

interesting that of the two closely related species of the same genus, one contained well-developed vestures while the other totally lacks it. *D. elata* is the only taxon of the tribe Caesalpiniae that has so far been reported to lack vestured pits.

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1. Bailey, I. W., *J. Arn. Arbor.*, 1933, 14, 259.
2. Quirk, J. T., *IAWA Bull. n.s.*, 1983, 4, 118.
3. Quirk, J. T. and Miller, R. B., *IAWA Bull. n.s.*, 1983, 4, 191.
4. Quirk, J. T. and Miller, R. B., *IAWA Bull. n.s.*, 1985, 6, 200.
5. Vliet, G. J. C., M. Van, *Acta Bot. Neerl.*, 1978, 27, 273.

## EFFECT OF STORAGE ON POLLEN GERMINATION AND POLLEN TUBE GROWTH

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POLLEN storage is advantageous not only in plant breeding programmes but also in many areas of basic studies such as viability, anhydrobiosis and germination. Extensive studies on pollen grains using a variety of methods have showed considerable success in their storage in a wide range of species<sup>1,2</sup>. In a majority of studies, on pollen storage, viability has been assessed on the basis of *in vitro* germinability. Pollen grains capable of *in vitro* germination are considered viable and capable of producing normal seeds. Lack of germination in stored seeds is generally the terminal manifestation of the loss of viability. Before this stage is reached the seedling vigour and its growth are affected. For effective pollination stored pollen grains should not only germinate but also produce vigorous pollen tubes.

Studies on the effect of pollen storage on pollen tube growth are limited. In a few systems storage has been shown to affect the division of the generative cell<sup>3</sup> and the ability of pollen to incorpo-

rate nucleic acid precursors<sup>4</sup>. This paper reports the effect of storage on *in vitro* germination and the rate of pollen tube growth. As the cytology of pollen (2-celled or 3-celled at the time of shedding) influences these responses<sup>5</sup>, studies were conducted on a 2-celled taxon (*Crotalaria saltiana*) and a 3-celled taxon (*Brassica campestris*).

Plants of *Crotalaria saltiana* L. and *Brassica campestris* L. var. brown sarson were grown in field and the pollen grains were collected from freshly dehisced anthers. A small amount of pollen was used for *in vitro* germination (control) and the remaining sample was stored in a petri plate kept in a desiccator containing dry silica (RH < 10%) or saturated solution of MgCl<sub>2</sub> (RH 40–45%). A small amount of stored pollen was tested for *in vitro* germination and tube length at 7, 15, 30 and 45 days of storage for *Crotalaria* and at 16 and 55 days of storage for *Brassica*. The pollen grains were cultured in drops of germination medium (Brewbaker and Kwack<sup>6</sup> for *Crotalaria* and Roberts *et al*<sup>7</sup> for *Brassica*), ca 50 µl each on glass slides kept in petri plates lined with moist filter paper and were maintained at 25 ± 2°C. At each observation period two cultures were scored under randomly selected microscopic fields. Over 200 pollen grains were scored for germination and tube length of over 50 tubes was measured. The experiment was repeated using 4 batches of pollen, collected on different days. Although there were day-to-day variations in germination and tube lengths, even in the control, the general response of storage was uniform. Statistical significance was calculated by using Student's *t* test (for mean pollen tube length) and *d* test (for per cent germination)<sup>8</sup>.

The responses of pollen of both the taxa are presented in table 1. In *Crotalaria* pollen stored for 7 days showed delay in germination. However, the germination and tube length were comparable to those of fresh pollen in 2 h. Pollen stored for 15 days showed germination value comparable to control only by 3 h. Tube length of 15-day stored sample was significantly lower throughout the periods of observation. Pollen sample stored for 30 days showed poor germination; only about 20% of the pollen germinated even by 5 h after culture. The pollen tube growth was also poor.

In *Brassica* there was no germination beyond 0.5 h in any of the pollen samples. Germination of pollen stored for 16 days was comparable to that of the control. Tube growth was considerably faster in pollen stored at lower humidity. In pollen stored at higher humidity, faster tube growth was observed

