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EFFECTS OF CHEMICALS ON THE GERMINATION OF POLLEN GRAINS OF *TORENIA ASIATICA* LINN.

E. M. V. Nambudiri¹ and M. K. Thomas²

ABSTRACT.— Germination studies of the pollen grains of a Scrophulariaceae plant, *Torenia asiatica* Linn. have revealed that the maximum percentage of germination and longer tubes were attained in 15 percent sucrose solution. Among the chemicals tried, calcium and boron enhanced germination. Less germination occurred among grains treated with potassium or magnesium salts. Abnormalities such as branched tubes with or without vesicles were found in chemically treated pollen grains.

Torenia asiatica Linn., a common garden plant belonging to the tribe Gratioleae of the Scrophulariaceae family is cultivated as an ornamental plant in and around Bombay, India. This small herbaceous plant produces bright violet flowers. Pollen morphology of Scrophulariaceae plants has been described by Risch (1939), Erdtman (1952), Ikuse (1952), Natarajan (1957), and others. Varghese (1968) described the morphology of pollen grains of *Torenia cordifolia* Roxb., along with certain other plants of this family.

Successful germination of pollen is a prerequisite for success in fertilization; therefore, many workers have studied the effect of different chemicals on the germination of pollen from various plants. Compounds of boron, calcium, potassium, and magnesium were selected for the study of their effects on the germination of the pollen of *T. asiatica*. Although the effects of these chemicals on germination of pollen grains of certain other plants have already been studied by previous workers, in this paper the authors are concerned with the study of various concentrations (20, 40, 60, 80, and 100 ppm) of boric acid (H_3BO_3), calcium nitrate ($Ca[NO_3]_2 \cdot 4H_2O$), potassium nitrate (KNO_3) and magnesium sulphate ($MgSO_4 \cdot 7H_2O$) on the germination and tube growth of *T. asiatica* pollen.

MATERIALS AND METHODS

Pollen grains used in the experiments were collected from flowers just prior to their opening to ensure that the material used was fresh and uncontaminated. Pollen grains of *T. asiatica* (Fig. 4b) show three colpae. Chemicals employed in the experiments were of AR-BDH. Culture media of sucrose (5, 10, 15, and 20 percent) were prepared in double-distilled water. After the sucrose concentration was standardized at 15 percent, different concentrations of four chemicals were added separately to the 15 percent sucrose solution. Concentrations of chemicals chosen were 20, 40, 60, 80, and 100 ppm. One drop of each of these solutions was then placed on a clean sterilized microslide, and pollen grains were dusted on these media. The microslides were then transferred to a moist filter chamber

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where pollen grains were allowed to germinate and grow for two hours. Experiments were conducted at a pH of 6.5 and a temperature of 26 to 28 C. Percentage germination was calculated by counting 100-200 pollen grains from different fields of the microslide. Mean tube length was calculated from 50 pollen tubes selected at random. All experiments were repeated until concurrent results were available.

RESULTS

During initial experiments, it was noticed that grains did not germinate in water but readily germinated in a sugar solution. Therefore, further germination tests were carried out with different types of sugars; namely, glucose, fructose, and sucrose. Sucrose gave maximum germination percentage and higher mean tube length. Hence, for further experiments with different chemicals, sucrose was selected as the basic medium.

Pollen germination in sucrose medium. Pollen grains were allowed to germinate in 5, 10, 15, and 20 percent sucrose solutions. The percentages of germination and tube growth in these media were recorded. Maximum germination percentage and tube growth were observed in 15 percent sucrose solution (Table 1, Fig. 1). A minimum percentage germination (10 percent) along with a lower mean tube length (222.5μ) were observed in 20 percent sucrose solution. A comparatively low germination percentage and shorter tubes were also found in 5 and 10 percent sucrose solutions (Table 1). It is therefore evident that 15 percent sucrose medium can be used for further experiments with different chemicals. Another benefit in selecting this sucrose concentration is that the bursting of pollen tubes in this medium is lower than in other concentrations. Bursting of pollen tubes before attaining required lengths is undesirable since such tubes cannot effect fertilization.

Pollen germination in different chemicals. Keeping 15 percent sucrose solution as the basic medium, different concentrations of the four chemicals were prepared separately. A comparative account of the results obtained is given in Table 2 and in Figure 2. The major effects of different chemicals on germination are given below:

1. *Boric acid* (Fig. 3b). A minimum concentration of boric acid enhanced pollen germination and tube length. Eighty-nine percent

TABLE 1. Percentage germination and mean tube length of pollen grains of *Torenia asiatica* Linn.

