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BOTANY

THE *IN VITRO* CULTURE OF EMBRYOS, OVULES AND OVARIES OF *PISUM SATIVUM* [1]

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Introduction: The nutrient requirements of growing ovules, ovaries or excised immature embryos of higher plants have been studied: by Maheshwari (1957) for poppy, by Asen and Larson (1951) for roses, by Gilmore (1950) for peaches, by de Capite (1955) for strawberries, by Nitsch (1949) for tomatoes, by van Overbeek (1942) for thornapple and by Dure and Jensen (1957) for cotton. The embryos (Asen and Larson, 1951, Gilmore, 1950; Nitsch, 1949; Dure and Jensen, 1957) or the ovules (Mahreshwari, 1957) or the whole ovaries (de Capite, 1955, van Overbeek, 1942) of various higher plants have been grown on simple nutrient media containing only minerals and sucrose, but attempts to grow immature ovaries of the leguminosae have not succeeded (de Capite, 1955, Nitsch, 1951).

Materials and Methods: The material for this investigation was *Pisum sativum* var. Alaska. Plants were grown in the greenhouse at about 22°C. The first flowers came to full bloom 30 days after planting and the pods (ovaries) contained mature seeds 25 days after flowering. White's (1943) medium containing 30 g/L sucrose, with or without 15 g/L agar and supplemented by vitamins or growth substances as indicated in the description of the experiments, was used. The ovaries were surface disinfected in 0.1% $HgCl_2$ and placed intact in test tubes with 15 cc. of solid nutrient medium. The ovules were removed aseptically from the ovaries and cultivated in small vials on 3cc. of medium.

Results and Discussion: Excised embryos grew on White's medium to maturity and yielded seedlings *in vitro* only if they were twenty days past the full bloom stage of the flower when removed from the plant. When ovaries were removed from plants ten days after the flowers had reached full bloom embryos in the partially mature ovules germinated while the ovule was still attached to the ovary wall. Neither supplementing the nutrient medium with vitamins, growth substances, yeast extracts, coconut milk, nor extracts from peas im-

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proved growth. Shaking the cultures also did not result in better growth.

Embryos from ovules removed from the ovary ten days after full bloom and cultivated on the basic medium containing only minerals, sucrose, and agar grew into seedlings. Different nutrients and vitamins had no influence during the incubation period of thirty-eight days. Gibberellic acid at 1 mg/L delayed the germination of these partially mature ovules for several days, compared to the controls.

Ovaries one day past full bloom grew on the basic medium with agar, but much slower than *in vivo* and only to a limited size. After seven days, when the ovaries had reached a length of 17 mm. and only a small amount of the nutrient available in the medium was used, the growth stopped. (Average length of pods at the beginning of the experiment was 10 mm.) This cessation of growth was not overcome by adding thiamine or yeast hydrolysate and only partially by gibberellic acid, coconut milk or a mixture of gibberellic acid, IAA, and kinetin (Fig. 1).

All experiments were repeated on media without agar. The ovaries were grown in test tubes with a hole in the bottom which were held in larger test tubes containing the nutrient. This made it possible to change the nutrient solution without danger of contamination and to exclude the possibility of an accumulation of inhibitory substances in the nutrient solution. The solution (5 ml.) was renewed every seven days. Ovary growth was increased and extended over a longer period compared with ovaries grown on the solid medium but after fourteen to twenty-one days growth stopped also and the ovaries did not reach maturity (Fig. 1).

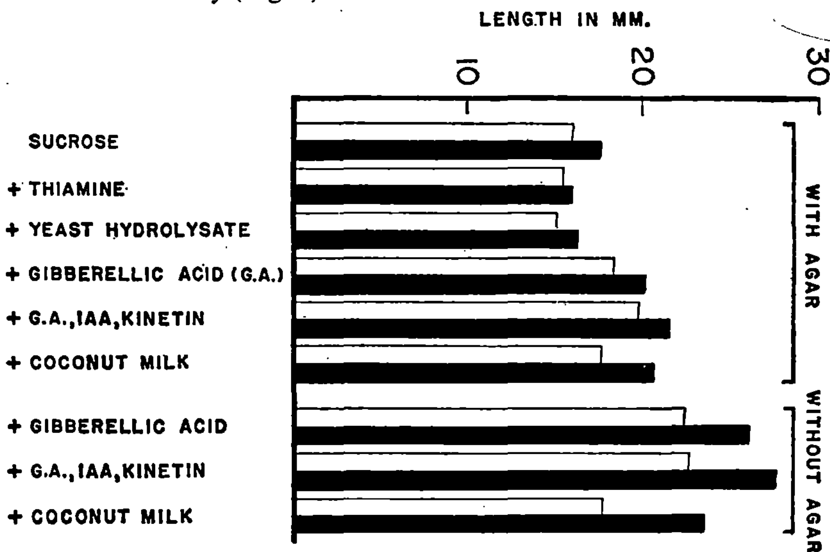

Fig. 1. Length of pea ovaries removed from plant one day after pollination, after cultivation *in vitro* for seven days (white columns) and twenty-one days (black columns).

With two, three, and four-day-old ovaries similar results were obtained. These ovaries grew to a certain length and stopped. Some of the immature seeds in the five-day-old ovaries germinated after thirty to forty days of incubation. At least one seed from nine-day-old ovaries germinated on water agar and on the basic nutrient medium. This was true of the six-day old ovaries. Gibberellic acid again inhibited germination. Since in six-day-old ovaries the embryos are smaller than 1 mm., considerable growth must have occurred. The same results were obtained with ovaries cut transversely into two halves, as long as the peas remained attached to the carpel. Ovaries cultivated separately from carpels in the same test tube did not germinate, however.

These results suggest that growing embryos require substances for their development which are partly supplied from the carpel. The fact that it was not possible to supply these substances entirely from an artificial nutrient medium suggests that the carpels may supply not only nutrients but other unidentified growth-regulating substances.

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