



STRUCTURAL CHANGES THAT OCCUR IN PLANT TUMORS ARE SIMILAR TO CHANGES THAT OCCUR IN TUMORS OF ANIMALS: MODEL SYSTEM FOR A CELL BIOLOGY CLASS

Josh Hall and Allison M.D. Wiedemeier
College of Arts and Sciences, Department of Biology, ULM Howard Hughes Undergraduate Research Program,
The University of Louisiana at Monroe, Monroe, LA 71209



Abstract

Cellular organization, division rates, and gene expression can be compared between normal and tumorous tissue; therefore, gall formation in plants can be used as a learning model for changes that occur to cells during cancer formation. *Agrobacterium tumefaciens* can be used to generate tumors or galls in certain plants. When a plant becomes wounded and subsequently infected, a Ti plasmid is transferred from the *Agrobacterium spp.* into the plant cell's genome where changes in protein production take place. In this study, we grew tomato (*Solanum lycopersicum*) plants for two weeks and inoculated the stems with *Agrobacterium tumefaciens*.

Our goal was to determine the structural changes that occurred in tomato stems during gall formation. The plants were placed into four experimental groups. The null group was not wounded nor infected. The second group was wounded only. The third group of plants was wounded and infected with a non-gall forming *Agrobacterium tumefaciens* (A136). The fourth group was wounded and infected with a gall-forming *Agrobacterium tumefaciens* (Wildtype). After another two weeks of growth, we dissected, fixed, infiltrated, embedded in paraffin, and sectioned the stems. Differences in the structure of the plants were noted. We found that only plants infected with the wildtype bacteria formed distinct tumors on the stems. Through our microscopic studies we determined that this was caused by an expansion in certain tissues of the plant. The most dramatic increase occurred in the area of the cortex. Other changes that were documented include the induction of new vascular tissue and nuclear abnormalities. These are similar to changes that occur in animal tissue, therefore this system can be used to model cancer for a cellular biology class.

Background

Agrobacterium tumefaciens (Agro) is one of nature's most successful plant genetic engineers. It is the organism responsible for the formation of crown gall tumors in many plants. *Agrobacterium spp.* is a Gram-negative soil bacterium which causes these tumors by the transmission of a Ti plasmid into a plant cell. The plasmid DNA is then expressed (T-DNA). The activity of enzymes from the T-DNA initiate the production of the plant hormones, auxin and cytokinin, which lead to uncontrolled cell division and subsequently a tumor (Binns and Campbell). Our study focuses on cellular changes that occur inside the gall. Typical changes that occur in tumor cells of animals include increased cell division, abnormal cell shape, abnormal nuclei and the induction of new blood vessels (angiogenesis) (Kleinsmith).

In order for the *Agrobacterium tumefaciens* to transmit the plasmid, the plant requires wounding, which creates an entry point for the bacteria, a suitable environment for bacterial growth, and access to receptor sites on the plant cell walls. The T-DNA also encodes for the production of "opines" that cannot be metabolized by the plant but can and are used by the bacteria as a nutrient source (Binns and Campbell). It has also been determined that in order for the tumor to grow, new vascular tissue is initiated (Ullrich and Aloni).

Our goal was to determine if we could see and score similar cellular changes within the gall tissue as compared to changes that have been reported in animal tumor tissue.

Abnormal Tissue Expansion

Figure 1



Figure 1: Galled Tissue. A: Galled Tomato stem 7 weeks post inoculation. B: Galled section (scale bar of 1mm). C: Galled section (scale bar of 1 mm.). D and E: New vascular tissue in the cortex of a gall (scale bar of 100 μm).

Table 1

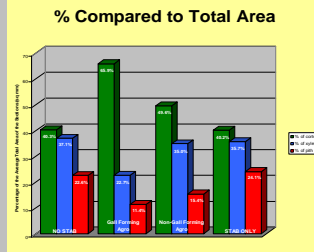


Table 1: Comparison of Tissues Within Cross Sections. Averages were calculated using between 2 and 7 sections for each group.

A large increase occurs in the Cortex of plants inoculated with gall forming *Agrobacterium*

Figure 2

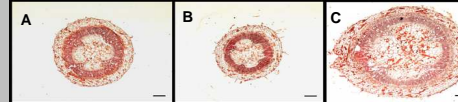


Figure 2: Experimental Controls. Part A and B show normal tissue distribution. A: Normal tomato stem not infected. B: Tomato stem wounded only. C: Tomato stem wounded and infected with *Agrobacterium tumefaciens* A136 (non-gall former). (All scale bars = 1mm)

The No Stab group on graph is represented by section shown in Figure 2A. The Stab Only group on graph is represented by Figure 2B. The Non-Gall Forming group on graph is represented by Figure 2C. The Gall Forming on graph is represented by Figure 1B.