Percentage concentration of sucrose	Percentage germination	Mean tube length in microns after two hours
5	43	290.0
10	55	351.0
15	73	443.5
20	10	222.5

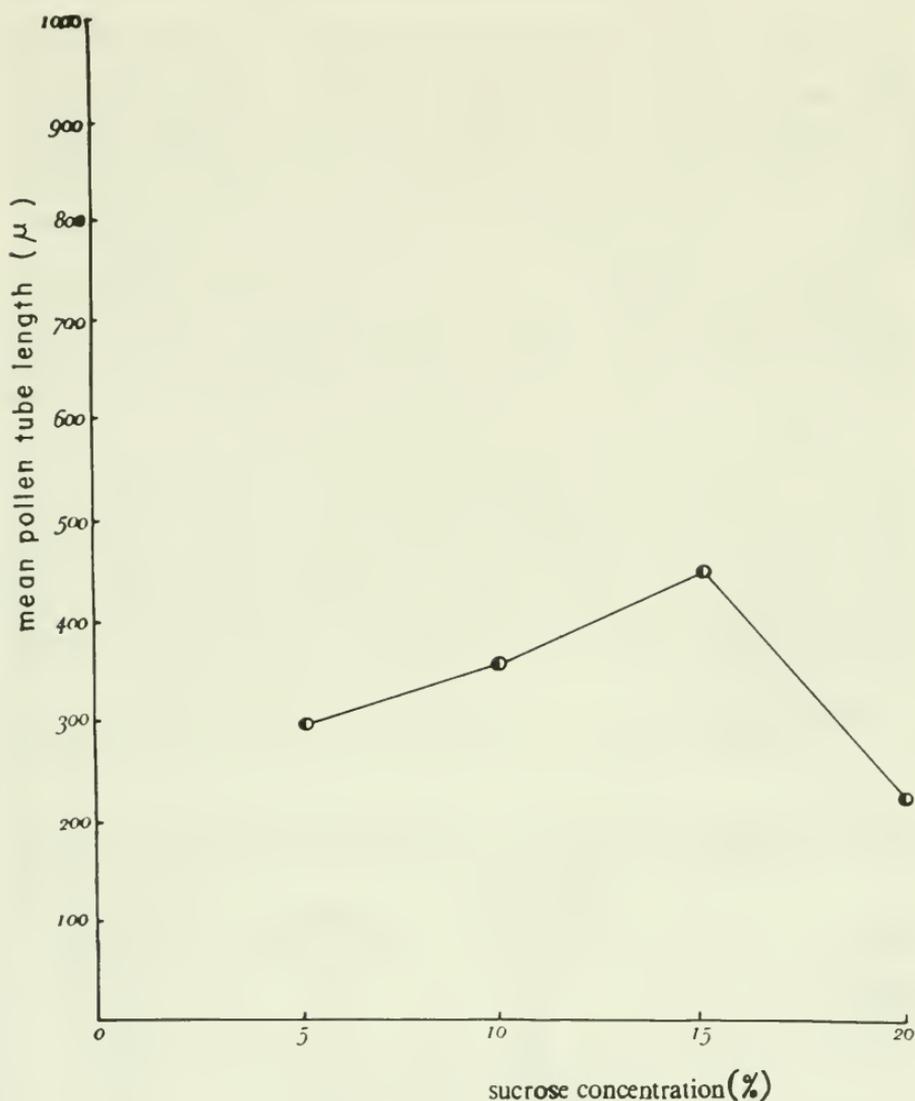


Fig. 1. *Torenia asiatica*. Graph showing mean tube length for pollen grains in control sucrose medium.

of the germinated pollen grains had a mean tube length of 755μ in 20 ppm of boric acid. Since the present work was confined to studying fixed concentrations of different chemicals, and not determining which concentration gave maximum tube length, the concentration of boric acid below 20 ppm was not tried. With an increase in the concentration of the chemical, percentages gradually declined—to a minimum of 70 percent germination and mean tube length to 403μ —in 100 ppm of boric acid.

2. *Calcium nitrate* (Fig. 3a). A condition the reverse of that for boric acid (as described above) was obtained when pollen grains were treated with calcium nitrate solution. A high germination percentage and longer tubes were noticed in the maximum concentration of this chemical (100 ppm), as can be observed in Table 2. Mean tube length gradually increased from 418μ in 20 ppm to 813μ in 100 ppm. Similarly, percentage germination progressed upwards from 66 percent in 20 ppm to 91 percent in 100 ppm.

