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The Technique of Giemsa Staining of Cereal Chromosomes

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Recently developed staining techniques have considerably enhanced cytogenetic studies in mammals. They result in differential banding of somatic metaphase chromosomes, permitting the identification of individual chromosomes. With these methods, all of the chromosomes have been identified in man, mouse, and many other animal genera; further, in mouse almost all the linkage groups have been correlated with specific chromosomes and chromosome arms.

Unfortunately, the application of these techniques to plant chromosomes has not been particularly successful. One of the differential staining techniques, Giemsa C-banding (C = constitutive heterochromatin), which was first applied to animal chromosomes, involves denaturation-reassociation of DNA, with the highly repetitive DNA reassociating faster and appearing as dark bands.

Attempts have been made to identify individual plant chromosomes with conventional staining methods, but the interpretation of the results is difficult. In this communication we report a Giemsa staining procedure on grass chromosomes that can be routinely used and by which the individual chromosomes can be easily identified.

TECHNIQUE

1. Germination of Seeds:

A. Place seeds on damp filter paper in Petri dish for 24 hours at 25°C, 24 hours at 2°C, and then 24 hours at 25°C.

2. Root Tip Treatment:

A. Collect root tips when 0.5 to 1.5 centimeters long.

B. Place the detached root tips in a freshly prepared saturated solution of mono-bromonaphthalene in tap water. This solution can be made by placing one

or two milliliters of monobromonaphthalene in 250 ml. of water and violently agitating. The time of treatment at room temperature (72°F) varies from three hours for diploid species to three and a half for polyploid species. Too long a treatment will cause over-contraction and will obscure faint bands.

C. Fix in glacial acetic acid overnight (twelve hours minimum, three days maximum) in refrigerator (2°C).

3. **Enzyme Softening:**

A. Wash roots in distilled water and transfer to tubes filled with enzyme solution.

B. Leave root tips in enzyme for one to one-and-a-half hours at room temperature.

4. **Root Squashing** (put 100cc of 2x SSC and dish in oven to begin warming to 60°C):

A. Transfer root tip to distilled water for washing.

B. Place two or three tips on slide and cut off meristematic portion of the tip. Wipe off excess water and the elongated portion of the root.

C. Add small drop of 45% acetic acid and macerate completely.

D. Place cover slip on slide and tap vigorously.

E. Heat slide till warm to touch and press under filter paper.

F. Remove cover slip:

Freeze the macerated material under the coverslip by either placing the slide in contact with the carbon dioxide or spraying it from below with liquid CO_2. Pry off the cover slip before the frozen material melts.

5. **Dehydration:**

A. Place slide in fresh 95% ethyl alcohol (see discussion).

B. Remove slide and air dry.

6. **Barium Hydroxide Denaturation:**

A. Place slide in barium hydroxide solution for five minutes. (Keep dish covered to prevent carbonation.)

B. Rinse slide in distilled water for ten minutes, changing the water three times.

C. Air dry.

(Start filtering Giemsa stock solution)

7. **2x SSC Renaturation:**

A. If slide has a film of barium hydroxide on it, place in cold SSC for four minutes.

B. Place in hot (60°C) 2x SSC for one hour.

C. Wash in distilled water for ten minutes and change water three times.

D. Air dry. (During the drying prepare the staining solution and remove the scum from the surface.)

8. Giemsa staining:

A. Stain in Giemsa for appropriate time.

B. Wash in distilled water. If it is not stained enough, restain. If it is overstained, decolorize in 95% ethyl alcohol.

C. Air dry. Place in xylene overnight. Mount with Canada balsam.

SOLUTIONS

Enzyme Solution

1. The enzyme solution is made up by adding 500mg of pectinase and 500 mg of cellulase to 10cc of distilled water to which six drops of 1N hydrochloric acid are added. This solution should not be used for 24 hours after being made, but it will keep for one to two months in the refrigerator. Tubes containing the enzyme may be reused but should be kept corked and at about 2°C when not in use.

Barium Hydroxide

1. 100cc distilled water + about 5 gm barium hydroxide till solution is saturated. Shake vigorously while preparing.
2. Use fresh solution every time.
3. Keep bottle well stoppered.

2x SSC (saline sodium citrate)

1. 8.716 gm sodium chloride + 4.410 gm sodium citrate + 6 drops 1N hydrochloric acid + 500cc distilled water.
2. Heat to 60°C before using.
3. Stock solution keeps about two weeks at 2°C.

Giemsa Stock Solution

1. 1 gm Giemsa powder + 66cc glycerin + 66cc methanol.
2. Dissolve Giemsa powder in the glycerin at 60°C for one hour with constant stirring.
3. Add methanol and continue stirring at 60°C for one day (24 hours).
4. Keep refrigerated when not in use.
5. Stock solution will keep one or two months.

Citrate Buffer

1. 2.1 gm citric acid + 100cc distilled water = A.
   14.2 gm sodium phosphate + 500cc distilled water = B.
2. Mix 4.55cc of A with 15.45cc of B to prepare stock citrate buffer.

Giemsa Staining Solution

1. 5cc filtered Giemsa stock solution + 1.5cc methanol + 1.5cc stock citrate buffer + 60cc distilled water.
2. Before using skim the top of the staining solution.

Sources of Chemicals

1. Cellulase (200 units/gm.)—E. Merck, catalog number 2329
2. Pectinase (1.1 units/gm., polygalacturonase)—Sigma Chemical Company, catalog number P-4625.
3. Giemsa powder—Fisher Chemical Company, catalog number G-146.
4. The source of all other chemicals did not prove to be critical.

DISCUSSION

This technique has been used to investigate a range of grass chromosomes. Giemsa stained karyotypes have been produced for *Secale, Triticum,* and some of its diploid and tetraploid relatives, and banding has been observed in *Fescue, Agropyron, Elymus, Hordeum,* and *Avena.*

Several aspects of the technique require greater attention than do others. The length of time in the mono-bromonaphthalene solution is important. During this phase of the technique the spindle is inhibited, and the chromosomes contract. Too long a treatment will result in overly contracted chromosomes and will obscure faint bands. Too short a treatment will not allow the easy recognition of individual chromosomes. The treatment is temperature sensitive and appears to be accomplished best at about 70° to 72°F.

The time in alcohol (stage 5B) has optimum values for different species, with a degradation of the staining occurring with divergence from the best time. The best time for wheat is between 1¼ and 1½ hours, and rye requires 2½ hours.

The length of the Giemsa staining also should be adjusted so that alcohol decolorization is generally not required. The times used have ranged from as little as 15 seconds to as long as one and a half minutes.

As with all techniques used in identifying individual chromosomes, there are limitations to the reliability of the data obtained. If it is possible to identify the chromosomes by other techniques, then the banding pattern can be assigned with certainty to that chromosome. For example, the satellited chromosomes are conspicuous in any case and thus their banding pattern is easily identified. It is better to construct karyotypes of species from single, whole cells rather than from assembling a number of individual chromosomes. By using a complete cell, each chromosome can be identified disomically and the possibility of confusion between similarly banded chromosomes is reduced.