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A NEW MEDIUM FOR POLLEN GERMINATION IN VITRO

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SUMMARY

Poly-ethylene glycol (PEG)-400 was found to be superior to sucrose as the major component in media for the germination of *Petunia hybrida* pollen. PEG promotes pollen tube growth considerably more than sucrose. Pollen tubes grown on PEG resemble the *in vivo* tubes much more than those grown on sucrose. The respiration rate of pollen germinating on sucrose is 33% higher than on PEG; the extra energy produced is clearly not related to tube growth.

1. INTRODUCTION

As pollen germination *in vitro* is a routine method in several research fields, many incubation media have been established during the last decades (STANLEY & LINSKENS 1974). In most media a soluble sugar, generally sucrose, is used both as an osmoticum and as a substrate for respiration. Because high concentrations of metabolizable sugars might interfere with the utilization of other substrates, several substances such as mannitol, sorbitol, penta-erythritol, ethylene glycol, propylene glycol and poly-propylene glycol, which are not or not easily metabolized in pollen, have been proposed as substitutes for sucrose. These compounds were tested on lily (DICKINSON 1968, 1978) and *Petunia* (STANLEY & LINSKENS 1964) pollen. It was found that on none of them did pollen tubes grow better than on sucrose.

Poly-ethylene glycol (PEG), which is also not metabolized in plants and can provide water potentials over a wide range (STENTER et al. 1981), has been tested by DICKINSON (1968) on lily pollen with unsatisfactory results. In this report we propose a simple PEG-400 medium for germinating *Petunia* pollen which yields about the same germination percentage but promotes tube growth considerably more than the conventional sucrose medium.

2. MATERIAL AND METHODS

Plant material – Plants of *Petunia hybrida*, clone W166H (incompatibility alleles S_2S_3) and clone W43 (incompatibility alleles S_1S_1) were grown in the greenhouse with artificial light (15,000 lx) at a photoperiod of 18 h.

Pollination and germination – Pollen of clone W166H was harvested on the day of anthesis, mixed thoroughly and pollinated to the surface of mature stigmas of clone W43. Four hours after pollination the pistils were collected and fixed in a mixture of formalin, acetic acid and ethanol, 70% (1:1:18 v/v/v). Stigmas

with attached styles were softened in 8 N NaOH, squashed and stained in aceto-carmine. The diameters of pollen tubes growing in the pistil were determined microscopically.

Pollen from the same clone was equilibrated for 2 h in air at a relative humidity of 100% and germinated *in vitro* at 25°C for 3 h with continuous shaking in solutions containing sucrose or PEG-400 (British Drug Houses) at different water potentials. The sucrose solutions yielding the water potentials desired (ψ = –2.5 to –40 bar) ranged from 0.1 to 1.1 M. PEG concentrations were between 0.08 and 0.65 M for the same water potential range. Boric acid, 100