3. *Magnesium sulphate*. A 40 ppm concentration of magnesium sulphate gave the highest germination percentage (88 percent) and the longest pollen tubes (mean length 556μ) of any of the concentrations of this chemical used. Higher concentrations, like 100 ppm, were toxic, and they produced minimum germination percentages (69 percent) and shorter pollen tubes (323.5μ).

4. *Potassium nitrate*. Potassium nitrate produced many abnormalities. The most favorable concentration of this chemical for pollen germination was 60 ppm. This concentration gave 74 percent germination with pollen tubes of 444μ mean length. At 20 ppm concentration of potassium nitrate, 4 percent of the pollen tubes were branched at their tips. However, rate of branching declined as the chemical concentration increased. Many of the branched pollen tubes had vesicles at the tip of one of their branches.

Morphology of pollen tubes: Pollen tubes in different media were studied for an understanding of the morphological variations,

TABLE 2. Percentage germination and mean tube length of *Torenia asiatica* pollen germinated in 15 percent sucrose solution mixed with different chemicals.

Chemical	Concentration of chemical (ppm)	Percentage germination	Mean tube length in microns after 2 hrs.
Boric acid (H_2BO_3)	20	89	755.0
	40	86	587.5
	60	78	492.7
	80	75	429.5
	100	70	403.0
Calcium nitrate ($Ca[NO_3]_2 \cdot 4H_2O$)	20	66	418.0
	40	68	484.0
	60	80	591.0
	80	83	707.5
	100	91	813.5
Magnesium sulphate ($MgSO_4 \cdot 7 H_2O$)	20	86	439.5
	40	88	556.0
	60	75	499.5
	80	75	379.5
	100	69	323.5
Potassium nitrate (KNO_3)	20	70	346.0
	40	72	354.5
	60	74	444.0
	80	66	302.0
	100	62	240.0

