USE OF RADIOACTIVE TRACERS IN THE STUDY OF POLLEN TUBE GROWTH

A KNOWLEDGE of the extent of pollen tube growth in the style is necessary for understanding the cause of failure of seed-set in self- and cross-incompatible combinations. Such information will be useful in devising means to overcome barriers to fertilisation which result from stigmatic inhibition of pollen germination or inadequate growth of the pollen tube in the style.[1] Pollen tube growth is usually studied by style dissection followed by suitable staining or by phase-contrast observations. In our studies in the genus *Nicotiana*, we found the following technique involving the use of radioactive isotopes like those of phosphorus (^{32}P) or sulphur (^{35}S), provides a rapid and reliable means of estimating the extent of pollen tube growth in incompatible crosses. A similar technique is described in a paper by Ar-Rushdi,[2] which appeared while the present study was under way.

The cross between *N. rustica* and *N. tabacum* succeeds if *rustica* is used as the pistillate parent but there is seldom any seed-set in the reciprocal cross. The style of *tabacum* is nearly thrice as long as that of *rustica* and we suspected that this difference in style length may contribute towards the failure of the cross in which *tabacum* is the pistillate parent. To ascertain this, some plants which had commenced flowering were transferred to glass jars containing ½ litre of Hoagland's Solution No. I, without P or S while using ^{32}P and ^{35}S respectively. ^{32}P in the form of H_3PO_4 or ^{35}S in the form of H_2SO_4 was added to the nutrient solution at the rate of 1 millicurie per ½ litre of solution and the pH was adjusted to 5·8. The amount of radioactivity shown by the pollen of plants treated in this way was measured with a pocket battery monitor; the maximum activity (50 to 100 counts per second) was shown 3 days after treatment in the case of ^{32}P and 5 days in the case of ^{35}S.

Pollen from *rustica* and *tabacum* plants treated in this way were used to make reciprocal crosses, using control plants without any treatment as the pistillate parents. The styles from crosses as well as parents were collected 24, 48, 72 and 96 hours after pollination and were exposed to Ilford X-ray film for 120 hours. The autoradiographs thus taken are shown in Fig. 1. The autoradiographs indicated that *rustica* pollen did not grow beyond one-third of the length of *tabacum* style. This observation was independently confirmed in style dissection preparations made according to a

modified schedule of Buchholz's method.[3] Thus, the tracer technique provides a correct picture of the extent of pollen tube growth.

FIG. 1. (*a*) Ovary, style and stigma of *N. rustica* (left) and *N. tabacum* (right) plants grown in ^{32}P solution. (*b*) Growth of *rustica* pollen tubes in *tabacum* styles, 72 hours after pollination. (Autoradiographs—Natural size.)

The dose of ^{32}P and ^{35}S added to the nutrient solution had no adverse effects on pollen fertility or functionability, as tested by germination in an agar-sucrose medium and stainability in aceto-carmine. Also, normal capsules with viable seeds could be obtained by selfing the flowers of treated plants. Though the absorption of ^{32}P by the plants is better than ^{35}S, it will be economical in such tracer studies to use ^{35}S since it has a half-life of 87·1 days and different plants can be kept in the same solution one after the other.

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