IN-VITRO POLLEN GERMINATION OF *CASSIA FISTULA* L.

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ABSTRACT

The germination of *Cassia fistula* L. pollen, ranging from fresh samples to those stored for various periods, is reported. At the time of writing, the oldest samples studied were stored for 4 weeks. It is intended to extend the observations for longer periods.

INTRODUCTION

Under the research programme at the Botanic Gardens, the author is involved in the crossbreeding of ornamental plants. Among the various projects is an attempt to produce new hybrids of *Cassia.* One of the species used is *Cassia fistula,* the Indian Laburnum, a leguminous tree which has spectacular inflorescences. In connection with this research, it is necessary to study the viability of the pollen, especially when it may have to be stored for varying lengths of time to await the anthesis of flowers of other species.

MATERIALS AND METHOD

1. PREPARATION OF CULTURE MEDIUM

A sucrose-agar medium was made by dissolving agar strips (5 g), sucrose (100 g) and boric acid (0.1 g) in 1 litre of distilled boiling water. The solution was poured into petri dishes which were then packed in aluminium foil, autoclaved, and allowed to set at ambient temperature.

2. COLLECTION AND STORAGE OF POLLEN

Cassia fistula has been observed to flower at least once a year, usually after a dry spell. The whole crown is often covered with flowers at the beginning of the flowering season, with sporadic flowering lasting up to 3 months.

Flowers were collected at anthesis, between 8 am and 10 am from 10-12 Nov 1981. On each day , eight flowers were collected from the same tree. Pollen was taken from the three anterior fertile stamens, distinguished by their long and curved filaments (Venkatesh, 1956). This decision was made after a series of viability tests of pollen from each of the ten stamens showed that those taken from the three anterior stamens were viable, whereas the other stamens yielded pollen which proved to be nonviable. Pollen to be tested immediately was sown on agar for germination, the rest were stored in tiny pill capsules. Silica gel was used to keep the vials containing the capsules moisture free. The vials were then stored in a refrigerator at 4°C. Pollen was taken out subsequently for testing: one week, two weeks, and four weeks after storage.

3. ASSESSMENT OF GERMINATION

Germination percentage was used to assess the viability of the pollen. Pollen grains were dusted and smeared evenly on the agar using a fine brush and left to germinate

at room temperature (about 24°C). Although some pollen grains were observed to germinate after half an hour, the assessment was not done until after 24 hours to ensure maximum germination.

The germination percentage was derived in the following manner: For each of the three pollen samples pertaining to a particular storage period, a viability test was done on only one petri dish. Counting was done under a microscope fitted with a grid in the eye-piece. A field was selected in which the pollen grains were not clustered together to facilitate counting. An even distribution in the microscopic field selected for counting generally produces lower variation between samples (Stanley, 1974). When such a field was obtained, the whole field was systematically counted using a tally counter. It was found that each field had between 100-150 pollen grains. A second count was made on only the pollen grains with germinated pollen tubes. The total number of pollen grains observed was then compared to the number with germinated pollen tubes.

RESULTS AND DISCUSSION

Fresh pollen showed the best viability. The average germination was 91.2%. Storing the pollen for one week reduced the average germination by 2.4% (Table 1). The decline was progressive after one week and by the fourth week the average germination was 66.9% (Fig 1).

