IN VITRO POLLINIAL GERMINATION IN *CALOTROPIS*: POLARITY OF TUBE GROWTH AND ACTION OF GROWTH SUBSTANCES

THE occurrence of bag-like pollinia enclosing pollen grains in some members of Asclepiadaceae offers a new dimension to *in vitro* studies on pollen germination and tube elongation. The pollinial wall is made up of sporopollenin[1-3] except for a specific region (referred as a 'furrow') through which the pollen tubes emerge during germination. This regional specificity of germination implies that tubes originating from all points inside the pollinium grow towards the furrow. It has been suggested[1] that the unidirectional growth of pollen tubes is due to the orientation of the germ pores of individual pollen grains towards the furrow or the germinating region. Alternatively, the polarity of tube growth may be due to the presence of chemotropic factors in and/or around the furrow. These assumptions are tested in the present study. Another aspect investigated here is the possibility of the pollinial wall altering or modifying the action of plant growth substances on the pollen grains it envelops.

The pollinia of *Calotropis gigantea* were selected for this investigation because of their large size and easy availability. Batches of 10 pairs of pollinia dissected out from fresh flowers of *Calotropis* were grown in distilled water, in the standard medium of Brewbaker and Kwack[5] and in standard media containing different concentrations (5, 10, 25, 50 and 100 ppm) of IAA, GA_3, NAA, 2, 4-D and kinetin. All experiments were conducted at $28^\circ \pm 2^\circ$ C. Emergence of the first (first few) pollen tubes outside the pollinium is taken as the initiation of pollinial germination. In each trial, individual pollinia showed little variation in the time taken for germination. However, tube length attained after 24 hr showed some variability and the values compared were the mean of 60 measurements on the longest pollen tubes from each culture. The germinating region of the pollinia was cut out under the microscope and this portion as well as the rest of the pollinium were extracted separately. The usual paper chromatographic techniques were employed for the analysis of the extracts.

The pollinium germinates even in distilled water. Within 15 min of its contact with water, a slit-like structure begins to develop at the germinating region through which amorphous exudates ooze out and collect along the opening. In the exudates, pollen protoplasts with or without tubes are also seen. The germination time of pollinia in water is shorter than that needed in standard culture solutions but the tube growth in the former is slow and virtually stops after 2–3 hr. The maximum tube length measured is 180 μm in 24 hr or only about 5% of the length attained in standard culture solutions.

The pollen grains are non-aperturate. When imbided with water, the thin exine breaks irregularly and the intine protrudes out as the pollen tube. Frequently, the opening of the exine is wide enough for the entire pollen protoplast to move out. Exineless protoplasts escape from the pollinia when they are cultured both in water and in culture solutions; more of them, as a rule, occur in the former.

Pollen germination and tube initiation in *Calotropis* pollinia appear to be a direct result of water uptake through the furrow. Pollinia with their germinating region covered with water-proof materials like Fevicol produce no pollen tubes either in water or in culture solutions even after 24 hr. However, a slit cut out in the closed pollinial wall will lead to the emergence of pollen tubes through the new opening. Pollinia with the usual germinating region *plus* a slit made on the opposite side were also cultured. Here, tubes emerge from the openings on both sides with equal ease and grow with comparable rates of tube elongation. Further, chromatographic analysis of the germinating region and the rest of the pollinia shows no significant differences in chemical composition. These results suggest that the tubes would grow through any opening in the pollinial wall offering accessibility to water and rule out the possibility of : (*a*) any special arrrangement of pollen grains with reference to the germinating region and (*b*) the presence of chemotropic factors in the furrow guiding pollen tubes towards it.

None of the growth substances tested here promotes pollinial germnination (Table I). However, except for

TABLE I

Effect of various growth substances on pollinial germination and pollen tube elongation in Calotropis gigantea

Concentration ppm	Time required for initiation of pollinial germination (min)				
	IAA	GA_3	NAA	2, 4-D	Kinetin
0	30	30	30	30	30
5	30	90	60	60	60
10	30	90	60	60	60
25	30	90	60	30	30
50	30	90	60	60	30
100	30	90	60	30	30
	Pollen tube length attained in 24 hr (μm)				
0	3509	3831	4313	3492	3492
5	..	3904	3289	5365	7792
10	4190	7100	1175	3958	1663
25	6230	3517	615	3300	1474
50	3257	4654	89	2974	1734
100	2735	1663	85	823	1550

NAA, all of them at low concentrations enhance the rate of tube growth. The extent of their effect on tube growth is not identical, but as a rule, higher concentrations induce inhibition of growth.

