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INOCOMPATIBILITY IN DIRECT AND RECIPROCAL CROSS BETWEEN *SESAMUM INDICUM* L. AND *S. ALATUM* THONN.

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The present communication details out the results of hybridisation studies in two Sesamum species, *S. indicum* L. and *S. Alatum* Thonn. Direct cross between *S. indicum* and *S. alatum* did not set seeds, while in the reciprocal cross seeds were set.

Key Words: *Sesamum Indicum*, incompatibility.

Sesamum (*Sesamum indicum* L.) is the oldest oilseed known crop to man having an oil content up to 60%. It is largely a rainfed crop cultivated in marginal lands, and its productivitty is severaly limited by phyllody disease caused by MLOs. Phyllody disease in sesamum is a highly destructive one against which resistant varieties were not available. Recently *S. alatum* Thonn has been identified to possess resistance to this disease (Srinivasulu and Narayanaswamy, 1992). However, though hybridisation between *S. indicum* and *S. alatum* has been attempted since 1954 (Kedharnath 1954) gene transfer between the two species was not possible because of interspecific inocompatibility (Subramanian, 1972, Ramalingam *et al.* 1992). Therefore, in the present study, direct and reciprocal hybridisation between five varieties/accessions of *S. indicum* and one accession of *S alatum* was made, *in vivo* pollen germination and pollen tube growth were studied to find out the exact stage at which the incompatibility mechanism operates.

MATERIALS AND METHODS

Four commercial cultivars (COI, TMV3, TMV6 and SVPRI) and one accession (VCR 75093) of *S. indicum* and one wild accession of *S. alatum* were used for hybridisation. Seedlings of parents were raised in raised beds. Flower buds, one day prior to opening were emasculated during evenings and bagged. Crossing was done by collecting the pollen from desired male parent between 7.00 and 7.30 a.m. and dusting on the emasculated flower. Crossed flowers were covered with muslim cloth to avoid contamination. Direct and reciprocal crosses were made, capsule and seed set were recorded.

To study *in vivo* pollen germination and tube growth, pistils were fixed in 3:1 absolute ethanol: acetic acid at hourly interval for eight hours, and then 12 and 24 h after pollination. The fixed pistils were dissected out, washed in distilled water and softened in 8 N NAOH fo 8 h at room temperature. The softened pistils were thoroughly washed in distilled water to clear off the adhering tissues, and then stained overnight in 0.1% aniline blue in IN tribasic potassium phosphate (K PO4. w/v). The stained pistils were mounted with glycerol on a glass slide, and examined under a microscope with UV illuminatiuon using a 320-400 nm exiter filter and 470 nm barrier filter (Leitz, Germany). Emasculated ovaries of *S. alatum* before pollination, after self pollination, and after cross polination with *S. indicum* were fixed separately after 48 h of pollination to observe the percentage of fertilisation *in vivo*. The ovules showing approximately two-fold increase in size over the ovules of unfertilised ovaries (emasculated but not pollinated) were considered as fertilised ovules. Viability of the seeds of *S. alatum* x *S. indicum* cross was tested using tetrazolium test.

RESULTS AND CONCLUSIONS

Sporophytic (pollen-stigma and pollen tubestigma interactions) and gametophytic (pollen tubestyle interactions) incompatibility result in prezygotic fertilisattion barrier. Incompatibility occurring after fertilisation and during different stages of zygotic development lead to postzygotic fertilisation barriers. Among the five female parents in *S. indicum*

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Table 1: Details of reciprocal hybridisation (*S. alatum* x *S. indicum*)

Female parent	Male parent	No. of flowers crossed	No. of capsules set	Capsule set (%)	No. of seeds set	Seeds set (%)*
S. alatum	*S. indicum* Col	210	15	7.14	315	25.6
S. alatum	*S. indicum* TMV3	140	8	5.71	176	26.8
S. alatum	*S. indicum* TMV6	170	8	4.71	184	28.0
S. alatum	*S. indicum* SVPR1	180	10	5.55	240	29.2
S. alatum	*S. indicum* Acc. No. 75093	192	10	5.21	234	28.5
		892	51	5.72	1148 **	27.6

* The average number of seeds in selfed *S. alatum* was estimated to be 82 per capsule. This was used as denominator to calculate the percentage of seed set in *S. alatum* x *S. indicum*.