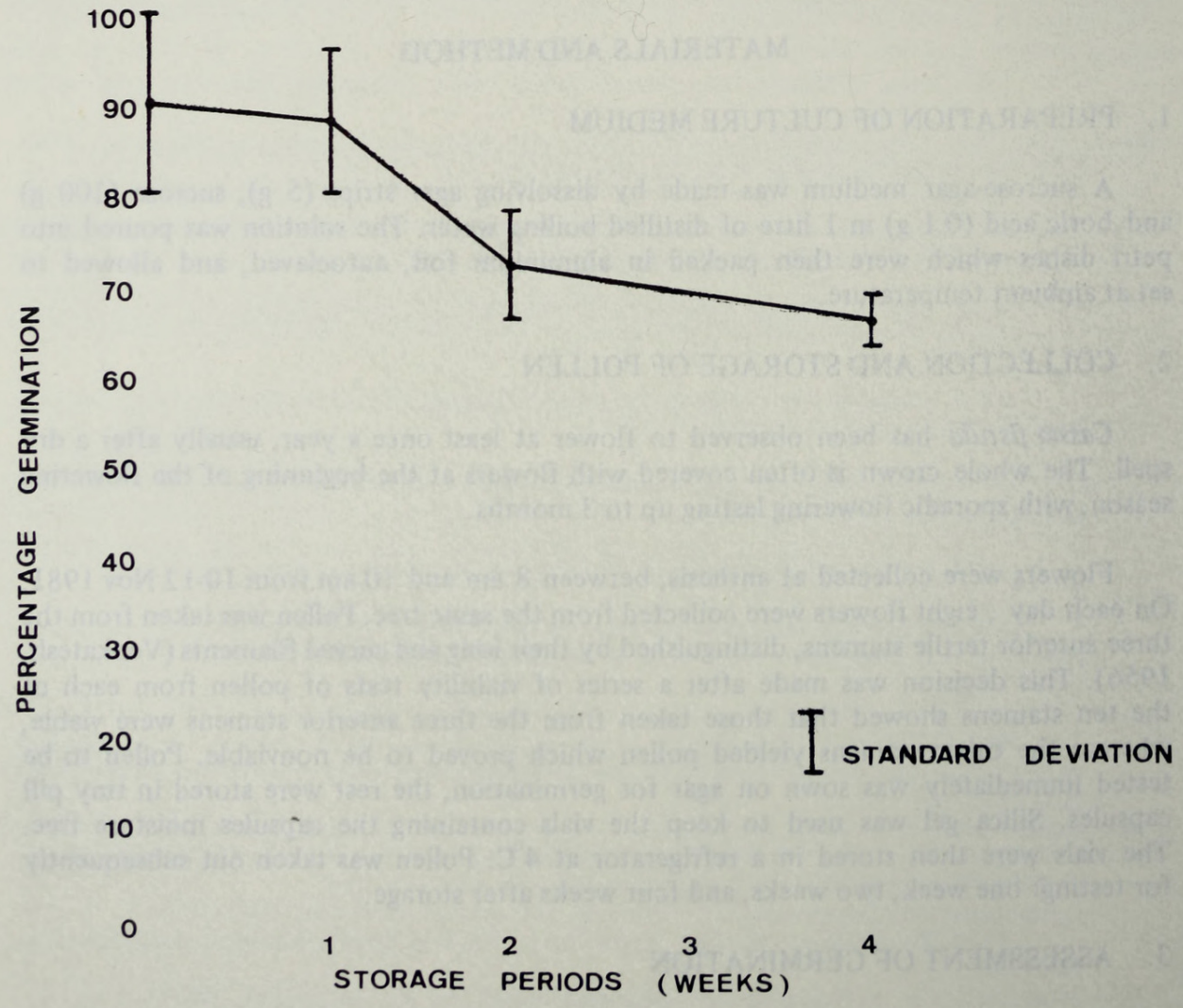

FIG 1 PERCENTAGE GERMINATION OF CASSIA FISTULA POLLEN STORED FOR VARIOUS PERIODS

Sample	Date of collection	Fresh pollen		Stored pollen					
				1 week		2 weeks		4 weeks	
		Number germinated/ Total	% germ	Number germinated/ Total	% germ	Number germinated/ Total	% germ	Number germinated/ Total	% germ
1	10 Nov 81	112/118	94.9	118/120	98.3	87/125	69.6	72/103	69.9
2	11 Nov 81	102/127	80.3	93/109	85.3	88/125	70.4	72/107	67.3
3	12 Nov 81	130/132	98.5	120/145	82.7	95/118	80.5	75/118	63.5
MEAN			91.2		88.8		73.5		66.9
STANDARD DEVIATION ($\sigma n-1$)			9.6		8.3		6.1		3.2

Table 1. *In-vitro* germination percentage of fresh and stored pollen of *Cassia fistula*

It was observed that germination was very rapid. After 3 hours most of the pollen grains had germinated. After 24 hours the pollen tubes had grown to an average length of 0.1 mm (Fig 2). The rapidity and high percentage of fresh pollen germination indicated that the sucrose-agar medium used was suitable for *in-vitro* assays of *Cassia fistula* pollen.

