Protocol Development for Enhanced DNA Visualization in the California Bay Laurel

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Abstract

California native plants provide a promising alternative to traditional model systems to study chromosome organization. While observational field studies exist for detecting and quarantining the pathogen *Phytophthora ramorum* (*P. ramorum)*, there is a lack of molecular tools to understand the pathogen and its foliar host, the California Bay Laurel (*Umbellularia californica). P. ramorum* is an invasive oomycete eukaryotic microorganism that is a pathogenic agent for the disease, Sudden Oak Death (SOD). This pathogenic agent is a cause for concern as it is responsible for the death and dramatic decline in coastal tanoak tree populations. In coastal rural regions, *P. ramorum* is a risk against the ongoing efforts of preservation of biodiversity, as 14% of Sonoma County has been affected by SOD. Observing chromosomes *in vivo* of *U. californica* is needed to develop molecular protocols for non-traditional host organisms. Our project aims to refine a histological DNA staining protocol for staining *U. californica's* chromosomes. This will provide the basis for future work to investigate the effects of *P. ramorum* on chromosomal organization in normal and infected states of the Bay Laurel. By using the Bay Laurel as a new model system to study native host-pathogen interactions, it will allow us to unravel the molecular mechanisms of infection and develop strategies to protect California's threatened coastal forest populations from SOD.

Cause and Impact of Sudden Oak Death in California

Figure 1. **Cause and Impact of Sudden Oak Death in California**. *Phytophthora ramorum* is an oomycete that is the source of Sudden Oak Death (SOD) that leads to mortality in tanoaks and oak trees (Rizzo, Garbelotto 2003). The spread of *P. ramorum* is mediated by rain and windfall dispersal from the leaves of one of its foliar hosts, the *Umbellularia californica* where infection is observable through identification of spores. **A)** Photo of California, SOD Blitz on a Google Earth map created by UC Berkeley. Shows Infected and Non-Infected California Bay Laurel. SOD has killed millions of Tanoaks and Oak species across 16 California Coastal Counties. **B)** Photo from the SSU campus using Google Maps to help visualize the infected *P. ramorum* Bay Laurel trees.

Importance of California Bay Laurel Apical Meristem

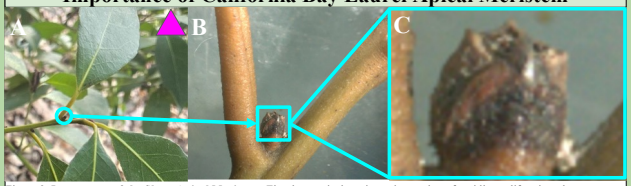
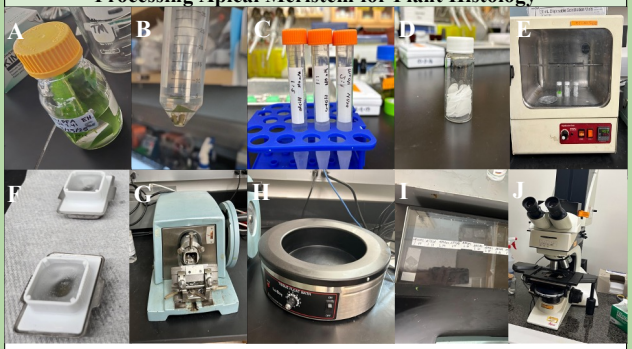


Figure 2. **Importance of the Shoot Apical Meristem**. The shoot apical meristem is a region of rapidly proliferating plant stem cells that are responsible for aerial plant growth (Kean-Galeno et. al 2024). This site is ideal to observe mitotic cell activity and observe immature plant cell walls that have greater permeability than mature cells. **A)** Photo taken from SSU campus, a normal California Bay Laurel sample attached to a tree. Blue circle is highlighting the apical meristem before collection and processing. **B)** Enlarged image of apical meristem captured using a dissecting microscope, after collection and preservation in 4% paraformaldehyde. **C)** Magnification from the previous image to emphasize the apical meristem.

