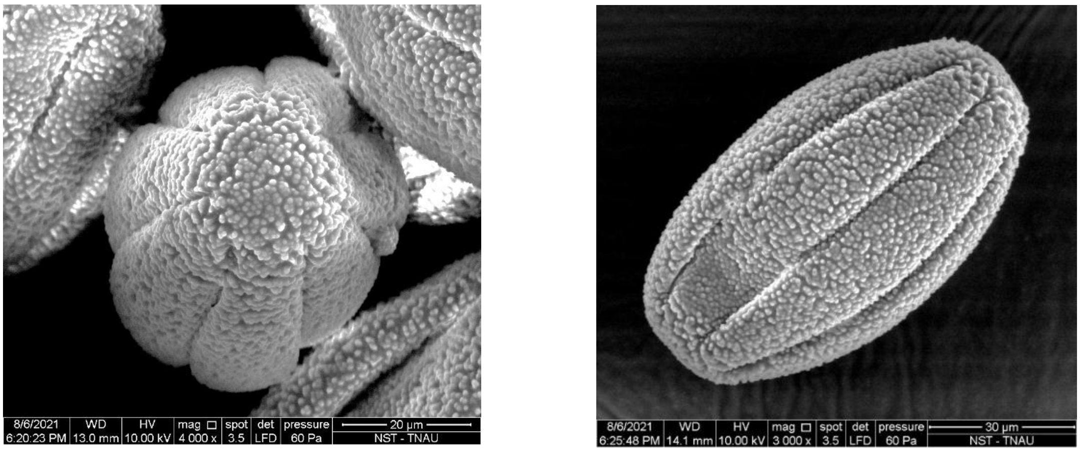
S. No.	Species	Number of colpi per pollen	Classification of pollen (based on number of colpi)	Ratio between the polar axis and the equatorial diameter
1	*S. indicum*	12	Stephanocolpate	1.39
4	*S. radiatum*	7-8	Stephanocolpate	1.76



1a. Polar axis of *S. indicum* pollen grain

1b. Equatorial axis of *S. indicum* pollen grain

Fig. 1. SEM images of pollen grains of *S. indicum*



2a. Polar axis of *S. radiatum* pollen grain

2b. Equatorial axis of *S. radiatum* pollen grain

Fig. 2. SEM images of pollen grains of *S. radiatum*

Erdtman (1952). In , *S. indicum* and *S. radiatum* the shape was identified as prolate type as the aforementioned ratio was between 1.33 and 2.00. The number of colpi recorded was greater in the cultivated species than in the wild species of sesame. A higher number of colpi was noticed in *S. indicum* (12) whereas it was lesser in *S. radiatum* (7-8). The importance of aperture in plant phylogeny was emphasized by Chung *et al*. (2010). Akhila and Beevy (2015) and Sruthi *et al.* (2021) stated that a rising number of colpi signifies that the species has progressed, and so the greater the colpi number, the more evolution has occurred in the species. Since the number of colpi in cultivated species is greater than in wild species, the former is presumed to have undergone more evolution than the latter. Although the genus falls under the same morphological type and possesses a similar shape, the number of colpi counted assisted in the identification of the species and hence the same would be rewarding for recognition of the species.

When *S. indicum* varieties CO 1, VRI 3, TMV 7 and *S. radiatum* were selfed, regular capsule development and seed set were observed. Out of the 50 flowers pollinated in each variety of *S. indicum* and *S. radiatum*, a highest of 48 capsules were developed and a successful seed set was observed with a capsule setting per cent of 96.00 , with 73.00 seeds per capsule in *S. indicum* variety CO1, while the lowest of 42 capsules were developed and successful seed set was observed with a capsule setting per cent of 84.00,with 62.00 seeds per capsule in *S. indicum* variety VRI 3 (**Table 2**).

The pollen germination in the stigmatic region of *S. indicum* (CO 1, VRI 3 and TMV 7) and *S. radiatum* were noticed one hour after pollination (HAP) upon selfing. The highest pollen tube germination per cent was seen in one HAP at the stigmatic region, whereas the lowest pollen tube germination per cent was detected in eight HAPunder selfing (**Table 3 and Fig. 3,4,5 and 6**). The pollen tubes that germinated in the stigmatic surface one HAP, took two hours to head towards the mid-stylar region and reached mid-stylar region four HAP and reached ovary after eight HAP under selfed condition. Ram *et al.* (2006) recorded self-compatibility in *S. indicum* (TMV 3), *S. alatum*, *S. lacinatum*, and *S. radiatum* . Kumari and Ganesamurthy (2015) reported normal capsule establishment and seed set in the Sesamum species *S. indicum* (TMV 3), *S. alatum*, and *S. radiatum*.