The reasonable germination percentage obtained after four weeks of storage indicated the capacity of the pollen to tolerate the storage conditions provided. The optimum storage factors, like temperature, relative humidity and atmosphere surrounding the pollen, would have to be determined for longer storage periods and better germination potentials.

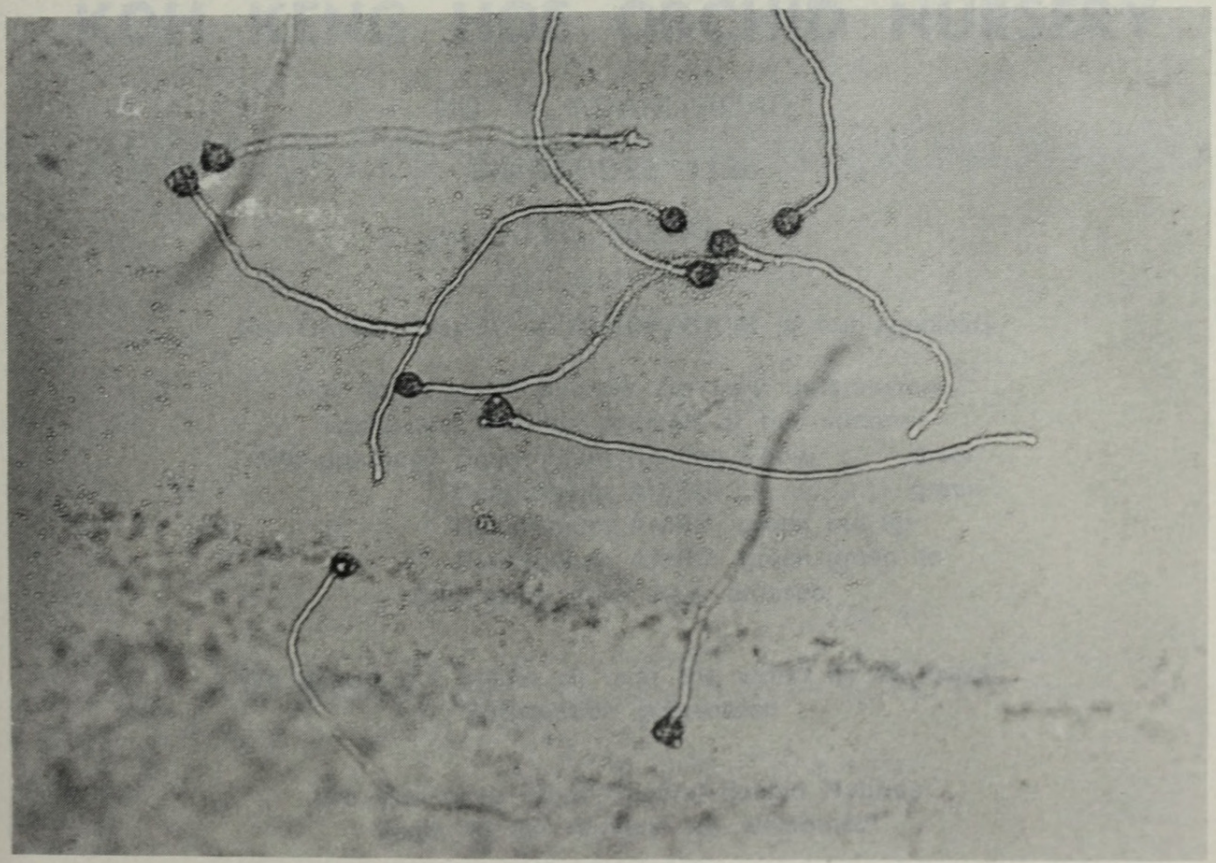

Fig. 2. *In-vitro* pollen germination of *Cassia fistula* on sucrose-agar (x 109). Note the long pollen tubes.

CONCLUSION

The storage conditions employed in this experiment were adequate in keeping the pollen reasonably viable after 4 weeks of storage.

These findings indicate that even if the other species of *Cassia* were to be out of phase in flowering by as much as 1 month and possibly longer, stored pollen of *Cassia fistula* could still be used for crossbreeding work.

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