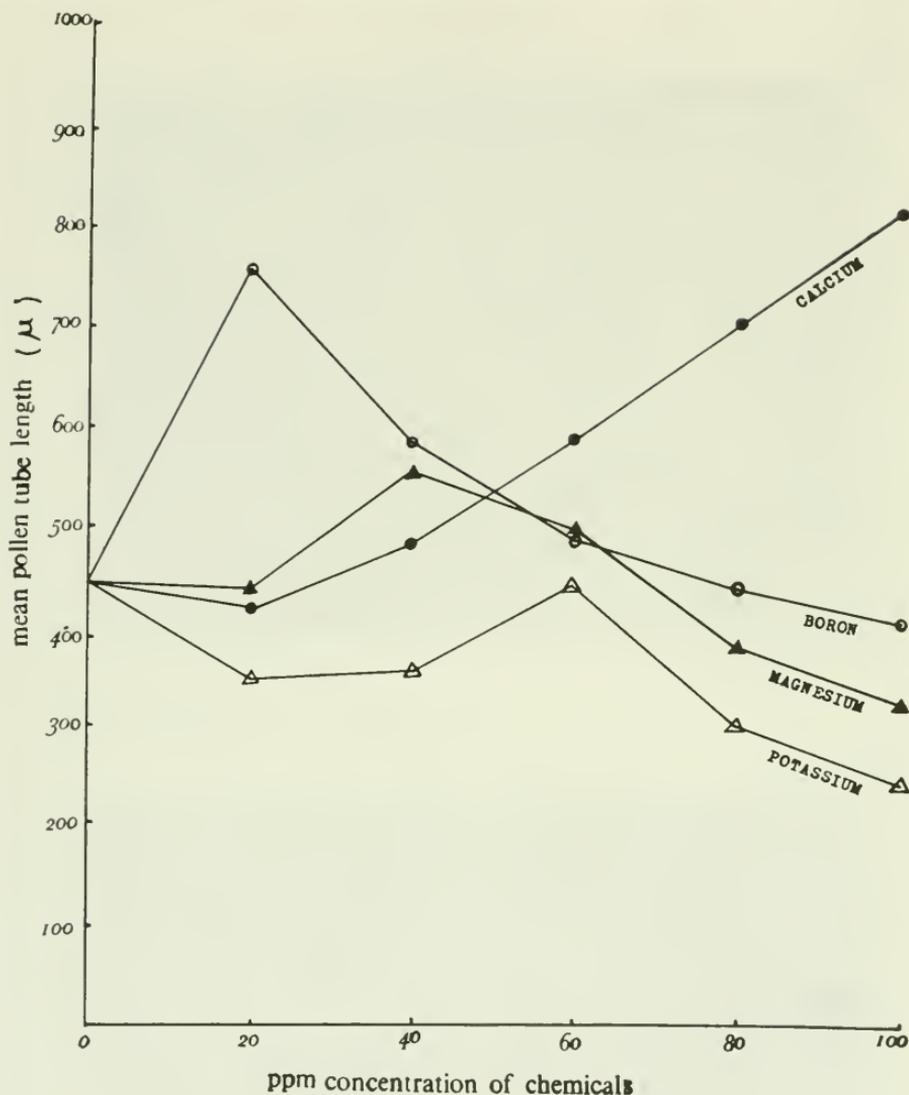


Fig. 2. Graph to show comparative effects of different chemicals on the mean pollen tube length for *Torenia asiatica*.

if any, shown by the growing tubes. Although pollen grains of *T. asiatica* consist of three germinal pores, only a single tube resulted. The protoplasm migrated into this tube. In the control sucrose medium and in the four chemicals, the pollen tube developed in either a coiled or a zig-zag manner (Fig. 4c). A combination of these growth characteristics also occurred in the same tube. Occasionally, tubes showed callose plugs (Fig. 4a). After its initiation into potassium nitrate, a pollen tube developed normally for a time and then branched. One of these branches had a vesicle at its tip.

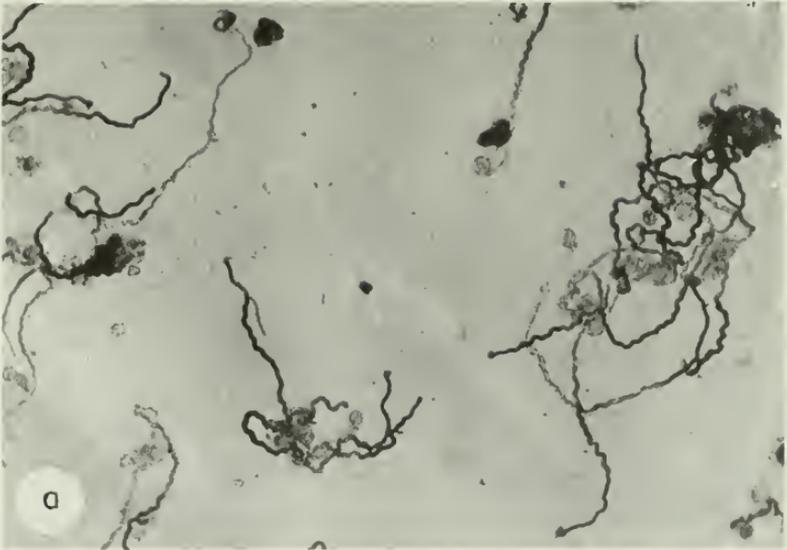


Fig. 3. *Torenia asiatica*: (a) germination of pollen grains in calcium nitrate-sucrose medium (60 X); (b) germinating grains in boric acid-sucrose medium (60 X).

DISCUSSION

Johri and Vasil (1961) noted that germinating media give varied results when used with pollen grains of different species. This study revealed that sucrose is the best sugar for germination of the pollen grains of *T. asiatica* and that maximum germination occurs in a 15 percent concentration. This conclusion is in accord with that of Goss (1962) who attained maximum germination in 10-15 percent sucrose medium for *Ornithogalum caudatum* pollen and Nair and Deshpande (1968) for pollen of *Luffa cylindrica*. Sucrose medium was also used for germination of pollen grains of various plants by Vasil (1961), Biswas and Datta (1964), Cook and Walden (1965), Premnath and Purohit (1969), and others. Linskens (1964) suggested that sucrose was better than any other organic compound as a medium for pollen germination. However, Premnath and Purohit (1969) found no pollen germination in sucrose concentrations below 40 percent for spinach beet pollen they studied. Biswas and Datta (1964) and Datta and Neogy (1965) observed maximum germination in 4 percent sucrose solution, while Singh (1957) reached a maximum in 10 percent sucrose solution, for *Crotalaria* pollen. However, as stated above, we determined that a 15 percent concentration of sucrose was the most suitable for pollen germination of *T. asiatica*. We consider this to be a specific difference depending upon the osmotic pressure of the cell which again may be dependent upon the locality.