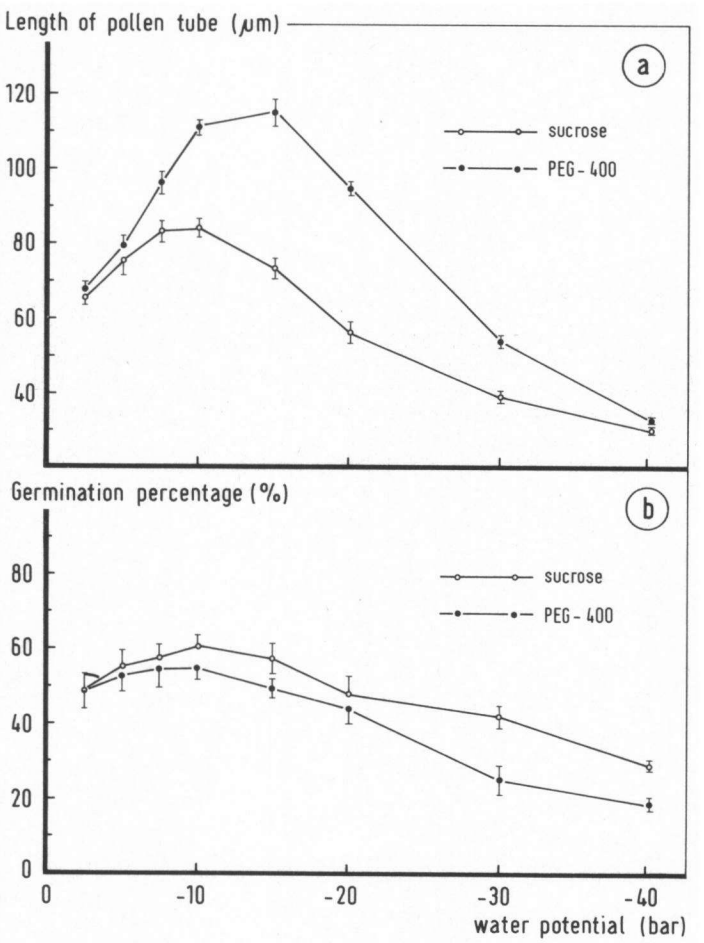

Fig. 1. Germination and tube growth of *Petunia* pollen in sucrose and PEG-400: Pollen of *Petunia hybrida* clone W166H was germinated at 25°C for 3 h. The data are means of 5 determinations. At least 100 pollen tubes were randomly selected for tube length measurement (a). For determining the germination percentage at least 200 pollen grains were screened (b). Vertical bars indicate standard deviations.

$\mu g.ml^{-1}$, was included in all media. The density of pollen was 5 mg (fresh weight) per ml. After culturing, the pollen was stained with cotton blue and the germination percentage was determined microscopically. From each medium 5 samples were counted. Pollen grains with tubes longer than half the diameter of the grain were considered to be germinated. Length and diameter of pollen tubes were determined under a projection microscope; at least 100 randomly selected tubes were counted in each determination.

Determination of water potential – All the media were carefully adjusted to selected water potentials determined by an osmometer. At the end of the incubation 1 ml of the culture was filtered and the water potential of the filtrate was determined.

Respiration – 10 mg of pollen from clone W166H were germinated in 2 ml of sucrose or PEG-400 medium at 25°C in a Warburg flask and the respiration rate was measured manometrically (UMBREIT et al. 1964). Three pollen samples per treatment were assayed in each experiment.

3. RESULTS

Pollen of *Petunia hybrida* clone W166H incubated at different water potentials in sucrose or PEG-400 medium started germination about 0.5 h after the onset of incubation. After 3 h germination percentages ranged from 29 to 61% (*fig. 1b*). Both curves show a broad optimum at about –10 bar (0.36 M for sucrose, 0.29 M for PEG). With sucrose the germination percentage is somewhat higher over virtually the whole range; at the optimum the difference is approximately 5%.

Much more difference is observed in the length of the pollen tubes (*fig. 1a*). In PEG-400 medium the optimal water potential is –15 bar, 5 bar lower than in sucrose medium. It is remarkable that pollen tubes are longer in PEG medium and that tube length varies over a much wider range than germination percentage.

Morphological differences between pollen tubes grown on sucrose and on PEG at water potentials optimal for elongation are striking. In general, the PEG-grown pollen tubes resemble more closely tubes growing in the style than those grown on sucrose (*fig. 3*). On PEG-400 the tubes grow faster and more uniformly and their appearance is less crooked than in sucrose medium. Moreover, the diameter of the tubes in PEG-medium (8.8 μm) is nearly as *in vivo* (8.2 μm), but greatly differs from the diameter of the tubes in sucrose medium (12.2 μm).

After 3 h of incubation the water potential has dropped from –10 bar to –11.5 bar in the sucrose solution and from –10 to –10.6 bar in PEG. This might indicate that with sucrose more material is released into the medium than with PEG-400.