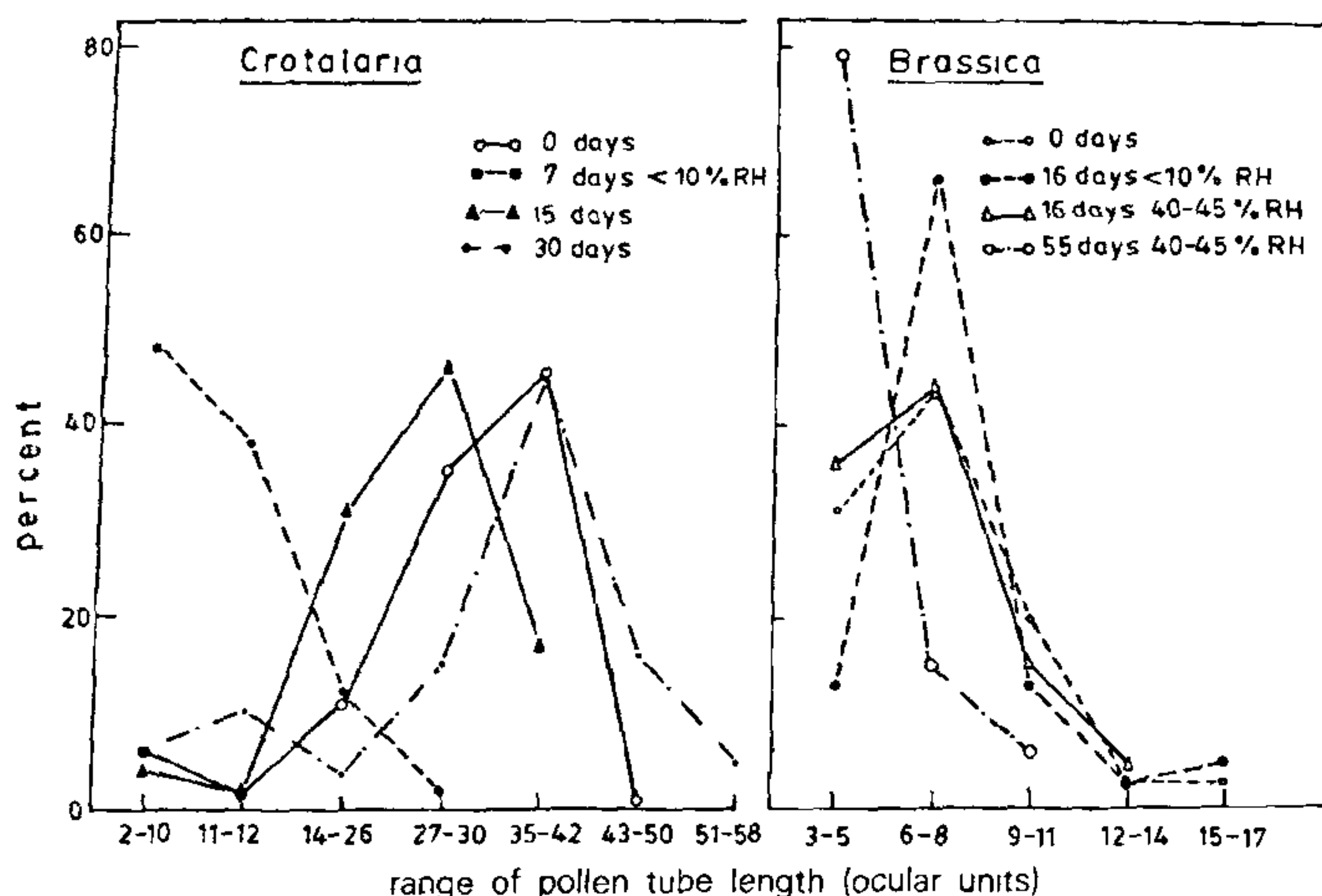


Figure 1. Frequency curves of pollen tubes from pollen samples stored for different periods.

only in the initial 1 h. Pollen stored for 55 days showed poor germination, and the tube growth, particularly in the sample stored at higher humidity.

Figure 1 presents the frequency curves of pollen tubes in different pollen samples. In *Crotalaria*,

although the mean tube length obtained in pollen stored for 7 days was not significantly different from that of the fresh pollen, a small proportion of pollen tubes grew longer than those of the control sample. Pollen samples stored for 15 and 30 days covered

Table 1 Pollen germination and mean pollen tube length (ocular units) in samples stored for different periods

Days of storage	RH %	1 h		2 h		3 h		4 h	5 h
		% Germination	Tube length	% Germination	Tube length	% Germination	Tube length	Tube length	Tube length
<i>Crotalaria saltiana</i>									
0 (control)		74.1	6.3	83.3	14.8	87.8	24.6	29.8	31.8
7	< 10	51.6*	5.2*	88.2	14.6	86.1	20.7	27.8	34.2
15	< 10	22.8*	2.5*	70.3*	6.4*	77.1*	13.7*	23.0	28.0*
30	< 10	0	—	5.1*	3.1*	7.4*	5.6*	8.1*	11.8*
45	< 10	0	—	0	—	0	—	—	—
Pollen tube length									
Days of storage	RH %	% Germination at 0.5 h	Pollen tube length						
			0.5 h	1 h	2 h				
<i>Brassica campestris</i>									
0 (control)		58.0	2.2	3.0	4.6				
16	< 10	54.5	3.3*	4.5*	5.6*				
	40-45	63.5	3.1*	4.1*	4.8				
55	< 10	24.4*	2.1	3.1	3.3*				
	40-45	24.6*	2.0*	2.5*	3.2*				

\* Values statistically significant over the control at,  $P \leq 0.05$ .

only the lowest 4–5 range and the peak was also shifted to a lower range.

