

viability but more than adequate numbers of pollen grains survive for genetic or plant breeding studies. Pre-treatment of pollen will augment the longevity. Pollen collections before 10AM should be cleaned and spread to dry for 1 - 2 hours before introduction into the storage environment.

Our studies of pollen storage in liquid nitrogen and via centrifugal freeze drying are being continued.

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3. Soluble-sugars and free amino acids of corn pollen.

Soluble sugars and free amino acids from the pollen of many genotypes have been studied by means of one and two dimensional paper chromatography. Pollen that has been shed, or whole anthers, have been squashed onto Whatman 3MM filter paper and developed by an ascending technique.

For the separation of amino acids the papers were run in the first direction in 3 parts methyl ethyl ketone, 5 parts butanol, 1 part ammonia and 1 part water and in the second direction in 12 parts n-butanol, 3 parts glacial acetic acid and 5 parts water. After drying they were dipped in 0.2% ninhydrin in acetone and heated. Soluble sugars were separated in one dimension using the butanol-acetic acid solvent described above, dipped in an aniline-diphenylamine-phosphoric acid reagent and heated.

Glucose, fructose and sucrose are present in corn pollen in large amounts and in addition trace amounts of other soluble sugars are recovered. The relative amounts of the above named sugars are dependent upon the immediate post-harvest treatment. For example, the amounts of sucrose increase if the pollen remains unrefrigerated for more than 30 minutes between dehiscence and analysis.

We have tentatively identified 9 amino acids in most corn pollen samples examined, including, proline, α -alanine, glutamic acid, aspartic acid, γ -amino butyric acid, serine, glycine, valine, threonine and 2 acid amides, glutamine and asparagine. Some additional unidentified spots have also appeared. Proline shows up in very large amounts relative to the other spots. Quantitative determinations on a dry weight basis are not yet completed.

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.