Respiration of pollen incubated at water potentials optimal for tube growth are shown in *fig. 2*. In both media the uptake of O_2 is almost linear with time. The rates of oxygen consumption as calculated from the regression lines are

10.0 $\mu l.mg^{-1}.h^{-1}$ in sucrose and 7.5 $\mu l.mg^{-1}.h^{-1}$ in PEG-400 medium. Thus possibly the sucrose is used as an exogenous substrate for respiration. Since the growth of the pollen tubes on PEG is faster, it is clear that the additional oxygen consumption in the presence of sucrose is not related to tube growth.

4. DISCUSSION

The initiation of the *Petunia* pollen tube *in vitro* is apparently affected by the water potential of the medium (*fig. 1b*). The similarity of the two germination curves indicates that sucrose primarily acts as an osmoticum. There is, how-

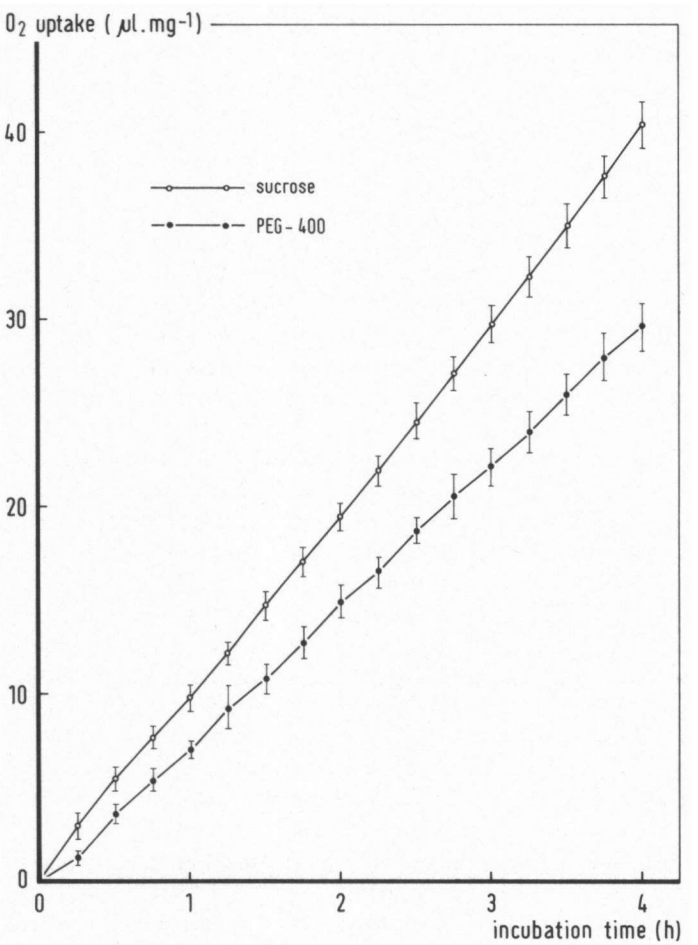

Fig. 2. Oxygen consumption: Pollen of *Petunia hybrida* clone W166H was incubated at 25°C in PEG-400 (ψ = −15 bar) or sucrose medium (ψ = −10 bar) and assayed by Warburg manometry. The data are means of 3 determinations.
Vertical bars indicate standard deviations.