** Tetrazolium test showed 62% viability for the hybrid seeds.

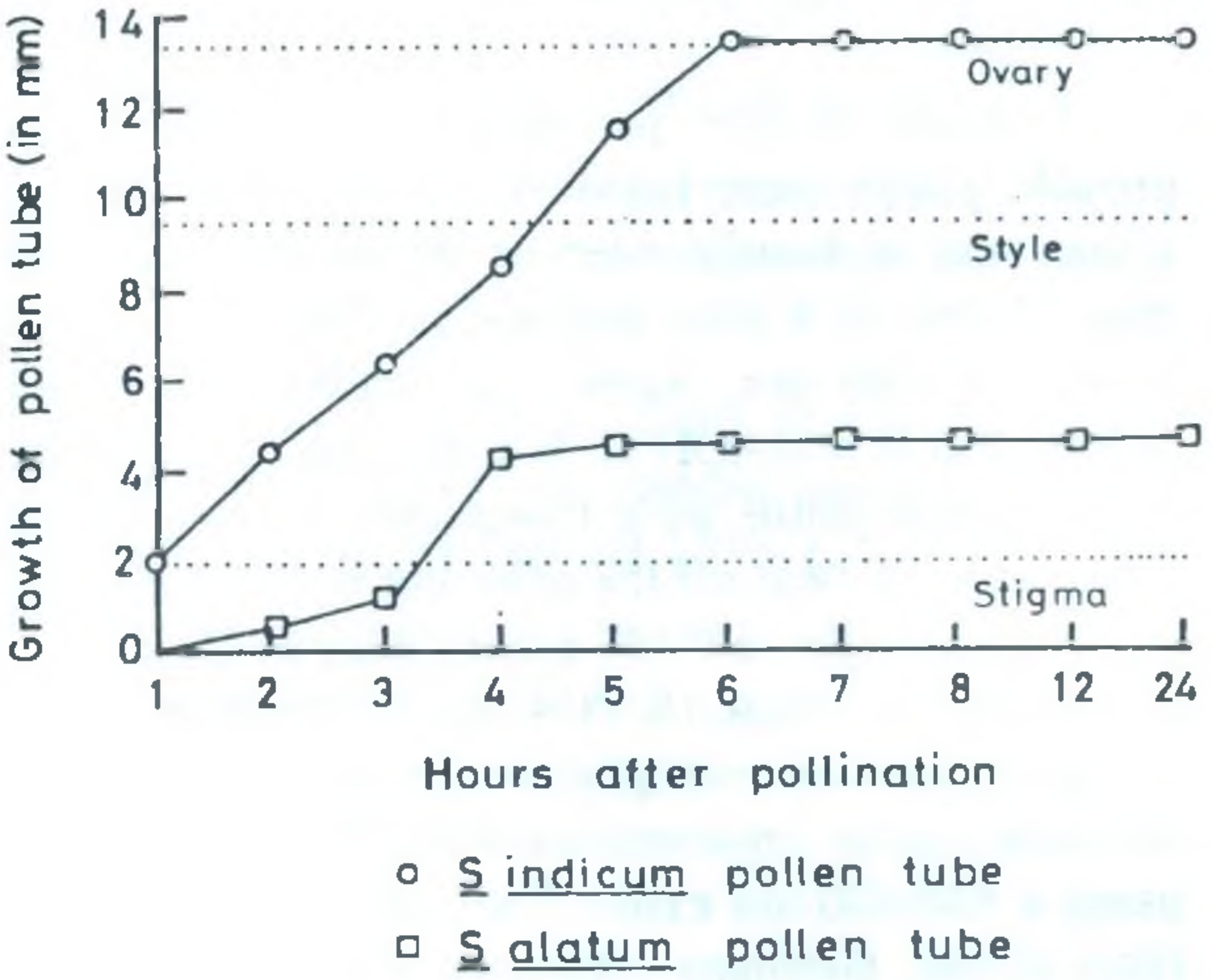

Figure 1. Growth rate of *S. alatum* and *S. indicum* pollen tube in *S. indicum* style.