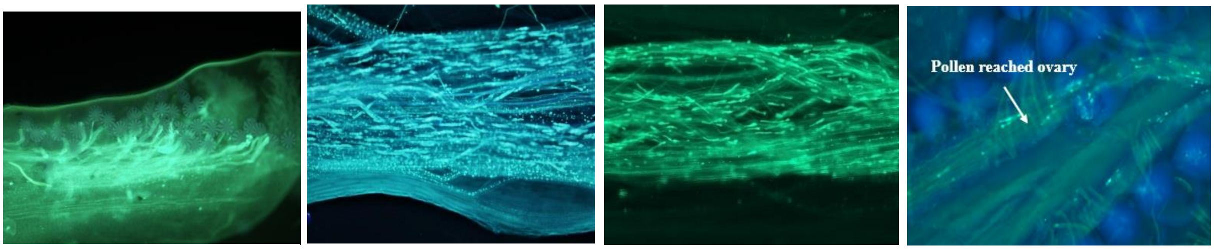
When *S. indicum* (CO 1, VRI 3 and TMV 7) was taken as the female parent and *S. radiatum* as male parent, the pollen tube germination of *S. radiatum* was first witnessed one HAP in the stigmatic region. Maximum pollen tube germination per cent was observed one HAP while the minimum pollen tube germination per cent was observed eight hours after pollination in all the direct crosses. (**Table 5 and Fig. 7, 8 and 9**). The pollen tubes

Table 2. Details of selfing attempted in cultivated and wild species of sesame

Species	Number of flowers pollinated	Number of capsules formed	Percent of capsules formed	Mean number of seeds per capsules	Remarks
S. indicum (CO 1)	50.00	48.00	96.00	73.00	Capsule set, and viable seeds
S. indicum (VRI 3)	50.00	46.00	92.00	63.00	Capsule set, and viable seeds
S. indicum (TMV 7)	50.00	47.00	94.00	58.00	Capsule set, and viable seeds
S. radiatum	50.00	42.00	84.00	62.00	Capsule set, and viable seeds

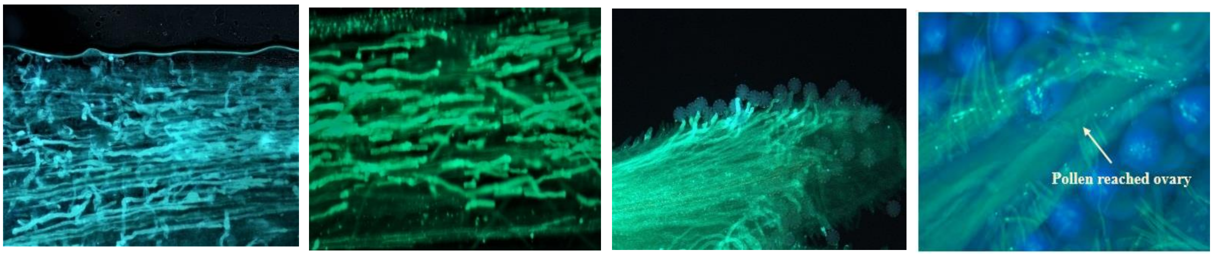
Table 3. *In vivo* pollen germination percent in selfed species of sesame

Species	Pollen germination percent				
	1 HAP	2 HAP	4 HAP	8 HAP	Mean
S. indicum (CO 1)	95.20	90.40	92.85	85.71	91.40
S. indicum (VRI 3)	93.70	88.50	86.50	82.00	87.67
S. indicum (TMV 7)	92.00	89.75	84.00	80.50	85.00
S. radiatum	89.50	85.50	88.00	81.75	86.18