Processing Apical Meristem for Plant Histology



A) Tissue collection. Healthy Bay Laurel samples were collected from SSU campus and fixed in 4% PFA to preserve tissue for histological processing. **B)** Fixation. Apical meristem was isolated from the stem and fixed in 100% methanol to preserve cellular structure. **C)** Clearing. Tissues were cleared using a methanol:Histoclear series (2:1, 1:1, 1:2) followed by 100% Histoclear (45 min each). This removes debris and prepares tissue for sectioning. **D–F)** Embedding. Tissues were infiltrated with Paraplast wax (melted in a hybridized oven), placed into steel molds with fresh Paraplast, and solidified at room temperature. **G)** Sectioning. Embedded samples were sliced into thin sections using a retracting microtome. **H)** Rehydration and staining. Sections were rehydrated through an ethanol series (100% to 30% EtOH, 1 min each; 10% PBS washes, 2 min each), then stained with DAPI to label DNA. Slides were coverslipped and stored in the dark. **I)** Imaging. Stained sections were imaged using a confocal microscope (Leica, 40x). DAPI was excited at 405 nm and emission detected at 461 nm (blue).

Modifications. Extended DAPI and methanol exposure (15 min each) were applied to enhance DNA binding.

Visualization of a California Bay Laurel Apical Meristem

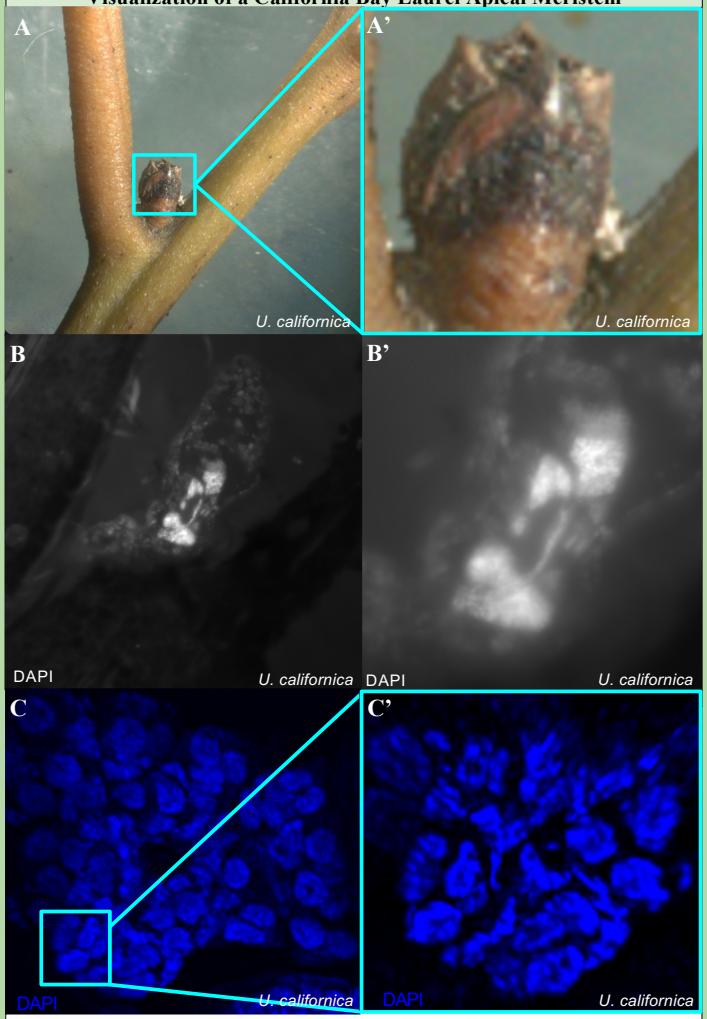


Figure 4. **Preliminary Data of Bay Laurel Apical Meristem. A–A')** Sample collection. A dissection microscope (Nikon E800) was used to view and capture the shoot apical meristem of a healthy California Bay Laurel sample, including a zoomed-in view of the meristem still attached to the stem. **B)** Epifluorescence imaging. DAPI staining revealed brighter fluorescence in the apical meristem, indicating possible mitotic cells. This is consistent with the apical meristem being a region of rapid cell division. In the black and white image, brighter white clusters of cells are visible in the center. **B')** Zoomed-in epifluorescence. A closer view of B' shows what could be individual mitotic cells grouped together with positive DNA staining. C) Confocal microscopy (3D). High-resolution 3D images (Leica confocal microscope) confirmed positive DAPI staining (blue fluorescence) in apical meristem cells. **C)** Zoomed-in confocal. A closer view of **C')** reveals structural features that may represent mitotic cells, possibly indicating tightly condensed DNA as chromosomes. Better visualization is needed for confirmation.