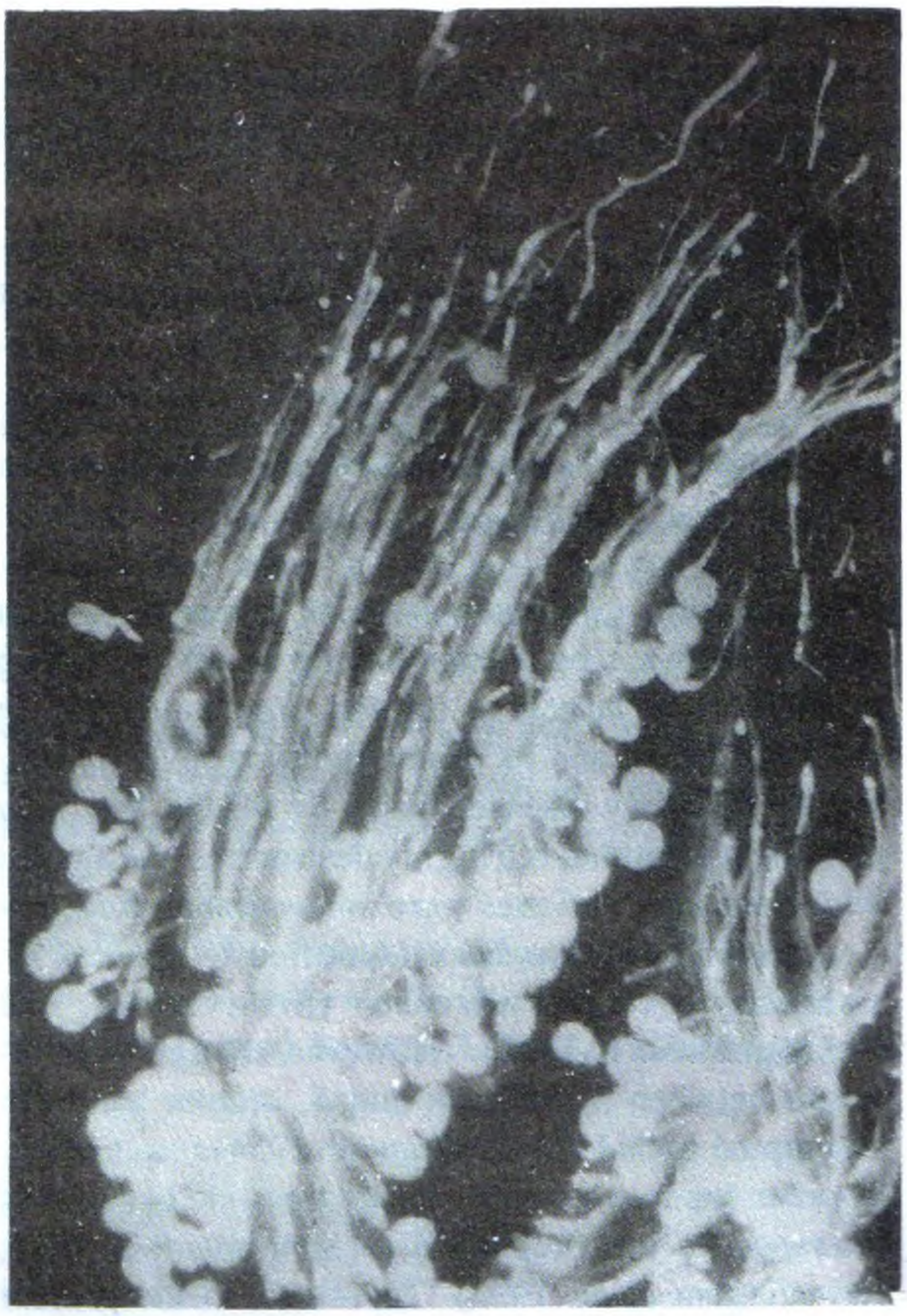

Figure 2 Fluorescence microscopy showing normal pollen germination in *S. indicum* x *S. alatum* cross.

x *S. alatum* crosses attempted in the present study, none set capsules (results not shown). All the pistils dropped in 36 to 48 h after pollination indicating that the crosses were completely incompatible. In the reciprocal cross (*S. alatum* x *S. indicum*) 4.71 to 7.14% capsule set and 25.6 to 29% seed set were observed (Table 1) which indicated that this cross was partially compatible.