Addicot[6] pointed out that pollen germination and tube elongation are distinct physiological processes depending on different factors. Later reports[7,8] on the effect of plant hormones on the pollen growth of loquat and tomato support this contention. The present findings also recognise two phases in pollen growth. As seen in Table I, most growth substances at low concentrations prolong the time needed for pollen germination but the same concentrations enhance tube elongation up to or over 200%. Thus, although the pollen grains of *Calotropis* are enveloped by the pollinial wall, they too exhibit characteristically different responses to growth substances during germination and tube elongation phases of early pollen growth.

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EVALUATION OF ROOTSTOCKS OF POME AND STONE FRUITS AND RELATED WILD SPECIES FOR RESISTANCE TO CROWN GALL

Crown gall, incited by *Agrobacterium tumefaciens* (Smith and Townsend) Conn, is an important disease of temperate fruit crops. In India, the writer has detected the prevalence of the disease on stone fruit trees in Himachal Pradesh[1] and Kumaon Hills of Uttar Pradesh. As the disease mostly attacks the underground and ground level parts of the plants, use of resistant rootstocks for commercial varieties of temperate fruits is deemed to be a suitable and effective control measure for the disease. A search for crown gall resistant rootstocks for pome and stone fruits was, therefore, considered an important and urgent manoeuvre to combat the disease in north-western hills of India. In order to identify sources of resistance, 24 stocks of pome and stone fruits and their related wild species were evaluated for their performance against the disease. The results of the evaluation are presented in this article.

Stocks of Malling Merton Series and species of the genera *Cotoneaster*, *Malus*, *Prunus* and *Pyrus* were included in the present investigation. A virulent culture of *A. tumefaciens*, isolated from galls on plum trees, was used as inoculum. At least 3 plants of each type were employed for evaluating the host reaction to the disease. Pot-grown, 2–3 year old plants were inoculated in the following manner: Crescentic annular wounds were first created on the stem at two sites—one at the soil level and another at 10–15 cm above soil—by knifing out a rectangular flap of bark and cortex of approximately 1 × 2 cm size. The wounded sites were then wrapped with absorbent cotton swabs soaked in aqueous suspension of 48 hr old culture of the bacterium (10^8 cells per ml). To avoid loss of moisture from the cotton swabs, the soil level and aerial inoculated sites were covered with soil and butter paper, respectively. Identically wounded plants but wrapped with water soaked cotton swabs served as checks. The inoculated and check plants were maintained in glasshouse at 17° to 28°C (24–26°C for about 18 hr a day). The inoculated sites were provided sufficient humidity for the development of the disease by keeping the cotton swabs moist. After 15 days, the cotton swabs were removed and regular observations were initiated for the development of the symptoms. The plants which failed to produce symtoms within 90 days of inoculation were reinoculated. Observations were continued for a period of 8 months.

On the basis of size and nature of galls produced at the inoculated sites, the plants were evaluated as susceptible, moderately resistant or resistant. In susceptible plants, the galls were characteristically active and attained conspicuously large size (diameter more than 1 cm) within 90 days. The galls continued to show peripheral meristematic activity for the whole period of observation. The galls produced on moderately resistant plants were comparatively smaller in size (less than 1 cm in diameter after 8 months) and did not show any peripheral meristematic activity. The growth of the galls was extremely slow on these plants. Failure of the plants to support any tumourous activity at the inoculated sites was designated as resistant reaction to the disease.

Of the 24 rootstocks tested, susceptible reaction was shown by the plants of *Cotoneaster microphylla* Wall., *Malus pumila* Mill., *M. sikkimensis* (Hook. f.) Kochne, Malling Merton 101, 102, 105, 106, 109, 110, 115, *Prunus amygdalus* Batsch, *P. armeniaca* L., *P. avium* L., *P. cerasus* L., *P. cerasoides* D. Don (syn. *P. puddum* Roxb.), *P. cornuta* Stend. (syn. *P. padus* L.), *P. domestica* L., *P. persica* (L.) Batsch., *P. salicina* Lindl.,