Comparing Onion Chromosome Squashes with Wheat Chromosome Squashes

Introduction

When preparing chromosomes for viewing, there are six main steps in the protocol. They are pretreatment, fixation, hydrolysis, processing, staining, and mounting. To save time, tissue samples have already undergone pretreatment and fixation. Today you will hydrolyze, process, stain, and mount the specimens for viewing. At the end of the session, you will be able to view the slides that you prepared, determine the number of chromosomes in each species, and discuss the importance of ploidy in plants.

Preparation of root tips samples

(This was done prior to today's session.)

To prepare the root tips samples for staining, they will need to be pretreated in ice water for 24 hours. Then the root tips will need to be fixed in Carnoy's solution I. Both of these steps may be done by students or they can be done by the teacher ahead of time. Protocols for pretreatment and fixation are from the Wheat Genetics Resource Center Electronic Lab Manual found at http://www.k-state.edu/wgrc/electronic-lab.html.

Step 1: Pretreatment

Purpose of pretreatment

The pretreatment of meristematic tissue serves two purposes. By preventing the formation of micotubuli, dividing cells are arrested at mitotic metaphase, thus, the number of cells in metaphase is increased. Second, the chromosomes are more contracted and shorter, making chromosome counts easier.

Ice water pretreatment

Cut roots when they are approximately 1.5 to –2-cm long. Place in glass vials that contain 2-mL tap water cooled to 1°C in an ice-water bath. Add a small piece of paper with the description of the material, cap tube, and return to the ice-water bath. Fix the material after 24 h. The mitotic index after ice water pretreatment is much higher compared to the other treatments, because the cells are arrested in metaphase during 24 h rather than only 3 h as with other pretreatment procedures.

Step 2: Fixation

Purpose of fixation

Fixation of meristematic tissue allows immediate death of the tissue. It also causes coagulation and precipitation of proteins to change the refractive index of the chromosome. The fixative will prevent bacterial growth and decomposition of root tissue. It will cause the chromatin to precipitate and make the chromosome visible. Finally, it helps with the adherence of acidic stain on the chromosome. In general, fixed material can be stored at 4°C for several months.

In the Carnoy's solution I (Farmer's solution), ethanol precipitates nucleic acid by causing irreversible denaturation of protein. Because of its dehydrating property, ethanol can also cause

undesirable hardening of the tissue. Acetic acid also precipitates nucleic acid. When tissue is kept in acetic acid for a prolonged period, the acetic acid dissolves histones on the chromosomes leading to their degradation.

Carnoy's solution I (Farmer's solution)

Carnoy's I is the most commonly used fixative and gives good results for a large number of different species and tissues.

1 part glacial acetic acid
3 parts 95% or absolute (100%) ethanol

For one test tube rack of 72 test tubes where each test tube is filled with 10mL of fixative, use the following:

180 ml glacial acetic acid
540 ml 95% ethanol

For today's session, you will follow the instructions below to prepare your root tip samples for viewing.

Step 3: Hydrolysis and Step 4: Processing

Participants should place the root tips into the test tube of fixative and incubate at 50 degrees Celsius for six minutes. Then participants should pour the heated fixative and tips into a watch glass. Next, using tweezers, participants should remove the root tips, one at a time, from the watch glass and place each in the middle of a microscope slide. Using scissors, participants should cut all of the excess from the root tips except for 2mm at the bottom of the root (*note: the end that was not cut from the plant*).

Step 5: Staining

Participants should place two drops of acetocarmine stain on top of the 2mm root tip. The stain should soak into the root tip for two minutes. Using the metal spatula, participants should squash the root tip, on each slide, pressing straight down so as not to overlap the cells. Participants should apply two more drops of stain upon the root tip and wait for another two minutes.

Step 6: Mounting

Next, participants should place the cover slip flat upon the root tip, making certain not to move the cover slip horizontally. Using a pencil eraser, student should press the coverslip gently straight down without moving the coverslip. Participants should soak up extra stain from the slide around coverslip with a paper towel without moving the coverslip.

Viewing of Tissue Sample and Analysis

Finally, participants should observe and record steps of cell division under the microscope (at 400x). Students should make a sketch of what they see in their lab notebooks and label the different structures they see on the chromosomes. (*Note: students will only see individual chromosomes with particular banding patterns. They will only be able to label the chromosomes and the chromosome bands.*)

Next, students should determine the length of the stages of mitosis by first locating the meristem region of the root tip. Students should started by looking at the squash using the 10x objective and find the region of active cell division. They should then switch to the 40x objective and begin observations at the lower end of this region. Student pairs should take turns as observer and recorder. The observer should call out the stage of mitosis of each cell that they observe for the first and second slide. These should be tallied by the recorder in the results table. Roles should be switched for the second and third slide. Since prophase and prometaphase are difficult to distinguish, classify these cells as prophase. Only count as prophase cells that contain distinctly visible chromosomes.