S. indicum x *S. alatum* cross

Though difference in percentage of pollen germination between selfs and crosses were not significant, further growth of *S. alatum* pollen tubes into the pistils of *S. indicum* was retarded (Fig 1). When *S. indicum* was self pollinated, the pollen tubes crossed the stigma in 2 h, the stylar tissue in 5 h, and fertilised the ovules between 5 and 6 h after pollination. In cross hybridisation, the pollen tubes of *S. alatum* took more than 3h to grow through the stigma, and growth was arrested in the upper part of the style 6h after pollination. No further growth of the pollen tubes was noticed even after 24 h of pollination (Fig 1). Normally, as the pollen tubes grow, the inner volume increases and turgor pressure decreases. To make up this, series of callose plugs are formed near the tip which restores the pressure. Sufficient turgor pressure at the tip is very essential as it provides virtual mechanical facilitation pathway for the tubes to proceed. But, callose is not usually found at the tip which would otherwise hinder its growth. In. *indicum* x *S. alatum* cross, callose plugs were not formed until the pollen tubes stoped growing, and callose deposits were observed at the tip of almost all the pollen tubes (seen as fluorescent blob at the tip of each pollen tube in fig. 2). Therefore, failure to restore the turgor pressure at the tip might be the cause poor cessation of tthe pollen tube growth, and callose could have been deposited after the growth was arrested. The same phenomenon has also been observed