In *Brassica*, pollen stored for 16 days at < 10% RH covered the same frequency range as that of the fresh pollen; the number of pollen tubes in the median range, however, was higher in stored pollen. Frequency range of the samples stored for 16 days (at 40% RH), and for 55 days (in both the humidities) was only up to 12–14 units and 9–11 units respectively. As in *Crotalaria* the peak was shifted to a lower range (3–5 units) in these pollen samples. Thus, in pollen samples stored for longer periods, the proportion of shorter tubes was higher and the fastest growing pollen tubes were far shorter than those of the fresh pollen.

It is obvious from the results of both *Crotalaria* and *Brassica* that *in vitro* germinability does not necessarily reflect the ability of pollen to produce vigorous tubes. The vigour of pollen tubes was not reduced when the storage was confined to a limited period (7 days for *Crotalaria* and 16 days for *Brassica*), which would vary between species and between storage conditions. Interestingly the pollen of *Brassica* stored for 16 days at lower humidity showed better tube growth when compared to fresh pollen. This agrees with reports of improved germination in pollen grains of *Brassica oleracea*<sup>9</sup> and *Zea mays*<sup>10</sup> stored for a few days. Beyond optimal period, stored pollen showed significant reduction not only in per cent germination but also in tube length. For effective assessment of stored pollen grains it is therefore important to assess, apart from germinability, post germination processes, particularly the vigour of the tube.

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1. Stanley, R. G. and Linskens, H. F., *Pollen: biology, biochemistry and management*, Springer-Verlag, Berlin, 1974.
2. Shivanna, K. R. and Johri, B. M., *The angiosperm pollen: structure and function*, Wiley Eastern, New Delhi, 1985.
3. Kamizyo, A. and Tanaka, N., *Cytologia*, 1982, **47**, 185.
4. Bellani, L. M., Forino, L. M. C., Tagliasacchi, A. M. and Sansavini, S., *Caryologia*, 1984, **37**, 323.
5. Johri, B. M. and Shivanna, K. R., *Phytomorphology*, 1977, **27**, 98.

6. Brewbaker, J. L. and Kwack, B. H., *Am. J. Bot.*, 1963, **50**, 859.
7. Roberts, I. N., Gaude, T. C., Harrod, G. and Dickinson, H. G., *Theor. Appl. Genet.*, 1983, **65**, 231.
8. Bailey, N. T. J., *Statistical methods in biology*, The English Universities Press Ltd., London, 1959.
9. Chiang, M. S., *Euphytica*, 1974, **23**, 579.
10. Frova, C. B. and Feder, W. A., *Ann. Bot.*, 1979, **43**, 75.

### EFFECT OF ALCOHOL AND CHEMICAL EFFLUENTS ON SEED GERMINATION AND SEEDLING GROWTH OF BLACK GRAM

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POLLUTION of water resources by domestic and industrial wastes impairs quality of water. Polluted water is used for irrigation as the effluent waters and sludges contain plant nutrients and trace metals in small quantities which are essential for plant growth. However at higher concentrations they are toxic<sup>1</sup>. Trace elements including zinc hinder plant growth by binding co-ordinately with fixed positions of enzymic proteins<sup>2</sup>.

The effluent of the Coimbatore Alcohol and Chemicals Factory, Periyapuliur, Bhavani is used directly for irrigating crop fields or mixed with Bhavani river waters. The pollutants contributed by various processing units of this factory are rich in inorganic constituents like ammoniacal nitrogen (18 mg/l), chlorides (1270 mg/l), sulphides (26 mg/l), sulphates (1000 mg/l), flourides (0.63 mg/l) and sodium (5%). The effluent is also rich in suspended solids (12600 mg/l), dissolved solids (211180 mg/l), volatile solids (78865 mg/l) and traces of heavy metals (copper 0.5 mg/l and zinc 1.1 mg/l). The dissolved pollutants contribute to high B.O.D. (34.650 mg/l) and C.O.D. (113095 mg/l) values of the effluent. During collection the pH of the effluent was 4.7.

The seeds of *Vigna mungo* (L.) Hepper var. Co.5 were procured from the Tamil Nadu Agricultural University, Coimbatore. Selected healthy seeds, divided into batches of 20 each, were soaked in different effluent concentrations (1, 2.5, 5, 10, 25, 50 and 100%). One batch of seeds soaked in distilled