Methods and Materials

The following methods produced optimum results in the least amount of time.

Fixation, Dehydration, & Infiltration

- Stem tissue was hand sectioned into 50 mM Pipes buffer
- Sections were then fixed in 4% paraformaldehyde/ Pipes buffer for 1 hour.
- Sections were then rinsed in 50 mM Pipes Buffer for 30 minutes.
- The samples were then dehydrated through an ethanol series consisting of 25%, 50%, 75%, and 95%. Each step was 1 hour.
- Then the sections were place in 100% ethanol for an hour, including a drop of gentian violet stain. Another 1 hour 100% ethanol incubation followed.
- The alcohol was replaced with 100% Xylene for 1 hour. This was repeated two more times. During the last Xylene step a few paraffin chips were added.
- This Xylene/Paraffin mixture was replaced by 100% paraffin and placed at 56–62°C for 1 hour. This was repeated twice with the second time increased to two hours.
- The sections were then removed from the paraffin and immediately place in molds in which more paraffin was poured. The blocks quick cooled over ice.

Sectioning

- The molds were trimmed under the dissecting scope
- An iron knob was heated and placed on the back of the mold for the adhesion of the block to the knob
- The knob was then placed on the microtome for the block to be sectioned
- The microtome was set to a thickness of 10 μm
- After a length of ribbon was sectioned, it was placed in a hot water bath to be placed on a clean slide for dewaxing

Dewaxing, Rehydration, and Staining Process

- Slides were dewaxed using three serial incubations of 100% Xylene, each 5 minutes in length.
- The slides were then treated with a decreasing ethanol series: 100%, 95% and 70%. Each incubation was 5 minutes long.
- The slides were then rehydrated in distilled water for 5 minutes and then placed in 50 mM Pipes Buffer Rinse for 10 minutes.
- Slides were then simple stained.

Conclusion

We consistently see similar changes in all galled tissue. These changes include an increase in cell number, abnormal nuclei, and the induction of new vascular tissue. All of these changes are very similar to the changes that occur in animals; therefore, we can use this as a model system in a classroom setting. Our future plans include using this in Cell Biology in the Fall and performing semi-thin sectioning on the gall tissue to analyze ultrastructure within the cells, specifically looking at the formation of spindles within dividing cells.

References

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Acknowledgments

We would like to extend a special thanks to a number of people including Mr. Kris Kelley, Dr. Tom Sasek, Dr. Joe Pankey, and Mr. Dennis Bell for their expertise. We would also like to thank Dr. Tom Phillips at the University of Missouri–Columbia for sharing his knowledge of microscopy techniques. This work was supported through the Howard Hughes Undergraduate Research Program and the ULM Biology Department.

Abnormal Nuclei

Figure 3A: DAPI stained nuclei found in the cortex of a normal section with no infection

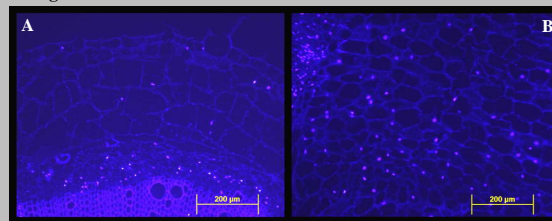


Figure 3B: DAPI stained nuclei found in the cortex of a gall.

Figure 4

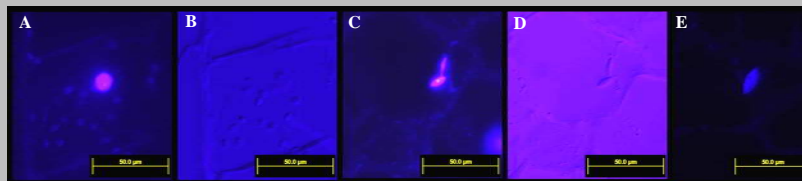


Figure 4: Nuclei Comparison Between Normal and Gall Tissue. A: DAPI stained nuclei found in the cortex of a normal section. B: Outline of the cell wall from same section in A. C: Two DAPI stained nuclei found in a single cell within gall tissue. D: Cell wall boundary of cell shown in C. E: Shows the shaped nucleus present in gall tissue.