The role of boron on germinating pollen was described by Stanley and Loewus (1964), Vasil (1964), and others as associated with sugar metabolism, as an inducer of oxygen absorption, and as a requirement for pectin synthesis. The effects of various compounds of boron on the germinating pollen have been reported by Stanley and Lichtenberg (1963). In the present experiments a lower concentration of boron (20 ppm) promoted a high percentage germination and longer pollen tubes. In higher concentrations of this chemical, germination was retarded. Higher germination percentage and longer tubes with boron have been reported by Thompson and Batjar (1950), Young (1958), and Bamzai and Randhawa (1969). Young (1958) found that effective germination was possible when pollen grains were allowed to germinate in boric acid concentrations of up to 50 ppm. However, experiments of Bamzai and Randhawa (1969) showed that higher percentage germination and tube length could be achieved with concentrations of up to 20 ppm. The results of the present investigation are in general agreement with those of Bamzai and Randhawa (1969) regarding the effect of boric acid. Therefore, we agree that the reduction in the germination percentages and a lower mean tube length in higher concentrations of boric acid may be caused by the toxic effect of this chemical in these higher concentrations.

Cook and Walden (1965) reported that the presence of a calcium ion is required for pollen germination. In the present work germination increased with an increase in concentration of calcium

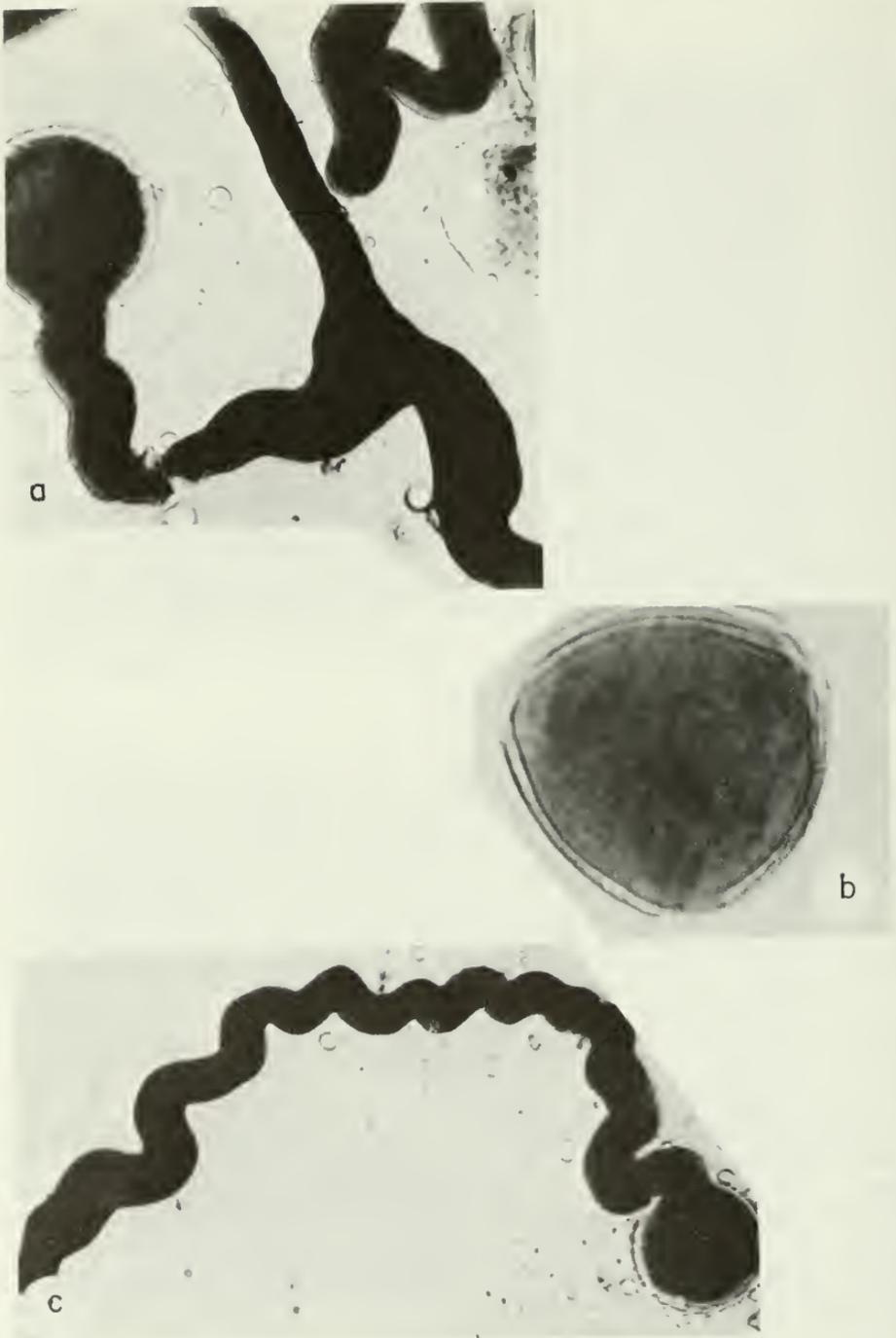


Fig. 4. *Torenia asiatica*: (a) branched pollen tube, showing callose plugs (150 X); (b) pollen grain of *Torenia asiatica*, showing tricolpate condition (250 X); (c) germinating pollen tube, showing the zig-zag nature (150 X).

nitrate (Table 2). Thus, in 100 ppm of calcium nitrate maximum germination was noted. The present experiments were intended to demonstrate only the effect of the known range of concentrations of different chemicals on pollen germination. Whether or not a higher concentration of calcium nitrate than 100 ppm would have enhanced pollen germination further was not within the scope of the present study; nevertheless, the study does provide a basis for further research. Cook and Walden (1967) also noted that concentrations of calcium higher than 100 ppm produced long pollen tubes and that germination does not occur in the absence of this chemical. Importance of calcium to the germinating pollen was given in detail by Faull (1955), Knawck and Brewbaker (1961), and Linskens (1964). Pfahler (1968) noted increased germination when calcium was added to pollen of some hybrid maize. As in experiments of Cook and Walden (1967), so in the present experiment: a relatively higher concentration of calcium gave better results. Most of the resulting pollen tubes did not rupture. This decreased incidence of rupture among the pollen tubes has been attributed to the capacity of calcium to give more rigidity to the cell wall (Brewbaker and Knawck, 1964).