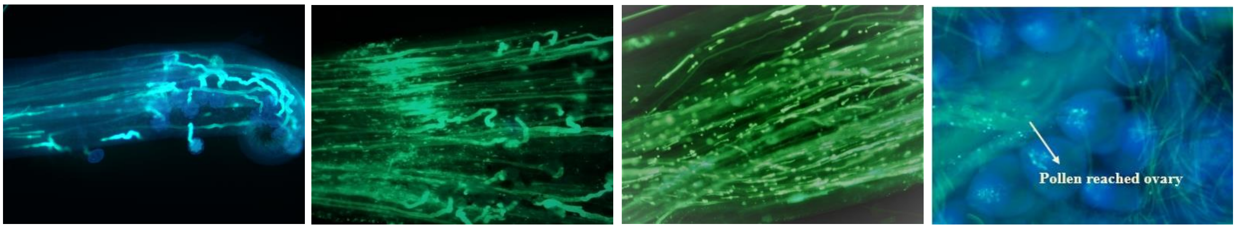
3a. 1 HAP 3b. 2 HAP 3c. 4 HAP 3d. 8 HAP

Fig. 3. *In vivo* pollen pistil interaction in selfed *S. indicum* (CO 1)



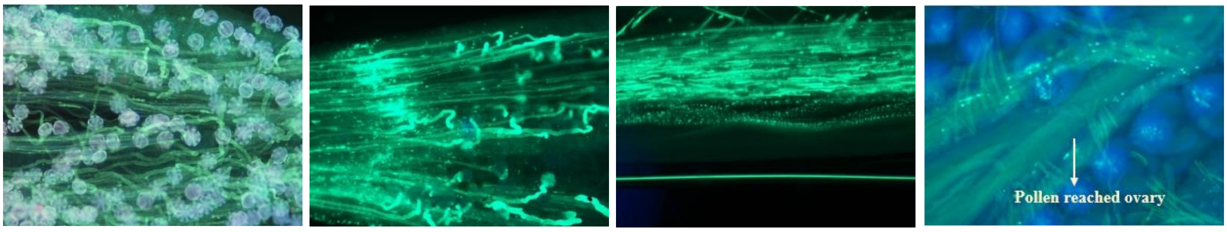
4a. 1 HAP 4b. 2 HAP 4c. 4 HAP 4d. 8 HAP

Fig. 4. ***In vivo*** **pollen pistil interaction in selfed** ***S. indicum*** **(VRI 3)**



5a. 1 HAP 5b. 2 HAP 5c. 4 HAP 5d. 8 HAP

Fig. 5. ***In vivo*** **pollen pistil interaction in selfed** ***S. indicum*** **(TMV 7)**



6a. 1 HAP 6b. 2 HAP 6c. 4 HAP 6d. 8 HAP

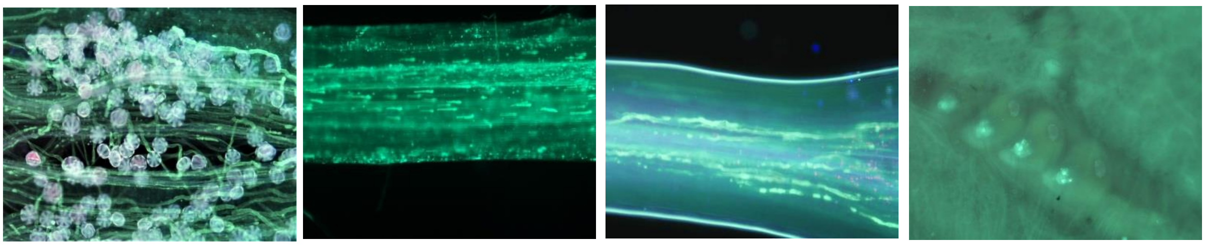
Fig. 6. ***In vivo*** **Pollen Pistil interaction in selfed** ***S. radiatum***

of *S. radiatum* failed to reach the ovary of *S. indicum* (CO 1, VRI 3 and TMV 7) even after eight HAP indicating prevalence of pre fertilization barriers and hence none of the pollinated flowers formed capsules or set seeds (**Table 4**). When the pistillate parent *S. radiatum* and the staminate parent *S. indicum* (CO 1, VRI 3, and TMV 7) were crossed, none of the flowers subjected to pollination established capsules or set seeds. (**Table 4**). The pollen tube germination of all varieties of *S. indicum* was first established in the stigmatic region one HAP. The highest pollen tube germination per cent was observed one HAP while the lowest pollen tube germination per cent was observed eight HAP in all the reciprocal crosses (**Table 5 and Fig. 10, 11 and 12**). Even after eight HAP, the pollen tubes of *S. indicum* (CO 1, VRI 3 and TMV 7) were ineffectual to reach the ovary of *S. radiatum*, exhibiting pre-fertilization barriers.

