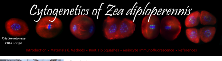

Zea diploperennis plant growing in the UGA greenhouse

Phylogeny of *Zea* genus, modified from Vollbrecht & Sigmon 2005

Introduction

Zea diploperennis is a member of the *Zea* genus, which contains maize (*Zea mays* ssp. *mays*) or corn, one of the most agriculturally-important crops grown today. Maize was domesticated roughly 10,000 years ago in modern-day Mexico from *Z. mays* ssp. *parviglumis* (Vollbrecht & Sigmon 2005). Collectively, all members of the *Zea* genus besides maize are referred to as "teosinte". Two teosinte species, *Z. perennis* and *Z. diploperennis*, are perennials unlike the other members of the genus that belong to a clade containing only annual species. These perennial teosintes therefore represent interesting organisms to study in understanding how perenniality was lost in other *Zea* species and if perenniality genes or alleles could be bred into cultivated maize.

Studying perenniality in *Z. perennis* is difficult due to the fact that it is an autotetraploid (2n=4x=40) and generally incompatible with diploid maize (2n=2x=20) (Tiffin & Gaut 2001). *Z. diploperennis* was thought to be extinct until 1979 when it was rediscovered in wild Mexican populations but has not been widely studied for its perennial behavior, despite being a diploid (2n=2x=20) (Doebley JF, Guzman RM, & Pazy B 1979). However, a mapping experiment using *Z. diploperennis* x *Z. mays* ssp. *parviglumis* from John Doebley's lab did reveal 38 quantitative trait loci (QTL) controlling aspects of perenniality, such as rhizome formation and tillering (Westerbergh A & Doebley J 2004).

Z. diploperennis has also been used to study pathogen resistance, cytogenetics, and comparative genetics, in part to understand questions about the systematics and origin of the *Zea* genus. In an interesting cytogenetic study, tassels from the trihybrid plant [*Z. diploperennis* x *Z. perennis* (2n=4x=40)] x *Z. mays* (2n=4x=40) were colchicine-doubled and analyzed for pairing. Tetravelents were observed, indicating recombination can occur between the *Z. diploperennis*/*Z. perennis* and *Z. mays* genomes (Molina et al. 2004). This result shows that genes from the perennial teosintes can be moved into maize by recombination to aid in crop development.

Here, I present work from my cytogenetic studies on *Z. diploperennis*. I performed root tip squashes to determine chromosome number and immunofluorescence (IF) of pollen mother cells to capture a meiotic series. To my knowledge, this is the first report of IF being performed in *Z. diploperennis*.

Next: Materials & Methods

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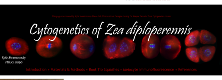

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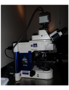
An immature *Z. diploperennis* tassel used for meiocyte immunofluorescence



The Dawe Lab Zeiss AX10 used for fluorescence microscopy.

Materials & Methods

Plant Materials and Growth

Root tips for squashes were obtained from young etiolated seedlings. *Z. diploperennis* seeds were punctured with a razor blade to penetrate the woody shell. Seeds were germinated in the dark for 2-5 days in a sealed container with vermiculite at 30°C.

An immature tassel was obtained in April 2017 for meiocyte immunofluorescence (IF) from a *Z. diploperennis* plant maintained by the Dawe Lab in the University of Georgia Dept. of Plant Biology greenhouses.

Root Tip Squashes

Small ends of roots (~1cm) were removed from seedlings and treated with a 8-hydroxyquinoline solution containing cycloheximide at 4°C for four hours. Root ends were then fixed in Farmer's (6 ethanol:3 chloroform: 1 glacial acetic acid) at room temperature overnight and stored in 70% ethanol at 4°C until staining. Root ends were hydrolyzed in 5M HCl for five minutes before the tips were removed and squashed in modified carbol fuchsin stain.

Meiocyte Immunofluorescence

Meiocytes were prepared and stained using a protocol developed for maize by past and present members of the Dawe Lab and University of Georgia (Li X, Lowry E, & Higgins D). Primary anthers were removed from florets on the tassel and fixed for two hours in a solution containing 4% PFA, 1.5X PHEMS (45mM PIPES, 18.8mM Hepes, 7.5mM EGTA, 1.5mM MgCl2, .26M D-Sorbitol, pH6.8), 1% Triton-X100. Anthers were then washed in phosphate-buffered saline (PBS) three times. Meiocytes were extruded from anthers and adhered to polylysine-coated coverslips by centrifugation at 100xG for one minute.