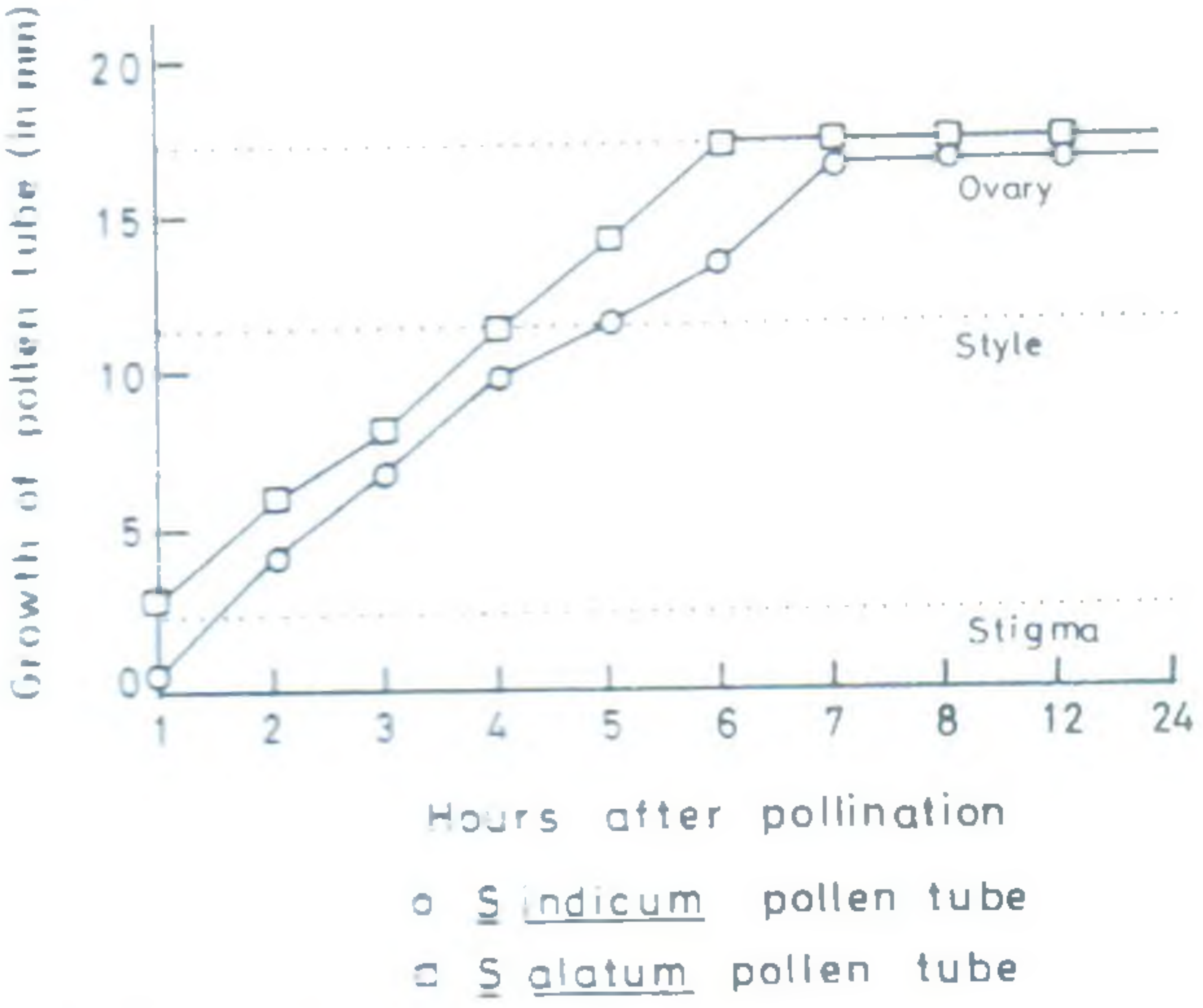

Figure 3. Growth rate of *S. alatum* and *S. indicum* pollen tube in *S. alatum* pistil.

Figure 4. Fluorescence microscopy of the neck and ovary of *S. alatum* showing a bunch of *S. indicum* pollen tubes and fertilisation of a few ovules.

in tomato (Dumas and Knox 1983). Failure of wide hybridisation due to prezygotic gametophytic incompatibility when wild species is used as pollen parent has been recently reported in rice (Sarkar *et al.*, 1993). This may be because certain combinations may lack crossability genes for compatibility as observed in other species (Jena, 1994).

S. alatum x *S. indicum* cross

In *S. alatum* x *S. indicum* cross also there was no significant difference in percentage of pollen germination between selfs and crosses. Salfed pollen tubes of *S. alatum* crossed stigma in 2h, the stylar tissus in 4h, and fertilised the ovules 6h after pollenation. In cross hybridisation, the pollen tubes of *S. indicum* crossed the stigma of S. alatum in 2h, but took 5h to cross the stylar tissue, and fertilised the ovules 6h after pollination (Fig. 3). Entry of *S. indicum* pollen tubes was observed in all the ovaries of *S. alatum* (Fig. 4). However, fertilisation was not successfully effected in all the ovules as revealed by the percentage of in vivo fertilisation (11.31 and 61.62) which indicated the presence of partial prezygotic barriers. Among the successfully developed capsules, the percentage of seed set was only 27.6 per cent which may be due to postzygotic fertilisation barriers. Tetrazolium test showed that 62% of the crossed seeds were viable.

The resultts indicated that the total failure of direct cross *(S. indicum* x *S. alatum)* was due to prezygotic gametophytic incompatibility. Therefore, it becomes obvious that technique like hybridisation followed by embryo rescue could bot be used to produce hybrids in this cross. Conventional hybridisation using large number of diverse genotypes of S. indicum may yield viable hybrids by chance recombination between right crossability genes. Classical methods like mentor pollen technique may be used, if the crossability genes could not be identtified from the gene pool. Somatic hybridisation could be used to avoid fertilisation barriers in sexual hybridization.

The present study indicated partial prezygotic and postzygotic barriers in the reciprocal cross (*S. alatum* x *S. indicum*). However, for practical breeding purpose 5.72% capsule set with 27.6% seed set (average) may be enoughh But, so far, hybrids in the reciprocal cross was not reported excepting

Ramalingam *et al.* (1992) who got a single hybrid plant out of 1102 flowers crossed. As the crossed seeds obtained in the present study did not germinate despite 62% viability as revealed in tetrazolium test, investigations on dormancy in selfed and crossed seeds may yield desirable results.

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