The mean pollen germination per cent was the highest in *S. radiatum* x *S. indicum* (CO 1) (73.81) followed by *S. radiatum* x *S. indicum* (VRI 3) (67.26), crosses, while *S. indicum* (TMV 7) × *S. radiatum* (51.47) crosses recorded the lowest mean pollen germination per cent (Table 5). The pollen germination per cent was highest at one HAP (77.27) followed by two HAP (75.29) and four HAP (56.22). The lowest pollen germination per cent was noted at eight HAP (37.37). The pollen germination per cent of *S. radiatum* × *S. indicum* (CO1) (85.00), *S. indicum* (CO1) × *S. radiatum* (80.00) at one hour after pollination and *S. radiatum* x *S. indicum* (VRI 3) (80.35) at two HAP were higher and were on par with each other . In case of *S. indicum* (CO 1) × *S. radiatum* (22.70), *S. indicum* (VRI 3) × *S. radiatum* (20.30) and *S. indicum* (TMV 7) × *S. radiatum* (20.15) at eight HAP, the pollen germination was lower. (**Table 5**). In all the crosses made, the pollen

Table 4. **Details of crosses attempted between *S. indicum* and *S. radiatum***

Cross	Number of flowers pollinated	Number of capsules formed	Percent of capsule formed	Mean number of seeds per capsules	Remarks
S. indicum (CO 1) x *S. radiatum*	100.00	0.00	0.00	0.00	No capsule set
S. radiatum x *S. indicum* (CO 1)	100.00	0.00	0.00	0.00	No capsule set
S. indicum (VRI 3) x *S. radiatum*	100.00	0.00	0.00	0.00	No capsule set
S. radiatum x *S. indicum* (VRI 3)	100.00	0.00	0.00	0.00	No capsule set
S. indicum (TMV 7) x *S. radiatum*	100.00	0.00	0.00	0.00	No capsule set
S. radiatum x *S. indicum* (TMV 7)	100.00	0.00	0.00	0.00	No capsule set



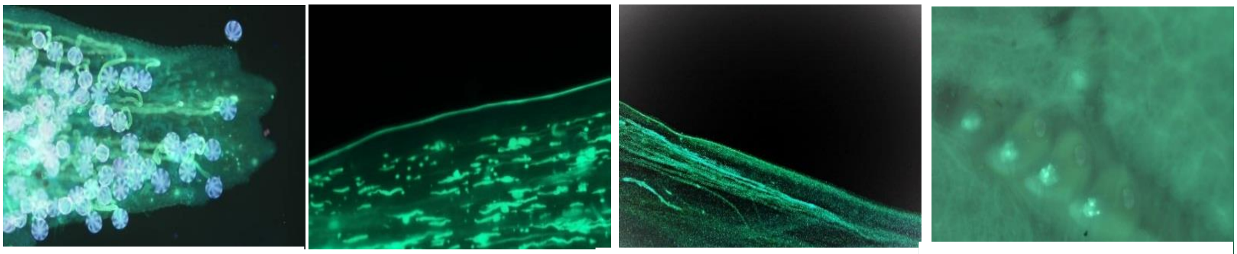
7a. 1 HAP

7b. 2 HAP

7c. 4 HAP

7d. 8 HAP-pollen tubes did not reach ovary

Fig. 7. ***In vivo*** **pollen pistil interaction in *S. indicum* (CO 1) x *S. radiatum***



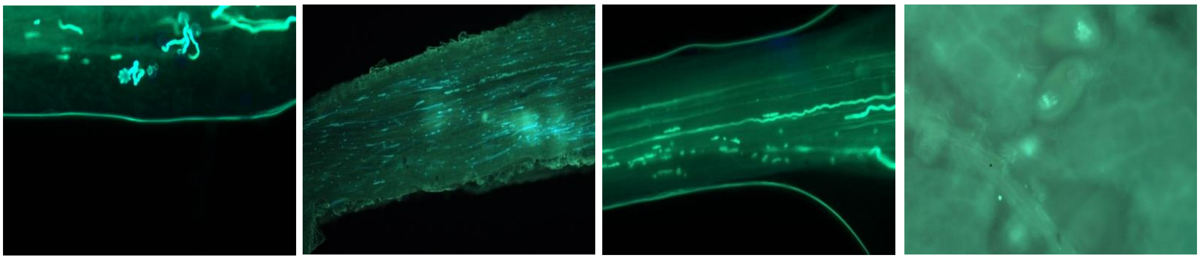
8a. 1 HAP

8b. 2 HAP

8c. 3 HAP

8d. 8 HAP -pollen tubes did not reach ovary

Fig. 8. ***In vivo*** **pollen pistil interaction in *S. indicum* (VRI 3) x *S. radiatum***



