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Additional study on the fine structure of maize microsporocytes

During the last year microsporocytes of a diploid maize inbred, maintainer of a cytoplasmic male sterile line, were examined with the standard techniques of electron microscopy. Particular effort was made to investigate the fine structures of bivalents, centromeres, the secondary constriction region, nucleolus and knobs of various chromosomes if available. At pachytene stage the synaptonemal complex was easily identified. By frontal view the diameter of the complex was approximately 2200 A.

Since the role of the synaptonemal complex in crossing over has been a subject of dispute, I had paid special attention to the finding of the existence of cross-elements in the complex. Up to the present, even though a large number of bivalents had been examined no cross-elements of any kind were definitely identified. If the synaptonemal complex is responsible for laying the ground for physical exchanges or crossing over between the two homologues, evidence of the formation of cross-elements between the two lateral elements should be expected. In view of this, once again the theory that the synaptonemal complex plays an important role in meiotic crossing over is questioned.

Chromosome 6, the nucleolar chromosome, is easily identified under both light and electron microscopes. It was observed that contrary to expectation, there was no synaptonemal complex beyond the secondary constriction region. Only darkly stained chromatin was shown in this region. As expected, synaptonemal complex was found in the rest of chromosome 6. In the centromere of this chromosome, only a lightly stained area was clearly observed. No microfibrils of any kind could be recognized. The synaptonemal complex was discontinuous in both the centromere and the secondary constriction regions.

In addition to the normal nucleolus, small nucleoli or nucleolar bodies varying in size and number from cell to cell were always present. These bodies were without any nucleolar cup and were unattached to any chromosomes. No fiber-like structures were seen in them; however, granular substances embedded in the homogeneous matrix were invariably observed. The appearance suggests their close relationship with ribosomes. Both the nucleolar bodies and the regular nucleolus possessed vacuoles which also varied in number and size but were always spherical in shape.

In the nucleolus of this inbred maize, two levels of structure could be revealed. One was the amorphous structure, the other, the fibrous. Fibers of the latter were about 350Å in diameter, and varied a great deal in length.

On the short arm of chromosome 9 there was a large terminal knob. This knob was frequently isolated and readily identifiable. No particular organizational characteristics were seen in the knob under the electron microscope. However, there were many fine fibrils radiating from the terminal region of the chromosome, and these fibrils measured less than 100Å in diameter. These fibrils are probably the basic components of the knob.

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Variations of maize anther callus in vitro

Since last summer 3950 maize anthers from 16 varieties have been cultured on defined medium. Callus growth from 201 anthers was observed. Morphological characteristics of these calli varied a great deal, from a size as small as 1-2 mm in diameter to as large as 3-5 times the original anther size. The small calli grew out from a part of the anthers, while the large ones grew out from the whole anthers. In the early stage of growth most of the calli were pale in appearance. When they were about two weeks old, they began to change from pale to brown. Chromosome constitutions of these calli have not yet been determined. However, it is expected that they vary from haploid to polyploid and aneuploid.

In order to induce embryoid development 182 calli of the above have been subcultured on an auxin-rich medium. It was found that after one week of subculture callus growth was almost completely stopped. Up to the present, no embryoid initiations were definitely identified. However, it is hoped that some of them will develop into plantlets before long. Previously it took 4-6 weeks for maize callus to differentiate into plantlets.

Nineteen of the calli were maintained by subculturing them on a 2, 4-D-containing medium.

It was also found that it did not make any difference in callus initiation and growth in the first four to six weeks of culture whether they were kept in dark or in light. Nevertheless, the calli were pale green if they were grown in light, while they were pale if they were grown in dark.

If this experiment will eventually lead to the production of plantlets, either haploid, diploid or aneuploid, it might become a new and useful technique in maize genetics, breeding, development and molecular biology.

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Methods of maize pollen germination in vitro, collection, storage, and treatment with toxic chemicals; recovery of resistant mutants

One vigorous corn plant sheds over 10^7 haploid, trinucleate pollen grains. Waxy and alcohol dehydrogenase-1 are known to be expressed after meiotic anaphase II, and the many correlations of duplication-deficient gametes with pollen abortion suggest that much of the pollen phenotype is encoded by its haploid genotype rather than the genotype of its pollen mother cell. For this reason, Nelson (commencing in 1958, Science 130: 794 with wx) and later, Freeling (1976, Genetics, in press, with Adh) were able to study intracistronic recombination and reversion, and Schwartz (1975: lecture at the International Maize Symposium, Urbana) was able to select Adh-deficient mutants among allyl alcohol-resistant pollen grains. Over the last two years, this laboratory has perfected numerous procedures involving maize pollen. Following Schwartz's lead, we have also recovered mutants via pollen selection. The methods and recipes we use follow. We hope they prove generally useful.

In vitro germination using "David's Bread Loaf": We have revised the pollen germination medium and conditions reported by Cook and Walden (1965, Can. J. Bot. 43: 779). Our concoction--called the Cook and Walden Revised Medium (CWRM)--is composed of 17% w/v sucrose, 300 mg/l $CaCl_2 \cdot 2H_2O$, 100 mg/l H_3BO_3 and 0.7% w/v Difco Bactoagar, to a pH of 6.4 after the addition of agar but before heating. After heating until just clear (120 C for 8 min), this medium is gelled in a Griffin beaker of the desired diameter and stored at 4 C for at least four days without change. This column of gel is called "David's bread loaf", from which 2 mm slices are cut and immediately used for pollen germination at 25 C and low (uncontrolled) humidity within an unsealed petri dish. Our methods differ from Cook and Walden's in sucrose concentration, pH and humidity requirement; the major difference is that our pollen grains germinate on a newly-cut solid surface. We achieve 75-95% germination of healthy pollen after 30 min for all of the seven different lines and inbreds we have tried. Before we devised the "bread loaf" technique, we experienced dramatic genotype-specific fluctuations; pH and sucrose concentration had to be continuously adjusted. The "bread loaf" technique affords the reproducibility necessary for determining kill curves for pollen pretreatments, and slices with gametophytes are easily moved to other dishes for staining, counting, fixing and the like.

Pollen collection and storage: The majority of our pollen viability studies utilized a 23± 2 C greenhouse, low humidity and natural January to March lighting. We find that our typical plant sheds over a five-day period. First-day pollen usually germinates poorly; second and third day collections 2-4 hrs after dawn are