

Khoo and Stinson (1957) and Britikov *et al* (1964) have reported marked reduction in proline content of anthers from male sterile plants (T-cytoplasm) or sterile pollen. Proline in only trace amounts is characteristic of our T and S cytoplasm stocks as well. In addition we have chromatographically identified γ -amino butyric acid in our T-cytoplasm stocks, but recover only trace amounts in the S-cytoplasm stocks. Restorer genes (NY16 and Ky21) convert both S and T to the normal amino acid spectrum. The significance of these amino acid differences is being further studied.

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4. An agar medium for the *in vitro* germination of corn pollen.

In a series of experiments designed to investigate various nutritional and environmental factors which influence the *in vitro* germination of corn pollen, a simple agar medium has been established which will consistently support germination of corn pollen in excess of 70%.

Plants used in these studies were a single-cross (su₁/su₁) hybrid, "Seneca 60" (Robson Seed Co., Hall, New York) grown in the field, in the greenhouse or in a controlled environmental growth room during 1963 and 1964. Day old anthers were removed from the plants the evening prior to pollen collection.

Germination required a carbohydrate and calcium ion. Sucrose and raffinose supported good germination. Lactose, D-glucose, D-galactose, melibiose, L-arabinose, maltose and D-xylose were inferior, and no germination was obtained with D-fructose, D-ribose, D-mannose or D-mannitol.

Although the magnesium ion could partly substitute for calcium, it and other ions that were tested had little or no effect in the presence of calcium. From amongst our data analyzed thus far, no evidence for enhancement (at the 5% level of significance) by boric acid in the presence of calcium can be obtained. However, differences approaching 5% germination have been consistently recorded and since a number of other workers have found boron to be effective in increasing the germination of several kinds of pollen, we have included it in our basal medium. Boric acid in the absence of calcium was ineffective. Phosphate salts at concentrations of 0.7 M and above suppressed germination completely.

The agar substratum influenced the effects of other components of the medium. The medium of Brewbaker and Kwack (Am. J. Botany, 50: 859-865, 1963) supported better germination on leached Special Agar-Noble (Difco Co.) than on unleached plain agar (Difco Bacto-agar). Presumably the addition of some ion(s) altered the concentration to an inhibiting level. The plain agar contained 1.6 times as much total calcium as the Noble agar.

Apart from the need for a high humidity (in excess of 90%), critical control of the environmental factors for germination was not required, although germination was characterized by optima for temperature, pH, tonicity and agar concentration.

All the pollen grains that were to germinate had done so in less than 30 minutes and a rapid assay could be undertaken without concern for contamination of the medium by microorganisms. Since no population effect, such as that described by Brewbaker and Kwack (1963), was demonstrated on the Noble agar medium the density of pollen on the plates was not a variable factor. The mechanics of counting was facilitated by the use of photomicrographs.

The pre-inoculation history of the pollen was important and remained a variable in our experiments. Pre-inoculation treatments did not standardize samples. Corn pollen from a single tassel tended to give successively lower % germination as the number of days from initial dehiscence increased, even though fresh pollen was collected each day.

Consistent estimates of variability (sampling error 2%, replication 3%, and experimental error 3%) suggest that 8-10 subsamples from 3-5 replicates of each treatment provide sufficient experimental units for employment of this bioassay.

A basal medium chosen for subsequent research with corn pollen consists of:

0.35 M Sucrose (12%)
 100 ppm H_3BO_3
 300 ppm $CaCl_2 \cdot 2H_2O$
 with or without 0.7% Difco Special Agar-Noble

The pH of this medium was 6.8 - 7.1. The agar surface in pyrex Petri plates (60 mm x 15 mm) was inoculated by shaking pollen from a camel's hair brush. The open plates were placed over water in sealed incubators,

held at room temperature for 30 minutes and removed for counting. Germinated grains were defined as those with intact tubes at least one grain diameter in length.

The authors gratefully acknowledge the assistance with this work of Miss D. E. Hamill.

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5. Germination of sh₂ pollen grains.

Using the medium described above, we have surveyed several genotypes among our stocks. We first noticed in 1963 a coded entry that consistently demonstrated higher % germination than the control (su₁) or its allelic stock. Repeated analysis in 1964 of material grown in the field, the greenhouse or the growth room showed that our sh₂ source stock surpassed significantly the germination of all other entries. Reciprocal crosses with several stocks have been prepared but not yet tested. The significance in this report resides not in the fact that our sh₂ stock performs better (we have not demonstrated yet that the performance is a precise function of the sh₂ locus) but that the possibility for differential pollen germination may be exploited.

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1. The metastable nature of paramutable R alleles.

Paramutable R alleles of different geographic origins may be characterized by their differing Rrr phenotypes in a common genetic background. These phenotypes form a continuous series with respect to degree of mottling, and range from forms lighter than characteristic for the standard allele commonly used in paramutation studies, to forms which are self-colored. However, this phenotype is not a suitable property for permanent