9a. 1 HAP

9b. 2 HAP

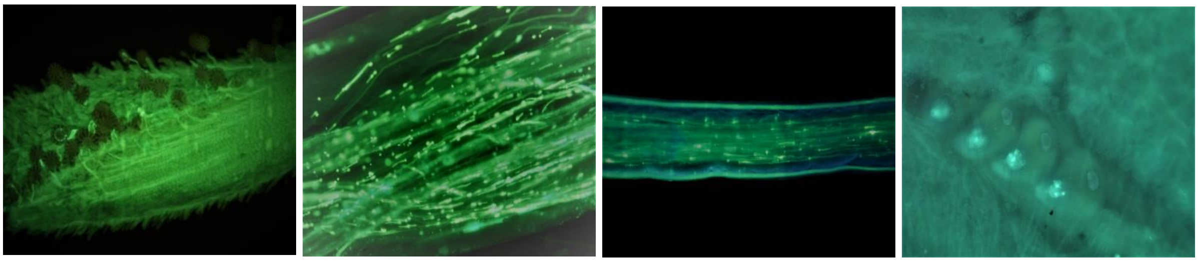
9c. 4 HAP

9d. 8 HAP-pollen tubes did not reach ovary

Fig. 9. ***In vivo*** **pollen pistil interaction in *S. indicum* (TMV 7) X *S. radiatum***

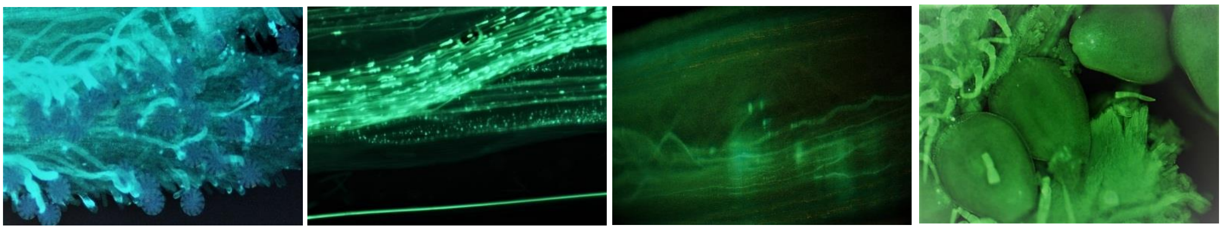
Table 5. ***In vivo*** **pollen germination percent in cross between** ***S. indicum*** **and** ***S. radiatum***

Species	**Pollen germination percent**				
	1 HAP	**2 HAP**	**4 HAP**	**8 HAP**	**Mean**
S. indicum (CO 1) x *S. radiatum*	80.00	75.71	50.60	22.70	57.25^{d}
S. radiatum x *S. indicum* (CO 1)	85.00	78.50	70.87	60.87	73.81^{a}
S. indicum (VRI 3) x *S. radiatum*	75.65	70.00	45.00	20.30	52.73^{d}
S. radiatum x *S. indicum* (VRI 3)	78.00	80.35	60.25	50.45	67.26^{b}
S. indicum (TMV 7) x *S. radiatum*	74.50	68.75	42.48	20.15	51.47^{c}
S. radiatum x *S. indicum* (TMV 7)	70.50	78.45	68.18	49.75	66.72^{b}
Mean	77.27^{a}	75.29^{a}	56.22^{b}	37.37^{c}	61.54
Factors	Species	Pollen germination percent	Species x Pollen germination percent	Pollen germination percent x Species	
S.E	0.8477	1.2367	2.7570	3.0293	
CD (P = 0.05)	1.8888	2.5085	5.6442	6.1445	



10a. 1 HAP **10b. 2 HAP** **10c. 4 HAP** **10d. 8 HAP-pollen tubes did not reach ovary**

Fig. 10. ***In vivo*** **pollen pistil interaction in the cross** ***S. radiatum*** **x** ***S. indicum*** **(CO 1)**



11a. 1 HAP **11b. 2 HAP** **11c. 4 HAP** **11d. 8 HAP -pollen tubes did not reach ovary**

Fig. 11. ***In vivo*** **pollen pistil interaction in the cross** ***S. radiatum*** **x** ***S. indicum*** **(VRI 3)**

tubes germinated in the stigmatic surface one HAP, took two HAP to head towards the mid-stylar region and reached mid-stylar region four HAP and did not reach the ovary even after HAP.