Optimizing DAPI Staining for Apical Meristem

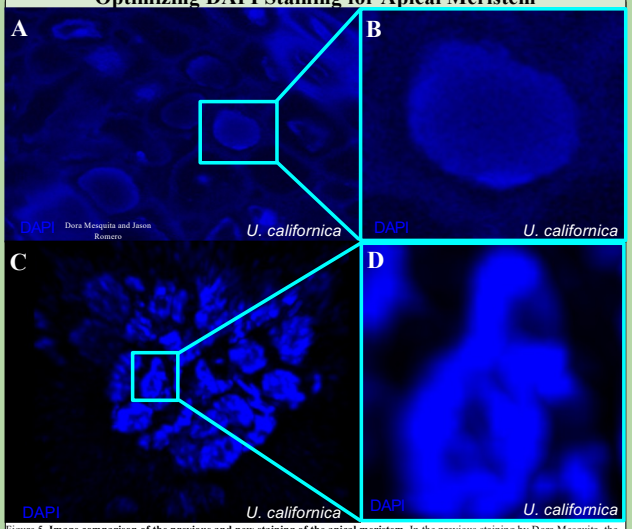


Figure 5. **Image comparison of the previous and new staining of the apical meristem.** In the previous staining by Dora Mesquita, the apical meristem was exposed to methanol and DAPI for 5 minutes. In our current modified procedure, a longer exposure time to methanol and DAPI was changed to 15 minutes to observe dehydration and penetration of the plant cell wall. **A)** We see the cell wall structure still present, and little to no detail of what may be the nucleus of the plant cell. This indicates poor DAPI penetration and binding to DNA. **B)** A zoomed-in image of what we suspect to be a plant cell and nucleus. **C)** Current staining protocol with increased exposure time of DAPI and methanol to 15 mins. More structural detail of what might be condensed DNA. No visible cell wall, indicating better dehydration and degradation of the sample. **D)** Zoomed-in image of the more detailed condensed DNA structure.

RPE-1 DAPI staining Comparison and Future Directions

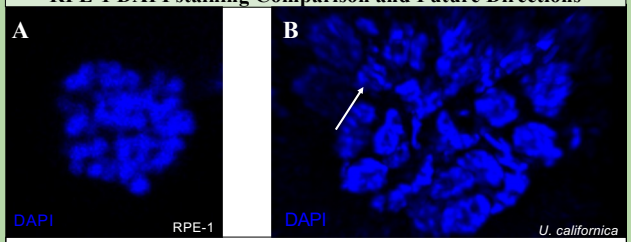


Figure 6. **Comparing DAPI staining of apical meristem to human RPE-1 cells. A)**When comparing a human cell, retinal pigment epithelial cell (RPE-1), imaged from our lab, we see a more detailed structure of DNA condensing down to chromosomes. **B)** When we compare this to our apical meristem sample, we see similar characteristics of what we suspect is condensed DNA. Our aim is to improve our native plant model so that we can observe more structural components of condensed DNA.

Future Direction:

- **Improve cell wall permeabilization**. Test vacuum infiltration and pectinase digestion to enhance stain entry past the cell wall (Nie-Can et al., 2013: Fal et al., 2021)
- **Compare healthy vs infected**. Apply optimized protocol to *P. ramorum*-infected apical meristems to assess changed in chromosome nuclear structure
- **Long-term goal.** Identify chromosomal markers of resistance for conservation and forest management.

Acknowledgements and References

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