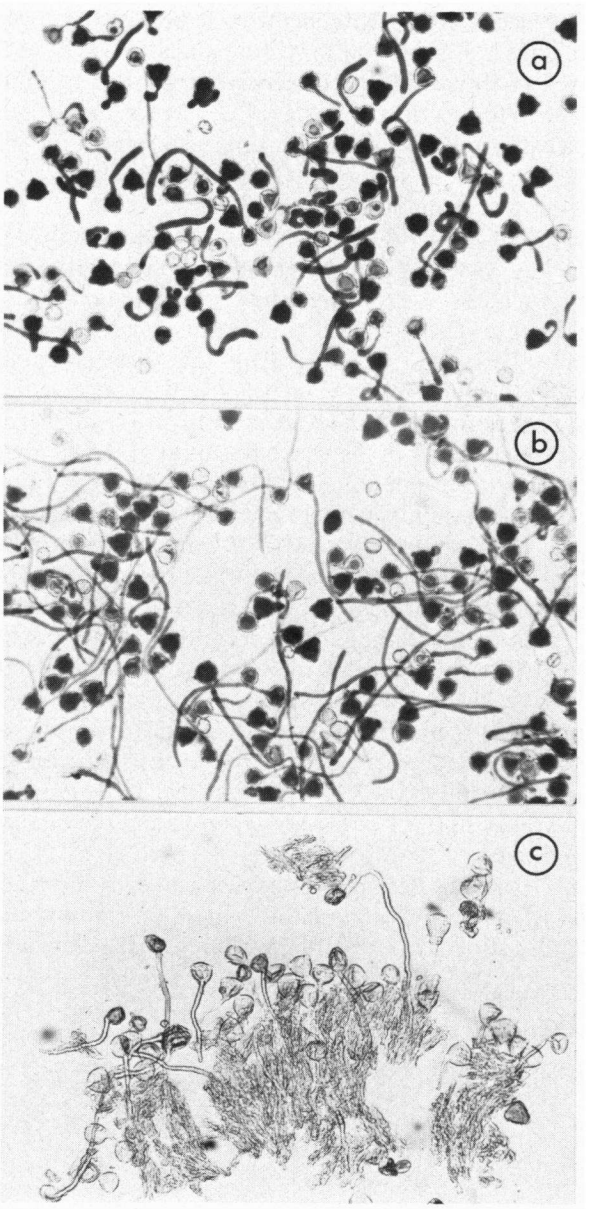

Fig. 3. Microphotographs of *Petunia* pollen germinated (a): in sucrose medium, (b): in PEG-400 medium and (c): om the pistil.
Magnification: 125 ×.

ever, some difference in germination percentage between the two kinds of media as the percentage is 5% higher with sucrose under optimal conditions. Before and during tube initiation ATP will be needed for protein synthesis and activation of enzymes, synthesis of wall material and other energy consuming processes. Thus sucrose might also serve as an energy source for germination. This view is supported by the observation that sucrose promotes respiration by 33% over the incubation without sucrose (*fig. 2*). In addition to a role of sucrose in germination the sugar might be involved in some way in the increase in volume of the pollen tubes which is considerably greater on sucrose than on PEG.

At all water potentials, the average length of the pollen tubes is greater on PEG than on sucrose (*fig. 1*). This could mean that concentrated sucrose is harmful for the tube growth. If this is true, the water potential optimal for elongation would be expected to be less negative with sucrose than with PEG. This is indeed what is found (*fig. 1a*). A harmful effect of sucrose might also be inferred from the changes in the water potential of the incubation medium. In the case of sucrose the water potential decreases 2.5 times more than in PEG during incubation, at least partly because more material leaks from the pollen into the medium. For instance, the release of amino acids into the sucrose medium is 32% larger than into PEG medium (data not shown). This might indicate that the permeability of the membrane is altered by the high sucrose concentration. Another factor leading to the decrease in water potential observed might be the hydrolysis of some sucrose by an extracellular invertase (TUPÝ 1960, DICKINSON 1967).

In the PEG medium there is no exogenous substrate for respiration. The endogenous substrate may be carbohydrate but also proline could be involved (BRITIKOV et al. 1965, BRITIKOV & LINSKENS 1970). The endogenous substrate supports ATP synthesis sufficient for germination and for tube growth during at least the first hours.

As *Petunia* pollen grains normally germinate and even produce much longer pollen tubes on PEG-400, this compound is more satisfactory than sucrose as the major component of the incubation medium for *in vitro* studies.

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