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1. Enzyme treatment of chromosomes.

A preliminary investigation into the effects of trypsin on maize chromosomes is being undertaken as an undergraduate research project. The experiments were suggested by the work of Trosho and Wolff (J. Cell Biol. 26, 1965), who succeeded in observing multistrandedness in metaphase chromosomes of Vicia faba root tips.

Modified Trosho and Wolff techniques were adopted to microsporocytes; the anther smear was made in a trypsin solution (0.1 mg/ml of 0.01 M phosphate buffer, pH 7.2), incubated for one hour at 32°C, air dried and stained in Feulgen. Compared to controls, incubated in the buffer and treated similarly, the enzyme treated chromosomes showed in all stages of division a "fraying" and "blurring", the effect being most noticeable for cells in diplotene. Although multistrandedness may occur, no cells demonstrated an organized arrangement of strands.

Studies are also being conducted on mitotic chromosomes and interphase nuclei from root tip and tapetal cells. A cursory examination has not revealed an effect as found for the sporocytes.

It is hoped that further observations may be performed on sporocytes exhibiting anaphase bridges and large translocation regions. Comparative digestion studies on metaphase chromosomes of mitosis and meiosis I and II are also underway.

Edward J. Ward

2. Refined smear technique for obtaining large numbers of metaphases in corn root tips.

This is an amended recipe of the technique which appeared in the Maize Genetics Cooperation News Letter, 40:146-147, 1966:

Step 1: The seeds can be grown on 2% agar or on vermiculite in a Petri dish. A 10 mm radicle will be produced within 40 hours.

Step 2: Transfer 10 mm radicles to a Petri dish containing a 0.2% aqueous tween 80 colchicine solution and incubate 8 hours.

Step 5: Hydrolyze in 1N HCl at 60°C for 25 minutes.

Step 6: Stain in leuco-basic fuchsin until the root tip is deeply colored.

Step 8: The enzyme is a 5% cellulase - 5% pectinase solution.

Step 11: Add a 22 x 50 mm coverslip.

New Step 12: Heat gently.

Step 13: Seal with gum mastic. The coverslip should be held down by a flat block of marble or other suitable material while sealing.

R. M. Brown

3. Karyotype of Zea mays.

Karyotypes of maize have been composed from several commercial varieties, translocation stocks, a trisomic 6 stock and normal stocks. Metaphase chromosomes from root tip cells were employed.

The relative lengths of the mitotic chromosomes are very similar to the meiotic chromosomes, with chromosome 10 being approximately one half the length of chromosome 1. Chromosome 1 in mitotic metaphase is easily distinguished because of its length. Chromosome 2 is also usually distinguished from all others. Chromosomes 3, 4, and 5 can be identified one from the other in exceptional preparations only. Chromosome 6 does not possess an easily discerned satellite at mitotic metaphase, although it is observed during mitotic prophase. Root tips germinated in .02% 5-bromodeoxyuridine contain cells with satellites at mitotic metaphase. Two chromosomes possess satellites, and the length of the stalk varies. From preliminary studies this appears to be chromosome 6. Chromosomes 6, 7 and 8 cannot always be distinguished. Chromosomes 9 and 10 can usually be identified.

Graphs have been made of 10 cells using the length and arm ratios as axes. The points were grouped, and compared with the karyotypes which had been grouped visually. The measurements for the graphs were taken from projections of the negatives. The groupings were in most cases similar in graphs and the karyotype.

Measurements of the longest two and the shortest two chromosomes in projected cells were taken and used to determine the mean, standard error, and variance among cells. A group of cells was measured independently eleven times to calculate the experimental error. Confidence limits were calculated; analysis of variance, and t tests were completed.

The mean arm ratio for the longest two chromosomes was 1.19. The standard error was .00279.

The mean arm ratio for the shortest two chromosomes was 1.59. The standard error was .0264.

A t test showed that the two samples (long and short chromosomes) were not from the same population.