Meiocytes were permeabilized by incubating in PBS, 1mM EDTA, 1% Triton X-100 for one hour, then washed three times in PBS. Meiocytes were then blocked using 10% goat serum in PBS for two hours, then washed three times in PBS. The coverslips were incubated in antibody solution (PBS, 3% BSA) containing mouse anti-tubulin mAb (1:200) and rabbit anti-CENPC pAb (1:200) primary antibodies overnight at 37°C. The coverslips were washed three times with PBS. An additional blocking as well as washing steps were repeated as above. The coverslips were incubated in antibody solution containing goat anti-mouse pAb Rhodamine (1:100) and goat anti-rabbit pAb FITC (1:100) secondary antibodies in the dark for two and a half hours, the coverslips were washed three times in PBS. The coverslips were mounted on slides using Prolong Gold with DAPI.

Microscopy/Imaging

Micrographs of root tip squashes were obtained with the Parrott Lab microscope using a color camera.

Fluorescence microscopy of meiocyte IF was performed on a Zeiss AX10 using the Slidebook software. The 100X objective was used and exposure times for each channel were as follows: DAPI 1000ms, FITC 1500ms, Rhodamine 750ms. Images were taken in the Z-plane at every 0.25µm through the entire cell and the maximum Z projection was taken for visualization. Contrast of each channel was optimized using the Slidebook software.

Non-microscopic photographs were taken using an Olympus TG-4 camera.

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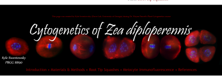

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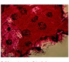
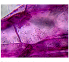


Z. diploperennis root tip cells in telophase.

In this image, the large cell near the center is undergoing **telophase** to complete cell division. The chromosomes have migrated towards each daughter cell and the nucleus is beginning to reform. Here you can see the chromosome number, 2n=2x=20.



Z. diploperennis root tip cells in prophase.

Here, this **prophase** cell has just duplicated its genome and 40 chromosomes are present. The cell is in the process of dividing, and will soon direct 20 chromosomes to each side of the cell for division.

Next: Meiocyte Immunofluorescence

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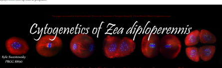

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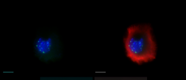
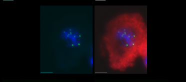
In all images below, colors are as follows:

Blue=DAPI (labeling chromosomes)
Green=FITC (labeling kinetochores using a CENPC antibody)
Red=Rhodamine (labeling the spindle/phragmoplast using a tubulin antibody)
DAPI/FITC channel overlay is shown on left, DAPI/FITC/Rhodamine channel overlay is shown on right

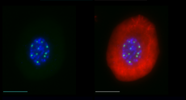
Scale bars are 10µm



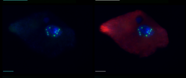
Zygotene: 17 centromeres are visible, indicating that chromosomes have not yet paired. Chromosome "bouquet" is visible.



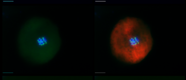
Pachytene: Chromosomes are beginning to condense and crossing over is occuring. Notice that the twenty centromeres are found in tight pairs, indicating that the chromosomes are paired.



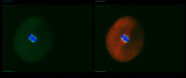
Diplotene: Crossing over has completed and the chromosomes are fully condensed. The nuclear envelope has not yet broken down.



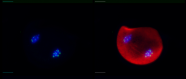
Diakinesis: The nuclear envelope breaks down and the nucleolus is now visible. The spindle begins to form at the poles.



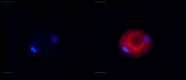
Prometaphase I: The spindle forms and begins to align the chromosomes in the center of the cell at the metaphase plate. The nucleolus has broken down.



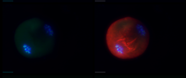
Metaphase I: The chromosomes have aligned at the metaphase plate and prepare for segregation towards the poles. The spindle has formed and connected to the kinetochores.



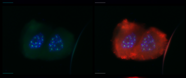
Anaphase I: The chromosomes move along the spindles to the poles. Notice the furthest part of each chromosome along the spindle is the centromere.



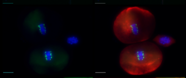
Telophase I: The chromosomes have reached the poles and the spindle is being restructured to form the phragmoplast.



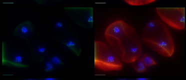
Cytokinesis I: The growing phragmoplast separates the cell into two daughter cells. Meiosis I is complete.



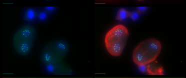
Prophase II: Now in two new daughter cells, the chromosomes await the second meiotic division.



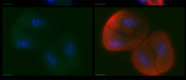
Metaphase II: The spindle has aligned the chromosomes along the metaphase plate.



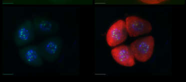
Anaphase II: The chromosomes move along the spindle towards the poles.



Telophase II: The chromosomes have reached the poles and the phragmoplast begins to form to divide the daughter cells.



Cytokinesis II: The phragmoplast divides two cells into four.



Tetrad: The meiocyte has successfully divided into a tetragonal tetrad. Meiosis is complete and microgametogenesis will occur.

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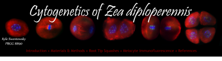

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