Karyotype of *Hordeum vulgare* (2n=14)

In this week's lab, we will expand on last weeks procedure by characterizing the chromosomes or karyotype of barley. Essential to the lab report is a good drawing with a scale added for measuring the length of the chromosomes (karyogram), including the exact location of the centromere and any secondary constrictions. These measurements and ratios will be used to pair the chromosomes and prepare an idiogram (a diagramatic sketch or interpretive drawing) of barley.

The protocols are essentially the same as in lab one, but we will switch to Feulgen stain, which is specific for the chromosomes only. Feulgen stain is a bit more tricky to work with than aceto-carmine. Feulgen stains everything it comes into contact with purple, has a limited shelf life and if it contaminates the ethanol storage solution or the HCl it will make future staining of the chromosomes impossible.

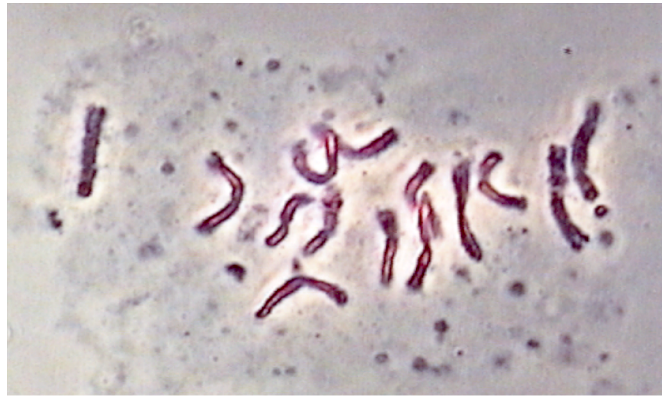

Figure 1 ***Hordeum vulgare stained with Feulgen stain. 2n=14, Magnification 1260X***

Procedure

Pre-treatment: Clean barley seed is placed on moistened filter paper in the dark until roots emerge and are 2 cm long. Root tips are harvested and placed in ice water overnight at 4ºC.

Fixation: Farmers solution at 4ºC overnight.

Storage: 70% ethanol at 4ºC.

Hydrolysis: Transfer the root tips to 1N HCL at 60ºC for 8-10 minutes. Rinse the root tips with distilled water and transfer to a clean vial..

Staining: Add the Feulgen stain and place the root tips in the dark. Staining will take between 10-20 minutes. Be sure to keep any tweezers that have come in contact with the stain separate or clean with Comet and rinse thoroughly. The chlorine in the cleaner will deactivate the stain.
Once the root tips have stained dark purple excise the tip and place in a drop of 45% acetic acid on a slide. Macerate carefully and apply a cover slip. Feulgen stain will fade over time so be sure to place your remaining root tips back in the dark. Check your slide carefully for a good metaphase cell. Remember the more detail at 40X the easier it will be to draw and therefore measure at 100X.

Photo: After finding the appropriate metaphase cell, take the chromosomes photos as carefully as you can under the Leica microscope. Take care to note the exact location of the centromere, the satellite length and the location of the end of each arm. Find the lines in the centre of the circle and focus under at 100X as you did with your photo. Carefully add the scale below your photo. Draw vertical lines below your photo spaced as they are on the slide, the space between two lines represents 10 microns. Use this scale by converting it to mm and measure your chromosomes and then convert back to microns for the final report. Include in your report this photo with the scale, a table showing the measurements and pairing and the karyotype represented as an idiogram. The idiogram is drawn longest to shortest chromosome. The labeling on your original drawing should also represent your pairing ie. change the original labels to represent the pairing you have made through the measurements and ratios.