The observer should systematically scan the root tip moving upward and downward through a column of cells. The recorder should tally each cell in a stage of mitosis that is observed (*note: participants should take careful not to record the same cell twice in the tally*). For each slide, twenty mitotic cells should be counted Tally the stages of those 20 mitotic cells in the table provided. Participants should calculate the percentage of cells at each stage in mitosis by using the data collected from all four slides.

The teacher should bring the class back together and have all student pairs pool their raw data with that of the class. The teacher should record the class totals in the table on the board. Student groups should calculate the percentage of cells in each stage using the combined class data. (*Note: the relative time span of each stage is equivalent to the percentage of cells found in mitotic stage.*) In their lab notebooks, they should compare and contrast their group's percentages with the entire class percentages.

(*Note: Activity is modified from Babich, H., Segall, M.A. and Fox, K.D. (1997). The Allium Test–A Simple, Eukaryote Geneotoxicity Assay. The American Biology Teacher. 59, 580-583.*)

Students should then find five cells that are at metaphase and count the number of chromosomes in each cell. Students should record the number they counted in student data table. (*Note: Students should find that onions have eight chromosomes and are considered to be diploid. The diploid number of chromosomes for the other plant tissues are as follows: Hordeum vulgare (barley) (14), Secale cereale (rye) (14), Triticum urartu (red wild einkorn) (14), Triticum aestivum (bread wheat) (42), and durum wheat (28)*)

Analyzing what you see

Teachers should give the number of chromosomes for *Hordeum vulgare* (barley) (14), *Secale cereale* (rye) (14), *Triticum urartu* (red wild einkorn) (14), *Aegilops speltoides* (goatgrass) (28), *Triticum aestivum* (bread wheat) (42), and durum wheat (28). The teacher should ask the relationship between the chromosome number between the different species and give them some time to determine any mathematical relationships. After giving students several minutes, the teacher should ask students what the specific number (prime factorization/greatest common multiplier) that the entire numeric set has in common. Explain that greatest common multiplier is the haploid number (N). (In this case, the haploid number is 7) The multiplier for haploid number is the ploidy (for example 2N=diploid, 3N=triploid, 4N=tetraploid, 6N=hexaploid).

Ask students to determine the ploidy for each of the grains, if the haploid number is 7. Then explain how scientists use the connection between the number of chromosomes within the Tricetacae tribe (ancestral grains, barley, rye) to develop the phylogenetic tree that they constructed several days ago. Further explain to the students that it is this understanding of the wheat genome that will enable insight into the standing genetic diversity for wheat improvement. This will enable genomics-assisted breeding on a level needed for developing high-yielding, climate-resistant wheat varieties. Scientists are finding that the size of the genome complicates things. If you compare rice, barley, and wheat in terms of genome size, there is a 35-fold increase in genome size from rice to wheat. Barley has a genome that is 10 times larger than rice. Wheat has a genome that is three times larger than barley. Wheat is five times the size of the human genome.

Elaborate on the Activity

The teacher should explain to student what does chromosome number tells us and explain the benefits of ploidy to plants. It is important to mention that plants have large areas of the genome make of repetitive elements (80%). Currently, only two percent of the entire genome represent genes that we can identify. The teacher should focus on how to increase genetic diversity (*Note: repetitive gene sequences are not diverse)*.

Evaluating the learning that occurred

Students should take the posttest.

An alternate protocol from the Wheat Genetics Resource Center Electronic Lab Manual found at http://www.k-state.edu/wgrc/electronic-lab.html.

Acetocarmine Staining

Acetocarmine preparation (1% solution)
Carmine is a basic dye that is prepared from the insect Coccus cacti. Dissolve 10 g carmine (Fisher C579-25) in 1 L of 45% glacial acetic acid, add boileezers, and reflux for 24 h. Filter into dark bottles and store at 4°C. This solution can be stored for a long time. Staining can be intensified by adding ferric chloride ($FeCl_2 \cdot 6H_2O$); add 5 mL of a 10 % ferric chloride solution per 100 mL of % acetocarmine.

Acetocarmine staining
To stain plant chromosomes, a 1% solution of carmine in 45% acetic acid is used. Freshly fixed material is transferred into 1% acetocarmine for at least 30 min and then analyzed by the squash method. If the material was fixed for a longer time, it requires a longer staining time (up to several days) to reach good contrast. If the material is to be analyzed immediately, fix and stain the tissue in one step using the 1% acetocarmine solution.

Chromosome squash technique
Drain off the fixative and place the roots in 1% acetocarmine for 1 to 3 h. Heat until the acetocarmine begins to boil. Cut off the root cap with a razor blade and squeeze the meristematic tissue out with a lancet needle. Add a drop of acetocarmine or 45% acetic acid. Place a razor blade (double-edged) to one side and add a cover slip. Tap the cover slip gently with the needle end of a probe. Slide the razor blade out and heat to a point just below boiling (steam will form beneath the slide). Then, quickly squash with thumb or forefinger between two layers of filter paper. Be careful to not move the cover slip at this point.