Compared with the control medium, potassium and magnesium do not improve germination percentage or mean tube length of pollen grains. However, potassium nitrate induced certain abnormalities, such as branched pollen tubes and vesicles.

In the pollen tubes that grew in a coiled or zig-zag manner, the wall was not straight as in many other plants. Such coiled or zig-zag tubes have been observed by Loo and Hwang (1944) and Vasil (1960). Loo and Hwang (1944) observed that when pollen grains of *Antirrhinum majus*, *Thea sinensis*, *Brassica juncea*, *Triticum vulgare*, and *Hordeum vulgare* were germinated in the presence of colchicine, the resultant tubes became either zig-zag or coiled. Loo and Hwang (1944) considered such an abnormality to be the direct effect of the treatment of colchicine, not of a change in the osmotic pressure, as had been suggested by earlier workers. Abnormal tubes were recorded by Brink (1924) and Vasil (1960) also. Vasil (1960) found in certain cucurbits abnormal pollen tubes having branched or swollen tips. Vasil attributed these modifications to hypo- or hypernutrition, high humidity, high temperature and/or stale pollen. However, Nair and Deshpande (1968), working on *Luffa cylindrica* pollen, noted that apparently there was no significant correlation between germination results and meteorological data. Because the coiled tubes are found in both the control and the chemically induced pollen, there might be some other hitherto unexplored factor that regulates the morphology of the developing tube of the pollen of *T. asiatica*.

Branched pollen tubes having a vesicle on one of their tips were also found by Loo and Hwang (1944), Vasil (1960), Nair *et al.* (1964), and Nair and Deshpande (1968). Loo and Hwang suggested that these swellings were the result of germination in a medium containing indole-3-acetic acid and that the abnormality

was the direct effect of this chemical. The branching of the tube and the subsequent swelling of one of these branches in the present experiments is observed only when the pollen grains are germinated in a potassium nitrate-sucrose medium. It is noteworthy that in lower concentrations of the above mixture there was a lower percentage of branching than in higher concentrations. Whether the abnormalities are a direct result of the addition of KNO_3 or an expression of certain other physiological phenomena in the plant cannot be ascertained until more pollen grains belonging to different taxa are studied responding to KNO_3 in the germinating medium.

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