Note

Stimulation of *Lilium* Pollen Germination by Pyrroloquinoline QuinoneLong Bao XIONG, Jiro SEKIYA*
and Noboru SHIMOSEDepartment of Agricultural Sciences, Faculty of
Agriculture, Okayama University, Tsushima,
Okayama 700, Japan

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Since Salisbury *et al.*¹⁾ reported the structure of pyrroloquinoline quinone (PQQ), there have been many reports on PQQ acting as a coenzyme of enzymes, dehydrogenases and oxidases that are found in microorganisms and animals.^{2~4)} PQQ also stimulates the growth of microorganisms such as *Pseudomonas*⁵⁾ and *Acetobacter*,⁶⁾ although the mechanism remains to be resolved. On the other hand, PQQ has been reported to be present in plants, as well as in microorganisms and animals.⁷⁾ However, the physiological effect of PQQ has not yet been reported, and the present report describes the stimulation of *Lilium* pollen germination by PQQ.

PQQ was purchased from Ube Industries Ltd., Tokyo, or from Mitsubishi Gas Chemical Co., Inc., Tokyo. Pollen grains were collected from *Lilium longiflorum* flowers purchased from local markets, and were stored in a desiccator at 4°C until use. Before use, the pollen was washed 3 times with petrol to remove pigments on the surface and dried for 5 min in ambient air. The pollen was spread in one layer on nylon mesh (60 μ m) and equilibrated with humid air in a moistened Petri dish for 30 min at 25°C. Then, the pollen on nylon mesh was transferred to a sterilized medium (pH 6.0) containing 1 mM CaCl₂, 1 mM H₃BO₃, 10% (w/v) sucrose, a given concentration of PQQ and 1% (w/v) agar, and cultured in a moistened chamber at 25°C in the dark. At the times indicated, the pollen was observed under an optical microscope, and the germination rate ($n > 200$) and pollen tube length of the germinated pollen ($n = ca. 20$) were determined.

Lilium pollen did not lose any germination ability, even after washing with petrol, this washing process being required to spread the pollen on the agar medium. When the pollen was cultured on the synthetic media containing PQQ, the germination of *Lilium* pollen was stimulated (Figs. 1 and 2). This is the first demonstration of the stimulative effect of PQQ on plant growth. Washing the

pollen with petrol did not affect pollen germination with or without PQQ. The stimulation of pollen germination was dependent on the PQQ concentration added to the media. At a concentration of 100 μ M PQQ, the germination rate was almost doubled compared to the control culture, while the stimulation rate was small at a concentration of 1 μ M (Fig. 1). The magnitude of stimulation by 100 μ M PQQ was, however, increased in a range of two to three fold, although the germination rate changed when different lots of pollen were used. When the pollen germination lasted for longer periods, the germination rate was increased and reached its maximum after 3 hr; ca. 80% of the pollen was germinated with

FIG. 1. Effects of Different Concentrations of PQQ on *Lilium* Pollen Germination.

The pollen was cultured for 1 hr at 25°C on media containing different concentrations of PQQ.

FIG. 2. Effects of PQQ and CHI on *Lilium* Pollen Germination and Pollen Tube Elongation.

The pollen was cultured at 25°C on media containing 100 μ M PQQ or 1 μ M cycloheximide. The germination rate and tube length of the germinated pollen were determined at the times indicated. Germination rate: control, (●—●); 100 μ M PQQ, (○—○); 1 μ M cycloheximide, (×—×). Pollen tube length: control, (●—●); 100 μ M PQQ, (○—○); 1 μ M cycloheximide, (×—×).

* To whom correspondence should be addressed.

100 μ M PQQ (Fig. 2). In the absence of PQQ, however, the germination rate was *ca.* 50% after 3 hr of culture. On the other hand, the pollen tube elongation of the germinated pollen was not affected by the addition of PQQ to the medium (Fig. 2). Cycloheximide inhibited both the pollen germination and the pollen tube elongation. The incorporation of 14 C-leucine into the pollen proteins was also examined. However, both pollen germinated with and without PQQ incorporated 14 C-leucine into the trichloroacetic acid-precipitated proteins at the same rate. Pollen treated with cycloheximide did not incorporate 14 C-leucine into the proteins. We have, therefore, assumed that PQQ activates a particular enzyme(s) relating to the pollen germination.

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