The attempt of crossing *S. indicum* and *S. radiatum* in both direct and reciprocal ways led to unsuccessful crosses. Capsules were not attained and seed sets were not observed suggesting the operation of pre fertilization barrier as pollen tubes in both direct and reciprocal crosses failed to reach ovary even after eight HAP. The failure of the cross can be presumed due to the differences in the ploidy level of both species. In the direct cross, the pollen tubes of *S. radiatum* were bigger due to its higher ploidy level which has led to disturbances of the former to travel in the pistil of *S. indicum.* The

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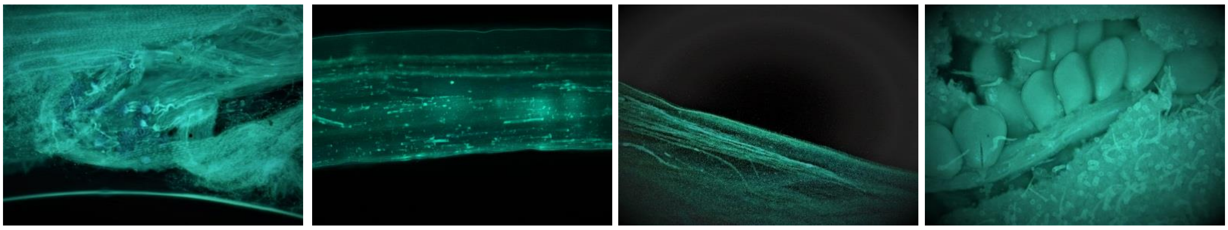

12a. 1 HAP **12b. 2 HAP** **12c. 4 HAP** **12d. 8 HAP - pollen tubes did not reach ovary**

Fig. 12. *In vivo* pollen pistil interaction in *S. radiatum* x *S. indicum* (TMV 7)

conflicting interaction between the pollen tubes of *S. indicum* and pistil of *S. radiatum* restrained the pollen tubes from reaching the ovary. Ramanathan (1950) reported shrivelled seed set in a hybridization programme involving *S. indicum* and *S. radiatum*. Tarihal *et al*. (2003) conducted similar research, observing underdeveloped capsules and shrivelled seeds,which failed to germinate upon sowing, indicating the presence of a post-zygotic barrier. Ram *et al*. (2006) observed twisted pollen tubes and germinated pollen with bulged tips in a cross between S. *indicum* and S. *radiatum* which concluded pre zygotic incompatibility. Dasharath *et al*. (2007) utilised ovary and ovule culture to successfully generate inter-specific hybrids between cultivated S. *indicum* and its wild relatives S. *radiatum*. Kumari and Ganesamurthy (2015) reported that the frequency of pollen germination and pollen tube growth was very slow in the interspecific cross between S. *indicum* and S. *radiatum* in direct and reciprocal crosses due to some inhibitory activity for germination on stigmatic surface and pollen tube development along stylar tissue establishing the presence of post zygotic barrier. The accumulation of callose, a β-1,3 glucan, in stigmatic papillae cells in interaction with incompatible pollen grains, as suggested by Kerhoas *et al*., 1983, could be the cause of pollen denial. Standard pollen tube metabolism may be obstructed in discordant situations, culminating in pollen tube degeneration, deterring it from trying to grow further down to the micropyle. In the cross *S. indicum* × *S. radiatum*, besides the pollen tube growth inhibition, numerous forms of abnormalities among alien pollen tubes were observed, that were not prevalent in self-pollinated pistils. Similar observation was reported by Ram *et al*. (2006).

Hence it could be concluded that operation of pre-fertilization barriers in the interpecific crossing attempted in the present study were hampering successful seed set. Strategies such as the use of bud pollination, irradiated mentor pollen technique, stump pollination, ovule culture method and application of growth hormones may lead to the development of interspecific hybrids and successful introgression of desirable genes from wild species to the cultivated species.

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