MASS CULTURE OF POLLEN TUBES

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Investigators of pollen germination and pollen tube growth in vitro have employed various methods of culturing, usually involving a thin liquid pollen suspension in petri dishes or drops on glass slides (LINSKENS 1964, 1967a, b). Effects of different substances on germination percentage and pollen tube length are tested under these conditions. For investigation of chemotropical activity, the pollen is often germinated on solid substrates such as gelatin and agar (ROSEN 1964; MASCARENHAS & MACHLIS 1962). In all of these techniques, the experimental measurements are made microscopically, so that only a small amount of pollen, less than 1 mg in each experiment, can be used.

However, for biochemical analyses of pollen and pollen tubes, larger amounts of germinating pollen grains are necessary (MASCARENHAS 1966; LINSKENS 1967a). At the same time, it is desirable that the medium remains homogeneous during the experiment, that optimal supplies of nutrients are available and that the germination process starts synchronously.

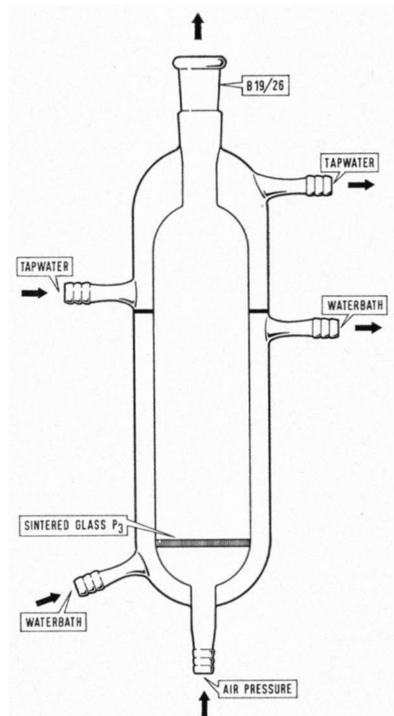

Fig. 1. Germination vessel for pollen tube mass culture. The germination chamber above the sintered glass, is penetrated by the air stream, which is pressed in through the lower entrance of the funnel. Germination chamber can be held at constant temperature by a streaming water bath. Condensation is caused by a cooling system (upper part) with tap water.

With these criteria in mind, we have designed a germination vessel as shown in *fig. 1*. It consists of a double walled cylinder of pyrex glass. The inner germination chamber is divided by a sintered glass filter (P3). The germination medium is in the upper part, which is aerated from below through the filter. The finely distributed air stream has a double purpose: (a) to maintain a homogeneous suspension through continuous mixing and (b) to supply the high oxygen demands of the germinating pollen, which can completely prevent border effects as they occur in drop cultures, for example. Circulation of water of the chosen temperature through the double wall enables one to vary or maintain the temperature of the germination medium. In addition, cooling of the medium by the constant air stream can be compensated for. Loss of the solvent by evaporation from the germination medium, which can have serious consequences especially in long term experiments, can be prevented by cooling of the upper walls with tap water to cause condensation.

Charging of the germination vessel is carried out in the following steps. The air stream through the filter is switched on and circulation of the heating resp. cooling fluids are started. About 10 ml of pure germination medium (e.g. 10% sucrose and 0.01% boric acid in quartz distilled water for *Petunia* pollen) is added through the upper opening (B 19/26). At time zero of the experiment, as much as 750 mg pollen is suspended in 25 ml of the germination medium in a test tube which is mixed for about 5 seconds on a Vortex mixer. The suspension is immediately poured into the germination chamber and the test tube is rinsed with 65 ml of medium alone which is also added to the chamber.

The described method delivers optimal germination conditions for many pollen species. Our experiments have yielded optimal results with *Petunia* pollen at a final concentration of 7.5 mg pollen per ml medium. As seen in *table 1*, after about 1 hour more than 70% of the pollen are germinated. Compared with other germination methods, such as hanging or lying drops, the germination percentages are higher. The growing pollen tubes are much straighter (*fig. 2*) and are not curled as occurs in all other germination conditions. This is true also

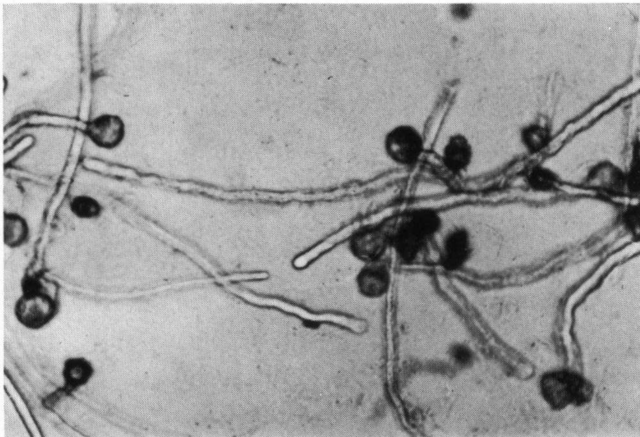

Fig. 2. Pollen tubes in mass culture after 6 hrs germination.

Table 1. Comparison of germination and tube growth in drop- and mass-culture.

time	% germination		length of pollination in μ	
	lying drop	mass-culture	lying drop	mass-culture
1 hr	59	75	25	40
3 hrs	64	73	80	130
6 hrs	65	81	200	350

after 24 hours germination. Growth rates of the pollen tubes are much higher in the mass cultures than in slide cultures (*table 1*). In contrast to all other methods of dispersal such as stirring or shaking, pollen tubes remain intact and do not break during germination times as long as 24 hours.

The germination chamber is, therefore, quite useful for mass culturing of pollen tubes which are necessary for biochemical studies of parameters of cell growth and studies on the synthesis of pollen tube wall materials.

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